Impact of scavenging versus predation on selected aspects of brown recluse spider, *Loxosceles reclusa* (Araneae:Sicariidae), biology

by

Robert "JR" Ewing

B.S., Kansas State University, 2013
B.S., Kansas State University, 2013

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Entomology
College of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2018

Approved by:

Major Professor
R.J. Whitworth

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Abstract

The brown recluse spider (BRS), *Loxosceles reclusa* (Gertsch & Mulaik), receives unfavorable publicity because of its common association with humans and the medical importance of its toxic bite. BRS range includes much of the south and central United States where they can be found in almost all structures, from homes and sheds to woodpiles and discarded materials. Typical management techniques for the control of BRS involve the use of residual contact insecticides and/or the use of glue traps. Contact insecticides rely on BRS remaining in contact with a treated surface for a length of time to achieve control and may not cause significant BRS mortality. However, if the insecticide kills another household pest that the BRS later scavenges upon, and thus results in the death or decreased reproduction of the BRS, the homeowner rids themselves of pests and potentially reduces BRS populations. This research was initiated with the objective of evaluating how feeding on live vs. dead vs. insecticide-killed prey impacts selected biological aspects of BRS as indicated by, mortality, and weight change over an eight-week period. Follow-on experiments evaluated the effect on fecundity of the adult BRS surviving the eight week trials. In four trials of juvenile and five trials of adult BRS, house crickets, *Acheta domesticus* (Linnaeus), used as prey, were exposed to one of four treatments: 1) Spinosad insecticide treated surface, 2) synthetic pyrethroid insecticide treated surface, 3) freeze-killed and 4) an untreated (live), and fed to spiders once weekly for eight weeks. BRS exposed to synthetic pyrethroid-killed crickets had significantly greater mortality than all other treatments.

Table of Contents

List of Figures

List of Tables

Acknowledgements

First my lovely wife Mary, whom I never thank enough, thank you for your patience and understanding. I could not have done it without your love, help and support.

My advisor, mentor and friend, Doctor Jeff Whitworth. You have “the patience of Job” especially when dealing with me, thank-you Boss.

The members of my committee: Doctor Phil Sloderbeck and Doctor Bob Pfannenstiel, thank you. You both were always there for questions and guidance, even if I did not ask.

The Kansas State University Insect Zoo, Kiffnie Holt. You shared knowledge, assisted in getting the brown recluse colony started, and were always there when I had a question or needed crickets, a very heartfelt thank you.

Last but certainly not least, the members of the Whitworth lab “KSUBugCrew”. Thank you all!

Doctor Holly Schwarting and Elise: Arthropod collectors and photographer without peer, your assistance was immeasurable!

The Whitworth lab “Spider Wranglers”: Keil Garey, Collin Sexton, Zach Nemechek, Breta Alstrom and Chloe Albin, a special thank you for your assistance in feeding, maintaining and taking care of the spider colony. My experiments would not have gone as well nor the colony have survived as well as it did without you. I will never forget your help or your exclamations of **“*recluse on the loose!*”**

Chapter 1 - Introduction

Impact of scavenging vs. predation on selected aspects of brown recluse spider, *Loxosceles reclusa* (Araneae:Sicariidae), biology

The brown recluse spider (BRS), *Loxosceles reclusa* Gertsch and Mulaik (1940), is largely considered a pest in homes and generally regarded negatively in newspaper and internet articles due in part to: 1) its close association with humans and 2) the medical importance of its toxic bite. Described as sedentary weavers by W.J. Gertsch (1967), the recluse spiders of the genus *Loxosceles* consist of more than one hundred species and have a wide distribution, both in temperate and tropical locations (Gertsch, 1967, WSC 2016). Gertsch, an arachnologist and former Curator of Arachnids at the American Museum of Natural History in New York, is considered by some researchers as one of the foremost arachnologists of the early 20th century (Kaston, 1981). Credited for describing nearly 1000 species of arachnids during his career, Gertsch also conducted extensive research on the genus *Loxosceles* (Kaston, 1981). The BRS, one of the species of this focus, has had a varied taxonomic placement history, being assigned at various times to the families Scyotedes, Sicariidae and Loxoscelidae by different researcher's dependent upon the characteristic being studied (Vetter, 2008). The genus *Loxosceles* originated when Heineken and Lowe created it for *L. citrigada* in 1835 (Lowe, cited in Vetter 2008); *Loxosceles* was placed in the family Sicariidae in 1880, by E.F. Keyseling (ITIS, 2016), where it still resides (WSC, 2016; ITIS, 2016). Sicariidae, a family of six-eyed spiders known for bites that may cause necrotic lesions, has nearly a worldwide distribution (WSC, 2016). Within the family Sicariidae, the genus *Loxosceles* was not well explored until Gertsch and Mulaik (1940) began describing members in their investigations of spiders in Texas. In their investigations, they described the genus *Loxosceles*, clarified species distribution, and began clearly designating separate species. *L. reclusa*, was originally identified in the early literature as *L. rufusans* (Vetter,

2008) and could be the cause of early misidentification and confusion within the medical, scientific and lay communities as the topic relates to distribution. The investigation by Gertsch and Mulaik (1940) resulted in clarification of the species and the new species designation of *L. reclusus*. However, the International Committee of Zoological Nomenclature (ICZN) required the masculine suffix *–us* to be feminine (*-a*), thus, the name was changed to reflect the feminine *Loxosceles reclusa* (Gertsch, 1958).

During the early 20th century, BRS were of little medical importance and considered just another member of the six-eyed spider family, Sicariidae (Hite, 1966). In 1928, a Halstead, Kansas, physician, L. F. Schmaus, was the first to record and correlate the bite of the BRS with resultant necrotic wounds (Schmaus, 1929). He documented the results of a patient that had suffered from the bite of a small brown spider, which he identified as *L. rufusans*, with the necrotic injury that ensued (Schmaus, 1929). It was not until the description of the BRS by Gertsch and Mulaik (1940) that interest in this small brown spider increased within the medical community regarding envenomation. Prior to the Gertsch and Mulaik (1940) investigations, the Black Widow spider, *Latrodectus mactans* (Fabricius), was the primary concern (Horner, 1967). In the early 1960's, Julia Maxine Hite began research on the biology of the BRS. Using observations from her Ph.D. research at Kansas State University, she co-authored an agricultural circular for the University of Arkansas (Hite *et al*, 1966). It was through her research, followed by Horner and Stewart (1967), that many aspects of the BRS biology were documented and illuminated. Researchers conducted studies and investigations involving other aspects of BRS biology in the following years. Of note is a study conducted by R.J. Elzinga, a Kansas State University connection as well as a member of Hites' graduate committee, involving BRS longevity and the possible influence of temperature. Elzinga (1977) built upon Hites' studies

with the additional focus of regulating the temperature to more closely represent those found in homes, (21-24°C (~70-75° F)), (Elzinga, 1977) rather than the higher temperatures, (21º- 40º C (70º - 104º F)), used by Hite *et al* (1966). Hite's laboratory was not climate controlled and the Kansas summer temperatures could be quite high. The 21st century brought renewed interest in the study of the genus *Loxosceles* and various papers: Martins *et al*, 2002, Fischer and Vasconcellos-Neto, 2005, Tambourgi *et al*, 2010, to list a few, were produced covering many aspects of different *Loxosceles* species, found mainly in South America. In North America, numerous research studies focused on the medical aspects of BRS venom, which is beyond the scope of this work. More recent research involving biology, management, cold tolerance, and predation vs. scavenging of the BRS, center primarily around three researchers: Rick Vetter, Dr. Jamal Sandidge and Dr. Richard Cramer, though others have also contributed work on the subject.

Description

Generally, BRS are medium sized, brown, and possess a body length measuring up to approximately 3/8 inch (10 mm) (Hite, 1966; Vetter, 2015). The actual size of mature spiders can vary, dependent upon diet and frequency of feeding (Vetter, 2015). Commonly referred to as the fiddle back, violin, or recluse spider, the spiders of the genus *Loxosceles* get their common name from the dark pigmentation found on the dorsal surface of the carapace (Figure 1.1). While well-known and typically the feature most commonly used, the violin marking is not the best characteristic to identify the BRS. Recently eclosed hatchlings and somewhat older juvenile (sub-adult) spiders may not have the dark pattern and the violin shape on recently molted spiders may not show immediately (Figure 1.2) (Vetter, 2008). The genus name, *Loxosceles*, meaning "slant legged" (Cameron, 2005), refers to the positioning of the legs while at rest (Figure 1.3),

and the species name, *reclusa*, refers to their propensity to remain hidden (Figure 1.4) in out of the way locations such as cracks and crevices (Gertsch and Mulaik, 1940). Definitive identification procedures include slight magnification to determine the number of eyes as well as the eye pattern. Most spiders have eight eyes, arranged in two rows; BRS have three pairs of eyes, in dyads, one pair at the front and one pair each at the sides of the cephalothorax (Figure 1.5) forming a u-shape with the arms of the "U" pointing back toward the spiders' abdomen. Typically, BRS have a light brown or tan body with somewhat darker legs and a dark brown inverted "violin" shape. However, again diet dependent, (Vetter, 2015) BRS can range from dark chocolate brown to light tan (Figure 1.6). Wingo (1969) stated, "*the body is covered by very dense but short hair and to the unaided eye appears to be bare*". Indeed, under magnification, fine dark hairs cover the BRS, as seen on the abdomen of the mature female (Figure 1.7). A sexually mature male can be identified by the palpal bulb, (ending segment of the pedipalp), which is used to transfer sperm to the female during mating. (Eberhard & Hubner, 2010) and a thin spine-like structure (embolus) (Figure 1.8) that is inserted into the female reproductive tract (Vetter, 2015). Female spiders lack the bulb and spine-like embolus; instead their pedipalps are long and "cigar-shaped" (Figure 1.9). The distinction between adult female vs. sexually mature male pedipalps (Figure 1.10) can be seen when comparing them side by side. Caution must be taken to ensure the BRS are mature when sexing if using the pedipalps as an indicator. Related to sexing, juvenile spiders can be easily misidentified. The pedipalps of a juvenile can closely resemble mature female pedipalps and mischaracterized as a mature female rather than a juvenile that has not reached sexual maturity.

BRS Life-cycle

BRS activity appears to cycle seasonally with the availability of prey. Cramer (2015) observed a population of BRS in an Illinois garage and determined their activity period as May to October. Hite (1966) observed mating of BRS in her laboratory colonies as early as February and as late as October, however the majority of mating occurred in June and July (Hite *et al*, 1966). During her observations of BRS mating, Hite (1966) determined that one male would inseminate multiple females demonstrating that one male in a population of females can produce large numbers of offspring. She also described the mating activity of the BRS in detail and determined that oviposition occurred, on average, 44 days after mating (range 6 to 208 days). However, Horner and Stewart, (1967) and Vetter (2015), reported oviposition within twelve days after mating. While a much shorter time than reported by Hite (1966), it is still within the range Hite originally reported. BRS usually undergo eight instars to maturity when reared at 21 - 32°C (~70 - 90° F) (Horner and Stewart, 1967; Vetter, 2015). Figure 1.11 shows three different stages BRS undergo outside the egg sac.

The first instar occurs within the egg (Figure 1.12) while all others occur outside the egg (Hite, 1966; Horner and Stewart, 1967; Vetter, 2015). Under laboratory conditions, using temperatures at times exceeding 37.5º C (~100º F), Hite *et al* (1966) determined the average number of days to reach the adult stage from oviposition to be 335.9 (range 266 to 444 days). During their study, temperatures in the lab varied seasonally, ranging from 21º- 40º C (70º - 104º F). Longevity studies conducted by Hite *et al* (1966) were based upon a laboratory colony without extreme cold temperatures normally found in winter. They determined an average longevity for male BRS as 543.2 days (1.48 years) whereas females averaged 627.9 days (1.72 years) with extremes of 796 (2.18 years) and 894 days (2.45 years) male and female, respectively

(Hite *et al*, 1966). They speculated that the maximum length of life of BRS may be extended should the spiders be subjected to lower temperatures during the winter (Hite *et al*, 1966). Horner (1967) determined that female spiders which over-wintered in a "near-natural environment" had a longer life span i.e. 1,420 days (3.89 years) compared to Hite *et al's* (1966), 894 days (2.45 years). Elzinga (1977) reared BRS at room temperatures 21-24°C (~70-75° F) and determined 25% of females survived more than 1,000 days (2.74 years) with a maximum of 1,755 days (4.8 years) and male maximum, 897 days (2.46 years).

Not only were longevity differences noted within the different studies, but differences in mortality due to molting were also recorded. Hite (1966) reported 46.5% developmental deaths associated with molting, whereas Elzinga, (1977) in a study of fifty BRS reported only 10% developmental deaths because of molting, and none following the fourth instar, at temperatures ranging 21º to 24º C (~70-75° F). His conclusion was the removal of higher temperatures, resulted in lower molting mortality and allowed sixty percent (30/50) of observed spiders to reach maturity. Elzinga's study is of interest because BRS populations, which may be found in occupied buildings, are kept at approximately the same temperatures year-round as the temperatures in his study. The lower mortality from molting results in the potential of an increased population in structures when kept at comfortable temperatures for humans. In most structures, BRS may not be directly exposed to the extreme cold temperatures that spiders in more "natural" environments are exposed to outdoors during winter. Even those BRS located in attics or unheated basements may avoid the extreme winter temperatures, however in unheated outbuildings and sheds BRS may be subjected to colder winter temperatures and was one of the areas explored by Horner (1967) in his study of fecundity. Fecundity, (a measure of fertility), is quantified in this study as either 1) the number of eggs produced per egg sac or 2) the number of

live hatchlings and unhatched eggs per egg sac. Elzinga (1977) did not explore fecundity, with his colony only focusing on BRS longevity.

Prior to oviposition, females begin the construction of the egg sac, first producing a circular mat of thick white webbing, then depositing the eggs. Eggs are small, round and slightly yellowish in color (Figure 1.13). Hite *et al* (1966) determined the average number of eggs produced per sac at ~50.5 and the average duration of egg stage at 13.45 days with temperatures ranging seasonally 21º- 40º C (70º - 104º F). Horner and Stewart (1967) determined the average number of eggs produced per sac was 23 and the average duration of egg stage was ten days at room temperature with a 70% hatch rate. With average temperatures, similar to those described in the Hite *et al* (1966) study, they also reported an average of 1.6 egg sacs from forty-four females in a given year, with twenty-three females producing more than one egg sac in a season. A major difference between the studies is, Horner and Stewart (1967) exposed overwintering spiders to "near-natural environment" temperatures by burying contained spiders in an unheated structure, unfortunately, the study did not report the subterranean winter temperatures. Once spiderlings emerge (Figure 1.14) from the egg sac, as second instar spiderlings, they are able to spin silk, feed and walk about (Hite *et al*, 1966). Development then occurs, as previously mentioned, taking approximately one year to reach maturity.

BRS sex ratios differ between Hite (1966), Horner and Stewart (1967), as well as the current study. Hite (1966) described male: female sex ratio as 1.6:1 of fifty-two adults surveyed in Arkansas/Kansas. In Texas, Horner and Stewart (1967) describe the sex ratio as 1:2 male: female for field collected BRS while laboratory raised BRS had a sex ratio of 2:3 male: female. Mature BRS in this Kansas study resulted in a sex ratio of male: female 1:1.6 field caught (eighty adults surveyed) and a sex ratio male: female of 1:1.8 lab raised (eighty-three adults surveyed).

Distribution

BRS range is predominantly in the south-central United States, (Gertsch, 1958; Hite, 1966, Gertsch and Ennik, 1983; Vetter, 2005) (Figure 1.15). They can be found in nearly every structure from homes to sheds as well as in woodpiles and discarded material throughout this range (Hite, 1966; Gertsch, 1967; Horner and Stewart, 1967, Sandidge and Hopwood, 2005; Vetter, 2008; Schwarting and Whitworth, 2015). Considered a true synanthrope, the BRS has adapted well to human habitats and can be quite successful increasing their population in homes and other structures (Guarisco, 1999). As an example, 2,055 BRS were captured in one house in Kansas over a six-month period (Vetter and Barger, 2002). Aptly named, BRS hide in dark undisturbed areas and typically only roam at night in search of food (Hite, 1966; Sandidge and Hopwood, 2005; Schwarting and Whitworth, 2015). BRS can be found in cardboard boxes, behind picture frames, in seldom-used clothing or in just about any areas of a structure where limited traffic occurs, (Hite, 1966; Vetter, 2008; Schwarting and Whitworth, 2015). The greatest concern is that BRS may hide in shoes or clothes left overnight on the floor (Sandidge and Hopwood, 2005). In natural conditions, BRS live under rocks, tree trunks, in holes and other natural openings (Hite, 1966; Gertsch, 1967). Hite (1966) also captured BRS in discarded materials and described capturing BRS in a hillside or bluff habitat in northwest Arkansas. Numerous authors have explained reports of BRS outside of the endemic range. Gertsch and Ennik (1983) created a comprehensive distribution map, marking the collection sites of verified BRS locations. Their explanation of BRS collected outside of the endemic area was that BRS may have been transported in packing crates or moving boxes, rather than that of an established colony. In their study on cold weather tolerance of recluse spiders, Cramer and Maywright (2008) suggested it unlikely that the current northern range will exceed its current placement.

BRS Feeding habits - Predators

It is generally understood that most spiders are predatory, whether trapping prey in a web or actively hunting. Sandidge and Hopwood (2005) describe the BRS hunting strategy as bite and retreat; allowing the venom to take effect, then return to consume the prey at their convenience. Cramer (2015) describes BRS as "sit-and-wait predators" relying on small networks of silk for prey detection while sitting motionless. Sandidge and Hopwood (2005) describe BRS as roaming hunters, wandering, typically at night or in darkened conditions, in search of prey and reportedly most active from approximately 8pm to 9am. In her dissertation, Hite (1966) describes finding numerous insect exoskeletons in the webbing of the BRS while investigating the bluffs in northwest Arkansas. She, along with later researchers, describes the feeding habits of BRS held in captivity. Hite (1966) caught insects while sweeping alfalfa and fed them, or chilled houseflies, to captive BRS. Cramer (2008) and Vetter (2005) used domestic crickets (*Acheta domesticus* (L.)) or *Drosophila spp,* for recently hatched juveniles, while Sandidge (2003) used wax worm larvae (*Achroia grisellar*) along with domestic crickets and yellow mealworm larvae (*Tenebrio molitor*). In these investigations, BRS fed on the live insects listed.

BRS Feeding habits - Scavengers

Sandidge (2003) determined that BRS actively feed (scavenge) on dead insects. His study showed that BRS fed overwhelmingly on dead prey (84% of the BRS tested in laboratory choice experiments) and appeared to ignore live prey. Furthermore, he stated that no adverse effect of feeding on 24-hour-old insecticide-killed prey was evidenced in those BRS that fed solely on prey killed with an insecticide. Sandidge's (2003) observation of BRS overwhelmingly preferring scavenging generated scientific interest and was investigated by Cramer (2008) and

Vetter (2011) in later studies. Cramer (2008) could not completely replicate Sandidge's results, and suggested the possibility that population origin caused the discrepancy in their differing results. Cramer (2008), suggested field collected BRS may feed differently than BRS raised under laboratory conditions, preferring live vs. dead prey, respectively. In a scavenging study involving recluse and other species of primarily cursorial hunting spiders, Vetter (2011) determined that 99 out of 100 spiders scavenge if given the opportunity. Vetter's study involved eleven different families of spiders in 31 genera and focused upon scavenging as the feeding response of the different spiders when presented a house cricket (*Acheta_domesticus*), killed by overnight freezing and thawed for 30 minutes, as prey. The results obtained by Vetter (2011) suggests that the scavenging behavior of the BRS is not unique among spiders nor is scavenging exclusive to BRS, finding that of the 100 spiders tested, 99 scavenged upon the dead crickets. Overall, these studies indicated that BRS appear to be opportunistic feeders and scavenge (consume) dead prey when presented.

Medical Importance

Often human/BRS contact occurs when putting on clothing left overnight on the floor, or clothing that has been stored for a period in storage areas. Other interactions can occur while the human is sleeping and rolls over, trapping the spider against the skin. Typically, BRS attempt to flee and avoid humans as much as possible, however, bites, while rare, do occur and in some cases, can be serious.

Loxoscelism is defined as the condition that occurs from bites of spiders within the genus *Loxosceles.* These spiders are considered medically important and are the only proven arachnological cause of dermonecrosis (Swanson and Vetter, 2006). Dermonecrosis, the potential necrotic lesions that are a result of the spider venom, are not the only resulting

complications (Figure 1.16). Systemic conditions such as fever, chills, and joint ache are also a common complaint among patients (Swanson and Vetter, 2006). Vetter (2008) described four categories of reaction to a BRS bite:

> "*Unremarkable (very little damage, self-healing)*
> *Mild reaction (redness itching slight lesion but typically self-healing)*
> *Dermonecrotic (necrotic skin lesion – considered by many to be the "typical" reaction)*
> *Systemic or vicerocutaneous (affect vascular system, very rare, potentially fatal)*"

With any bite or sting, the victim's sensitivity to the venom must be considered as an important variable regarding the severity of the reaction. However, there are numerous other variables that possibly contribute to or effect the severity of reactions: from the age of the victim, and their current level of health, to the location of the bite or sting and the amount of venom received, all may be considered when judging severity of reactions. Thus, there is a wide range of reactions dependent upon these variables, especially susceptibility and venom amount, when considering the medical importance of the BRS and its bite to humans (Vetter, 2008). Numerous medical studies have been conducted involving the investigation of loxoscelism but are beyond the scope of this study.

Management Strategies

Contact pesticides

The reclusiveness of this spider is one of the difficulties associated with trying to manage them, especially in homes. Different management techniques include the use of contact insecticides/pesticides, sticky traps, residual sprays, aerosols, and exclusion methods. Hite (1966) determined certain insecticides worked well on BRS if applied directly, however, the most effective insecticide she tested (lindane) has been banned by the Environmental Protection Agency (EPA). Agreeing with Hite, Vetter *et al* (2014) stated that much of the toxicological

research involving the BRS centered on topical or residual application of insecticides, some of which have been banned by the EPA.

The EPA separates general-use and restricted-use pesticides (GUPs and RUPs), respectively, principally based on their EPA toxicity class. GUPs can be sold to the public for unrestricted use, while RUPs can be sold to and used only by certified applicators (Rand.org, 2016). In a recent study involving GUPs and common household substrates, Schwarting and Whitworth (2015), explored the toxicity of two synthetic pyrethroid pesticides on separate household substrates to BRS. Results indicated the pesticides, labeled for BRS, did have some toxic effects dependent upon 1) length of time the spider was in contact with the treated substrate and 2) specific type of substrate involved. They determined that pesticide treated tiled surfaces resulted in greater BRS mortality than carpeting sprayed with the same pesticides, further determining the longer a BRS was in contact with the treated substrate the greater the mortality.

One problem for homeowners is to determine the best placement of a residual pesticide relative to BRS movement within the structure and ensuring BRS remain in contact with the pesticide treated surface for the longest exposure time possible. It is generally agreed that most homeowners do not want large treated areas of residual chemicals in their homes just to ensure the longest exposure time for the BRS. Aside from the danger involved with the presence of residual pesticides to pets and small children, the frequency or amounts of pesticides used may violate the usage restrictions outlined on the label.

Sandidge and Hopwood (2005) suggest the use of dusts in cracks, crevices and wall voids as a BRS control method. Some of the dusts, used in pest control, are composed of the exoskeletons of microscopic organisms called diatoms, (hence the name diatomaceous), and collect on the BRS when contact is made. These diatoms are sharp and abrade the spider,

especially at leg joints, causing injury with resultant loss of body fluids. Dusts tend to collect at the lowest part of the wall void or crack unfortunately, and thus do not always adequately control BRS populations. Diatomaceous dusts are not the only dust product for use in the control of spiders. Other dusts on the market contain active pesticide ingredients such as a suspended concentrate with a 4.75% deltamethrin based synthetic pyrethroid pesticide and a silica gel that works by dehydrating pests. These and other dusts are readily available for purchase online and from local merchants. Though popular and sometimes included in pest control measures, dusts were not included in this study.

Fumigants

Fumigants may also be used to manage BRS populations. Vetter *et al* (2014) tested the efficacy of the fumigant sulfuryl fluoride, to caged adult BRS and brown widow spiders (*Latrodectus geometricus*). Their research determined that fumigation, which was directed at termite control, if conducted at approximately 1.7 times the dry wood termite dosage at 21.7º C (~70º F) for 25 hours, was able to kill the contained adult spiders of both species. Commercial fumigation is not typically the first choice of pest control companies or of home owners for a variety of reasons, including fumigation dosage, and costs associated with the issues of sealing the structure and possibilities of the fumigants not penetrating all areas in which the BRS reside (Vetter *et al*, 2014). While fumigation appeared effective for adult spiders that were unable to escape the fumigant, 100% mortality for male BRS and 72% mortality for female BRS ~30 hours after the building was cleared for reentry, the issue of egg sacs and future hatchlings was not examined, thus population control may not be obtained. It should be noted the remaining female BRS in the trial died one week after fumigation (Vetter *et al*, 2014). The use of fumigants is not

always feasible in BRS management and, if possible, should be considered only after other methods of pest management have failed.

Glue Traps

A management technique used in monitoring for BRS is the use of glue traps (also called sticky traps). Glue traps consist of a piece of cardboard or heavy paper in various shapes and sizes covered on one side with an extremely adhesive coating (Fig 1.17). The pest comes into contact with the coated surface and gets stuck. Typically, these glue traps are placed in out-of-the-way, low-traffic areas along baseboards, etc. and as the BRS roam, hopefully, they are caught on the traps. Sandidge and Hopwood, (2005) outlined guidelines for the placement of glue traps to determine the extent of a BRS infestation in a structure. Paying close attention to the direction of travel, including the position and orientation of spiders caught on the traps allows the homeowner insight to the potential origin of the pest and can be an aid in determining locations best suited for the use of insecticides. Glue traps can easily be repositioned into other areas of the structure, should no pests be trapped at the location, and are effective for the length of time the adhesive is "sticky" (Fig 1.18).

Glue traps are sometimes recommended as a control strategy however, a major issue with the use of glue traps when used as a management technique alone is the sex of the spiders trapped. According to Sandidge and Hopwood (2005) males are typically the roamers (Fig 1.19) searching for food and mates. Females roam but typically not as much as males. Trapping males prevents them from mating. However, if a female has already mated and produced an egg sac, the capture of a male on a glue trap will not lessen the future population per se. As has been noted, females can produce multiple egg sacs from one mating which allows population increase with minimal male involvement.

Sanitation

While potentially not the most popular or easiest method, sanitation is an effective tool when attempting to manage BRS. Hite (1966) described BRS habitat as out-of-the-way locations. Vetter (2008), Sandidge and Hopwood (2005), as well as Schwarting and Whitworth (2015), also describe areas of clutter as well as storage areas as good locations for BRS infestations. The removal of old newspapers, magazines, cardboard boxes and general clutter from unfrequented traffic areas such as storage areas and attics, along with cleaning windows to allow as much light in as possible may reduce the possibility of BRS finding habitable locations. The use of topical or contact pesticides may kill those adult spiders that come into contact long enough with the chemical (Schwarting and Whitworth, 2015), however the chemicals will have little to no effect on eggs within sacs (Figure 1.12) (Sandidge and Hopwood, 2005). These must be physically removed and/or destroyed. An easy yet effective method for removal of egg sacs is a household vacuum with a wand. Homeowners can place a new bag into their vacuum, clean the infested areas of webbing and egg sacs, then remove and discard the bag. Physically removing egg sacs prevents hatching in that location and thus continuing populations. After cleaning the area of webbing and egg sacs, exclusionary methods should include spraying cracks and crevices with pesticides labeled for BRS, then filling cracks or crevices with caulk or sealing compound preventing BRS movement.

Other exclusionary methods include the use of weather stripping around doors and windows along with close examination of screens for needed repairs. While not necessarily an immediate method of controlling BRS populations, when compared to the use of pesticides, limiting access of potential prey into a structure can be an effective deterrent. Without a food source BRS populations will eventually diminish and die out.

Biocontrol measures

Another potential management method for BRS, suggested by Guarisco (1991), is the use of other predatory spiders commonly found in and around structures. He described *Steatoda triangulosa* (Triangulate cobweb spider) webs, all inhabited by immature *S. triangulosa,* from which he recovered the remains of fifteen BRS of various sizes. Sandidge (2004) investigated the possibilities of biological control using other spiders found in most homes. He suggests the use of three common web-building spiders (*S. triangulosa, Achaearanea tepidariorum* (American house spider), *and Pholcus phalangioides* (Cellar spider)) which he says are natural enemies of the BRS, to control populations of BRS, and recommends, when developing management plans for BRS control, leaving existing spider populations intact. While potentially a method for controlling a medically important spider, it leaves other spider populations to increase in the home, which may not be well received by most homeowners.

Definitions and terms

Throughout the study, the term "prey" is used to describe small (1/8-1/4") house crickets, *Acheta domesticus*, or flightless fruit flies, *Drosophila heydei*, both of which were obtained from the Kansas State University (KSU) Insect Zoo. Prey usually refers to an organism that is hunted and used for food leading one to surmise the "prey" is alive. In this study the term prey refers to the crickets used as a food source, which have been treated in one of the four treatment regimens; in only two of the cases (controls) is the "prey" alive (live and water treatment). The question arises then: if BRS are scavengers and utilize prey killed by pesticides will the BRS then suffer any detrimental effects because of feeding on "dead prey"? Acute effects can be defined and observed as: mortality, which is determined by observation of legs tucked up under the body and lack of response to gentle probing; erratic movements of the legs, abdomen, or pedipalps; or

increased/continuous movement of the spider itself. Chronic effects could be reduction in or loss of reproductive capability, either fertility/fecundity, or weight loss due to potentially lower nutritive value of the dead prey.

Research objectives

The objective of these research studies centers on the effect that scavenging vs. predation has on BRS biology, ultimately to give insight on better management techniques for control of the BRS. Research on BRS may be difficult, in part because of the toxicity of their venom, but also because they can become dormant for considerable lengths of time and can be cannibalistic (Hite, 1966; Sandidge and Hopwood, 2005; Vetter, 2008). The BRS can go months without food and water and it was even found that when kept in a lidded container, BRS were able to survive approximately ten months (Hite, 1966; Sandidge and Hopwood, 2005).

In this study, when provided insecticide-killed prey vs. prey killed by freezing vs. live prey, the preference of BRS for predation vs. scavenging may be determined. This study also compares BRS weight gain fed on live prey vs. those fed insecticide-killed prey to help clarify the question of predation vs. scavenging and may also assist in determining the rate at which BRS feed when presented prey at regular intervals. In the study by Cramer (2008), the issue of predator satiation was of concern. He suggested that BRS did not feed when satiated and ignored the prey offered. Determining how often BRS feed may provide a better understanding of BRS feeding/roaming habits. The ability to survive by scavenging does not appear to be unique to BRS, as previously stated by Vetter (2011), however the effects that scavenging has on the biology of BRS has not been explored.

Management techniques for BRS control typically involve the judicious use of pesticides and/or glue traps. If the efficacy of a pesticide relies on the BRS contacting a specific treated

substrate for a certain length of time, the "chances" of killing any number of BRS may not be good. However, if the contact pesticide kills a pest that the BRS later scavenges upon and scavenging results in the death of the BRS, then the homeowner is in a "win/win" situation in which they rid themselves of a pest and the BRS as well.

Sandidge (2003) described the BRS as an "*opportunistic feeder rather than obligate predator or obligate scavenger…prefer[ring] dead over live prey*", this research focuses on the premise that the BRS, as an opportunistic feeder, will derive greater benefit as a predator feeding on live prey than that of a scavenger feeding on dead prey. Furthermore, in the same paper, Sandidge (2003) observed BRS feeding upon insecticide-killed prey with "*no obvious negative effect".* The research we conducted attempted to determine if BRS scavenging on insecticide-killed prey has an acute effect on the BRS population by killing the BRS, or chronic effect by reducing other aspects of biological fitness, i.e. fertility/fecundity. The goals of these studies are to determine the effect of scavenging on selected aspects of BRS biology, using prey killed by three different methods (pyrethroid insecticide, Spinosad insecticide and freezing). Additionally, the effect scavenging has on fecundity; (through egg production and hatchling emergence) using prey killed by these three different methods is examined.

Figures Chapter 1

Figure 1.1 Darkening of cephalothorax

The dark "violin" pattern is evident on the carapace of the two mature females above. The "neck" of the violin points toward the abdomen of the spider. Darkening on cephalothorax may be difficult to determine on juveniles

Figure 1.2 BRS coloration

Juvenile BRS with *Drosophila hydei*

Figure 1.3 BRS displaying "slant legged" behavior.

Brown recluse spiders draw their legs up toward the body in a distinctive manner, during inactive periods or while at rest, as seen in the mature male above.

Figure 1.4 BRS hidden in out of the way spaces

Low traffic areas (left) and cluttered unused outbuildings are excellent locations to find brown recluse spiders.

Figure 1.5 BRS have three pairs of eyes (dyads)

Under magnification, the eyes can be clearly discerned as three separate pairs.

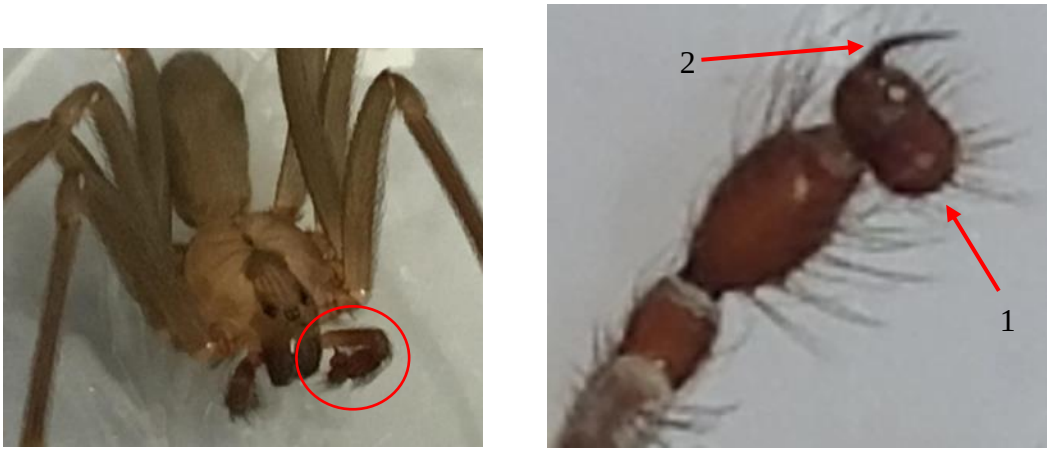
Figure 1.6 BRS range of color light to dark brown

BRS coloration (differing shades of brown) appears to be diet related.

Figure 1.7 BRS covered in fine dark hairs

Fine dark hairs cover the BRS Left- ventral view male Right – dorsal view female.

Figure 1.8 Male BRS



Mature Male BRS Identified by the bulb like palpus (1) and thin spine-like embolus (2).

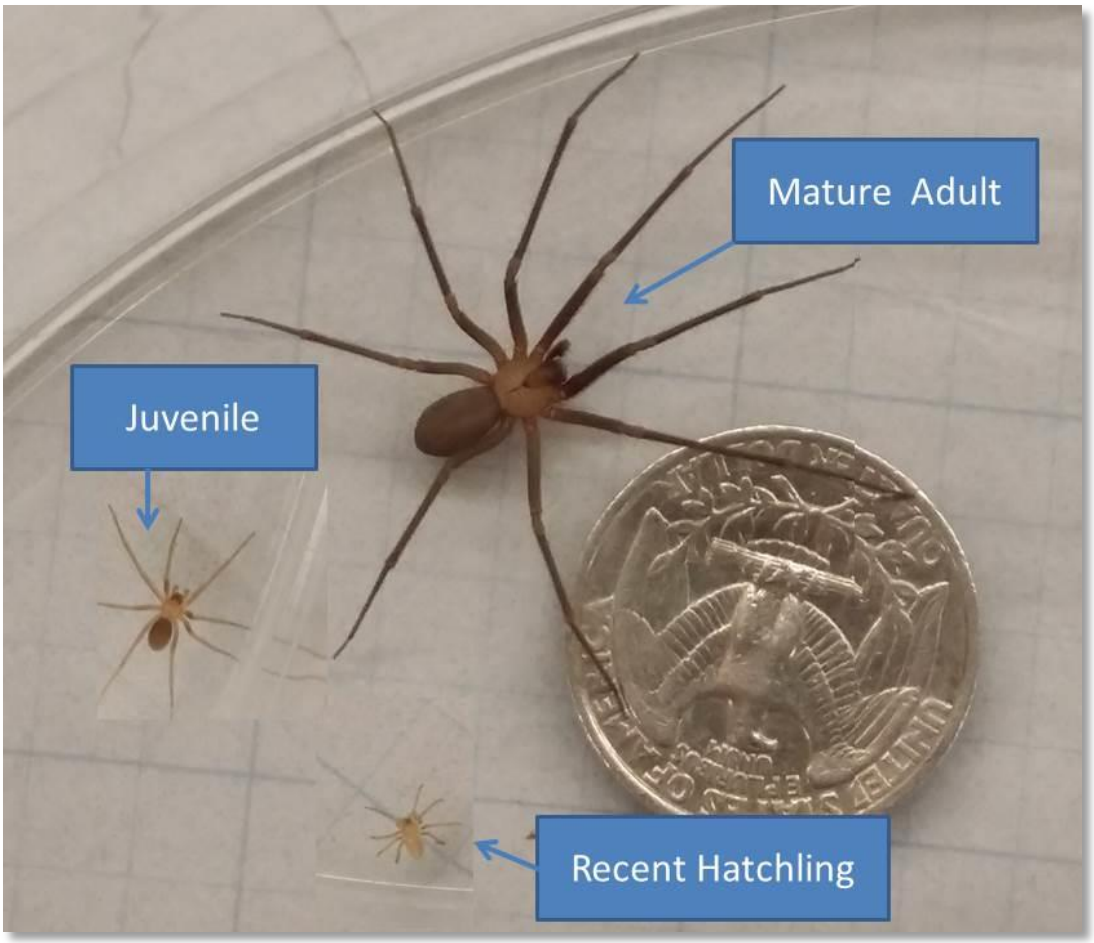
Figure 1.9 Female pedipalps

Female without the bulb-like palpus, pedipalps long cigar shaped

Figure 1.10 Side by side comparison of adult pedipalps

Left - Female long filiform "cigar shaped" pedipalp. Right - Male with bulbous pedipalps and spine-like embolus

Figure 1.11 BRS instars



Hatchlings may or may not show the darkening on the cephalothorax, and initially are light tan in color eventually darkening.

Figure 1.12 Exuvia from recently eclosed spiderlings

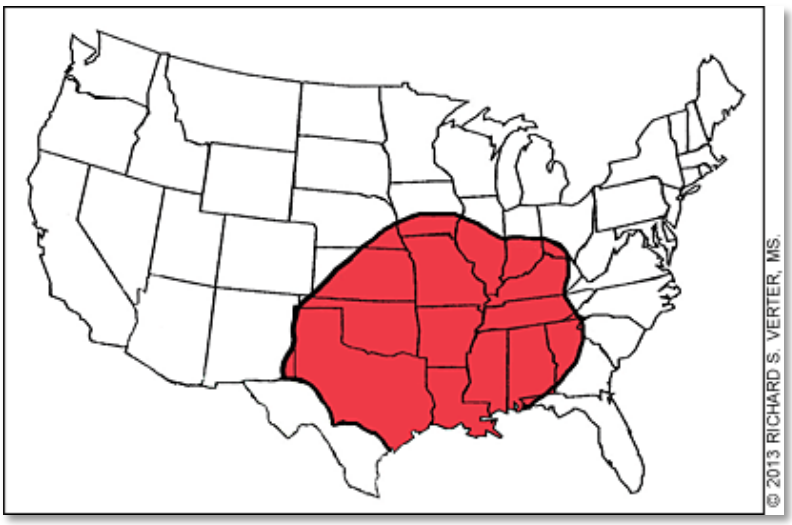
BRS first molt is within the egg sac as indicated by the arrows

Figure 1.13 BRS eggs within sac

BRS eggs are round with a yellowish coloration.

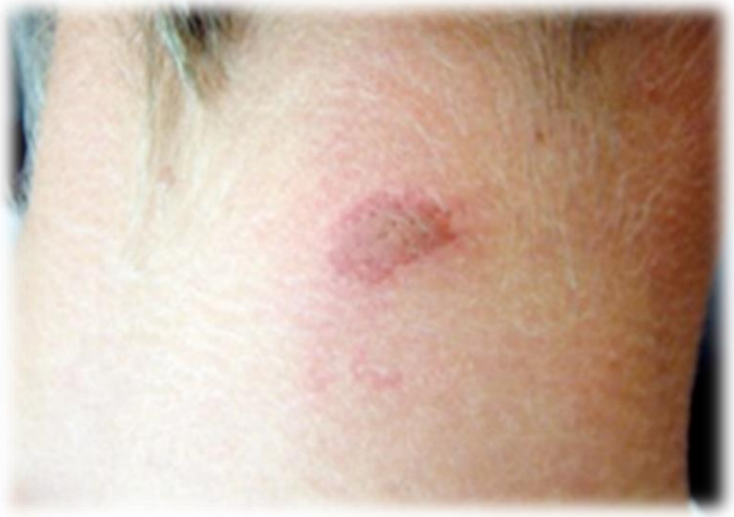
Figure 1.14 Female BRS with recently eclosed spiderlings

Figure 1.15 Map of BRS indigenous range



BRS map courtesy of R.S. Vetter (2013)

Figure 1.16 Verified BRS bite

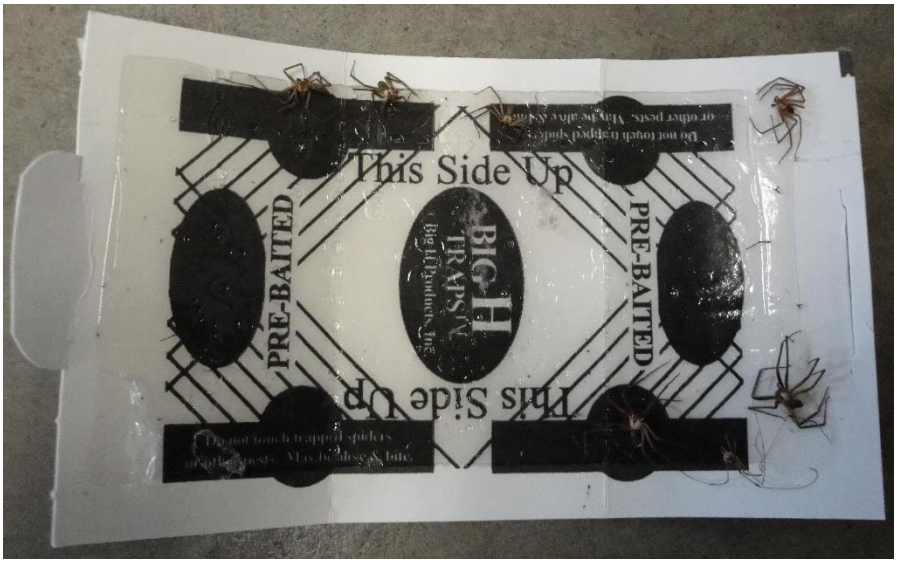


Physical reaction is dependent on amount of venom and sensitivity of the individual. Photograph by William V. Stoecker, M.D. (Swanson & Vetter, 2005)

Figure 1.17 Sticky Trap with spiders

Glue trap with trapped BRS

Figure 1.18 Sticky Trap opened



Glue trap opened to show trapped BRS

Figure 1.19 BRS captured on trap

Close up of BRS trapped on glue trap, the “violin” is clearly seen

References Chapter 1

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Chapter 2 - Colony Establishment: Capturing, rearing and handling

Objective 1

- *Establish and maintain a viable colony of BRS:*
- *Develop acceptable housing, feeding and handling techniques to ensure a reliable supply of BRS for consistent research*

Overall, nine-hundred seventy (970) brown recluse spiders (BRS), captured from various locations around North-Central Kansas, or raised in the lab, from 2013 thru 2017 were used in the laboratory studies. All laboratory studies were conducted in Waters Hall Annex, Room 110, on the Kansas State University campus, Manhattan, Kansas. Temperature was climate controlled and light was indoor ambient unless otherwise stated. Climate controlled, ambient temperatures ranged from ~20 - 23° C (~68 - 74° F) annually. Lighting was provided by overhead fluorescent tube lighting with outside lighting limited by covering the windows with black plastic sheets.

Colony establishment

The first objective was establishing a colony of *L. reclusa* suitable for research. A colony of eighty-three indeterminate aged BRS, collected from northeast Kansas or purchased from the KSU Insect Zoo, remained from a previous research project and became the nucleus of the experimental colony. Literature made reference to colony management and feeding practices (Vetter, 2015) as well as a warning from Hite's (1966) experiments that the addition of water in spider containers was not necessary and in fact was found to be detrimental to spider health. The coordinator at the KSU Insect Zoo provided information (Holt, personal communication) from her experiences with BRS management and care, which resulted in the ultimate choice for rearing containers; Fabri-Kal® polystyrene 4oz. portion cups, 7.8 x 4.1 x 5.8 cm (3.1 x 1.6 x 2.3 inches) with clear Fabri-Kal® lids (Kalamazoo, MI), were the most efficient for BRS rearing (Holt, pers com). Food sources in the form of (¼" - ⅛") house crickets, *Acheta domesticus*

(Figure 2.6) and flightless fruit flies, *Drosophila heydei* (Figure 2.10) in the quantities necessary for maintaining a large colony of research spiders were obtained through cooperation with the KSU Insect Zoo.

Capturing BRS

There are no references in the literature, which explain the best methods when attempting to live-capture BRS, but does give an idea of natural habitats. Northeast Kansas does not have the bluff complexes described in Hite's dissertation but does have adequate amounts of cluttered outbuildings, abandoned sheds and old homes available for exploration. Obtaining permission from the owners of properties to search for spiders typically was not a problem despite their initial reaction of disbelief. Typical instructions from the property owners included a statement to the effect of "take as many as can be found".

The first outbuilding searched in Abilene, Kansas (Figure 2.1), was definitely one that fit the description of "good" spider habitat. Discarded cardboard boxes, piles of wood, old tack for horses along with other piles of "clutter" filled this two-room storage shed. Once used as a shop, it had long ago degenerated in to a storage location and began to collect all manner of clutter. Being careful not to break anything or cause damage to the property initial spider collecting began. Immediately, a few questions arose, namely once found, how can the spider be safely captured without injuring the spider or the researcher? BRS are quite fragile and possess venom that can be toxic to humans, so picking them up bare handed was not the best method, even wearing gloves to prevent envenomation would not prevent the accidental crushing of the spider. Initial capturing techniques included placing the small cup on its side and "coaxing" the spider with the lid in an attempt to encourage the spider into the cup. More than a few spiders avoided capture by running past the cup to safety or running up the hand holding the cup and being flung

away by human reaction. Ultimately, it was decided to attempt to capture spiders with forceps by a leg and then place them into the cups. This process was slow and resulted in numerous traumatic amputations of spider legs. Another method was to lift the piece of whatever the spider was clinging to and attempt to "knock" it into the cup and quickly place the lid. A spider finding itself in a plastic cup immediately attempts to get away so the lid has to be readily available or the spider would escape sometimes crawling on the human on its way to freedom (another opportunity for spiders to become airborne).

An issue made quickly apparent was that of lighting. An unused shed does not always have the cleanest of windows or electricity, so attempting to find small brown spiders in dark and dirty corners is not everyone's idea of a great activity. Nor is the moving of clutter that had not been moved for years in an attempt to determine if a spider was present. Heat, lack of air circulation, dust filled air, clutter which slowed movement, and lack of illumination were the order of the day for the first spider-collecting foray.

Lessons taken from the initial outing:

1.) Better illumination techniques (flashlights at least!)
2.) Capturing techniques had to be improved, (limit the stress to the spider and to the human!)
3.) Teamwork essentials (someone to hunt, someone to trap and someone to run cups both filled and empty).
4.) A container to hold the cups that were filled with a spider.
5.) Lastly, identification.

Lighting was usually an issue within the areas and this problem was solved with the purchase of headlamps. Headlamps allowed hands-free lighting with the light directed where

heads were turned, allowing illumination where the eye was directed. The use of these lamps was always a necessity, however, as with anything there is a caution. Care must be taken when speaking to another person while wearing a lit headlamp, due to the light shining in another's eyes and temporarily blinding them.

Identification of the BRS is not very difficult in a lab environment; as a member of Sicariidae it has six eyes in three sets of two (dyads) formed like a U with the arms of the U pointing toward the abdomen. Another common indicator is the darkening of the carapace into what is commonly called a violin shape. In a dark shed however, if it crawls it gets caught, the first hunt ended with 34 adult and 35 juvenile (sub-adult) BRS and five egg sacs, as well as wolf, grass, and several jumping spiders.

Specific capturing techniques are not available in the literature and for the establishment of a colony it was imperative that captured spiders were healthy and uninjured. Plastic cups came in two sizes, for smaller spiders the smaller cups (Fabri-Kal® polystyrene 2.5oz. portion cups), 6.4 x 4.1 x 3.8 cm (2.5 x 1.6 x 1.5 inches) with clear Fabri-Kal® lids (Kalamazoo, MI) were used, and for the larger (usually adult) spiders the larger cups were used (Fabri-Kal® polystyrene 4oz. portion cups). This aided in the sorting process once returned to the lab and limited the need to move spiders between cups. For collecting, a pack of 3x5 cards was used in conjunction with the plastic cups. The spider was trapped with the cup on a flat surface, the 3x5 card was slid under the cup, effectively sealing the spider in the cup. The collector flipped the cup right side up, and once the spider was in the cup, quickly placed the lid on the cup to secure the spider in the cup. This method became quite effective as far as capturing the spiders safely as well as increasing the number of spiders caught. When a spider was discovered, a cup would be placed over it keeping it in place. If several spiders were in the area they could be captured as fast as

possible by trapping with different cups, then it was a simple matter to slide the card under and lid the cups one by one. Utilizing this capture method, more spiders were captured in a shorter time.

Trial and error resulted in the most effective method of capturing spiders; to have teams of at least two: one hunter/trapper, while the other provided cups, annotated the capture date/location and returned filled cups to a central location for storage/sorting. While collecting BRS, the less movement and vibration created, the better the chance of capturing more spiders. If too many people were moving around in an area, stepping on boards or boxes, and moving clutter for a better look, the spiders flee from the area and hide. Slow methodical movement of large items with a meticulous search of the area prior to the movement of any object was found to be most effective. Cardboard boxes appeared to be great places for BRS, under flaps, inside as well as outside. However, cardboard was not the only place to find BRS. Spiders were found hiding in old tack (leather and rope), paper sacks, as well as under sheets of plywood and in stacked piles of lumber. Whatever out of the way location a BRS could establish itself and form a nest was a probable place to look. Not many BRS were found inside metal cans but were located in old cabinets, behind furniture, hiding inside rolled carpeting as well as piles of what is best described as "clutter"; discarded items that appeared not to be of any use.

The best way to determine BRS inhabited areas is find exuvia (cast skins). BRS molts are distinctive. They molt "splayed" with all legs adhered to the substrate by tarsal claws and unlike molts, of other spiders like tarantulas, the molt does not curl upon itself, instead remains in the splayed position. This is very characteristic of the *Loxosceles spp* and can assist in determining the relative density of an infestation in an area.

Colony maintenance

Colony augmentation occurred as needed, typically in late May and early June, spiders of different instars were captured at several Kansas locations. Field collected spiders, as well as colony spiders, were placed in individual Fabri-Kal® polystyrene 4oz. portion cups, with clear Fabri-Kal® lids (Kalamazoo, MI); a rectangular 2.5 x 1.5cm (~ 1 x ½ in) piece of cardboard, creased in the middle, was added to each container to provide shelter and traction. Typically, the spiders webbed the cardboard using it as their daytime retreat or "refuge". Originally, crushed walnut (Figure 2.4) was used as a substrate in the rearing cups along with the cardboard strip, however it was determined that the walnut substrate was unnecessary and an added complication when moving spiders between cups. (Figure 2.5). Thus, the practice was discontinued.

Each spider was kept in an individual container and provided with a small (¼" - ⅛") house cricket, *Acheta domesticus*, weekly (Figure 2.6) from March to October, and once every two weeks, November to February. Crickets, used as food for the BRS, were killed by freezing, ~24 hours prior to feeding the colony spiders. Following the freeze killing, crickets were thawed for 30 minutes at room temperature prior to placement in the cup with the spider. Freeze killing the prey ensured the cricket was no threat to the spider and allowed the spiders to scavenge on recently killed prey. BRS were kept in a darkened environment at ~70° F (21° C) (range 68 to 72°) under ambient relative humidity and exposed to light only when being fed or during rearing container maintenance (Figure 2.7). Captured juvenile BRS were also kept in individual containers (same as adults) and fed 4-6 *Drosophila* weekly until large enough for smaller crickets. BRS obtained fluids from feeding and were not provided an external water source (Hite, 1966). Typically, BRS are found in attics, basements and abandoned/seldom used outbuildings where the environment is hot and dry during the BRS "active" season anyway. Hite (1966)

determined a moistened cotton ball placed within the spider's container actually increased spider mortality, thus she stopped the use of moisture in her colonies. Therefore, moisture was never used in the rearing containers.

In an attempt to prevent cannibalism or injury, spiders were housed individually, therefore colony maintenance required a significant amount of time maintaining rearing cups (Figure 2.8) and feeding spiders. Vetter (2015) suggested half-grown spiders need to be fed once or twice a month, which allows them to continue to grow and prevents starvation. The feeding regime initiated in this study was part of the experimental protocol. Weekly feeding of the colony spiders kept them on the same feeding regime as spiders being tested. Changing the feeding interval from once/week to once/two weeks in the winter months was simply a matter of logistics and had no apparent fitness cost to the spiders. Spider feeding on prey was determined through actual observation or through the desiccated remains of the prey. Colony spiders fed continuously throughout the year if prey was available. Raising BRS from egg to maturity was not difficult under this scenario.

Rearing

Egg sacs obtained during BRS collecting trips were kept in Fabri-Kal® polystyrene 32oz., tall containers (PK32T) with vented lids (Kalamazoo, MI) measuring 11.7 x 14.2 x 8.6 cm (4.6 x 5.6 x 3.4 inches), with two or three sheets of single-ply paper toweling as a substrate suggested by Vetter (2015) and referred to as a "nursery" (Figure 2.9). The paper toweling was crumpled to provide numerous hiding places for the BRS hatchlings. The number of *Drosophila hydei* (Figure 2.10) introduced weekly to each nursery, varied by the number of hatchlings. Hatchling numbers ranged from eight to forty-seven in the fourteen active nurseries. Spiderlings were kept together with the mother for six-weeks after eclosion, then transferred to individual

rearing containers and fed individually. Cannibalism in nurseries was a concern; however, it was kept to a minimum through the judicious feeding of nurseries with *Drosophila hydei.* After eclosion, spiderlings may feed on each other unless a sufficient food source is available (Hite, 1966). One to three freeze killed crickets were introduced weekly into the nursery container to feed the female and lessen the chance of her feeding on the spiderlings while kept together. A female feeding on young was never observed, however, plentiful food was provided to ensure cannibalism did not occur.

Mating

Whether for colony growth and maintenance or as a follow-on to studies conducted with prey treatments, the successful mating of BRS and subsequent egg sac production was vital. Initially, colony expansion occurred through 1) finding egg sacs during field collection, or 2) capturing females previously inseminated. Field-collected females did produce egg sacs in the lab, however knowing when insemination occurred or if it was a first or subsequent egg sac for the season was impossible to determine. As has been previously noted and observed in the current study, females can produce multiple egg sacs within a season. The use of field-collected egg sacs solely to expand the colony was ineffective and determining an effective method to mate BRS in the lab with a resultant egg sac was a challenge, initially ending with few if any egg sacs.

Several early attempts at mating BRS occurred with mixed results: females would kill the male or vice versa, cohabitation occurred with no mating, consequently no egg sac production, or rarely, a successful mating. Hite (1966) along with other researchers clearly describe the mating process, therefore when a successful pairing was observed it was clearly apparent (Figure 2-11). This figure is of interest in that the male has the female on her dorsal side (back) rather than

going below her and inserting his palps from below (or ventrally), which is the method of insemination we typically witnessed in the lab. Descriptions from Hite (1966) and Horner & Stewart (1967) concerning the ease of mating led to the belief that "nature would take its course" however, it was not as clear-cut as putting a male and female together. The initial lack of successful pairing and egg production raised the question of mating procedures, seasonality and the amount of light to which the mated pair were exposed. A small trial using colony raised virgin females was conducted in June 2014 to determine if light exposure had an effect on mating and egg production.

Methods and Materials – Mating

Virgin females from the colony, fed prey killed by freezing (scavenging), were paired with colony males for one-week in natural light (12:12 L:D) or one-week in darkness (0:24 L:D). Following the exposure to the males, the females were removed from the mating chamber and placed into their original cup. The date of mating was annotated upon the lid of the cup and the container was then placed into a cabinet drawer separate from other females, and monitored daily for egg sac production. The cabinet drawer was locked open allowing exposure to natural light.

Mating chambers were clear SOLO® polystyrene 16oz., containers (DM16R-0090) with lids (Urbana, IL) measuring 11.68 cm (4.6 in.) top diameter, 9.65 cm (3.8 in.) bottom diameter and 7.62 cm (3 in.) tall (Figure 2.12). Transfer of male and female spiders was accomplished by (Figure 2.13) using the refuge in each rearing cup. Transferring both sexes to the mating chamber was accomplished by picking up the refuge with forceps and quickly moving cardboard and spider into the mating chamber. After transfer, mated pairs were placed in a closed cabinet 0:24 L:D or placed upon the lab benches 12:12 L:D and exposed to ambient temperature and relative humidity during the one-week mating. Following the one week exposure to the males,

females were removed from the mating chamber and placed into their original cup, placed into a cabinet drawer separate from other females, and monitored daily for egg sac production. The cabinet drawer was locked open allowing exposure to natural light.

Once an egg sac was produced, the date of production was annotated and monitoring for hatching began. Once hatchling emergence began, the female was placed into another cup and the hatchlings counted. To count the hatchlings one of two procedures was used dependent upon the status of the hatchlings: 1) if experimental hatchlings, (requiring an accurate count of both eggs and hatchlings), the cup was placed in a freezer and all hatchlings freeze-killed. The spiders were then counted and the egg sac opened to determine if any of the eggs did not hatch. 2) If the hatchlings were for colony building, the hatchlings were placed individually into cups and fed drosophila.

Results – Mating

Spiders exposed to light during the mating process produced more offspring than those continually kept in a darkened cabinet as seen in tables 2-1 and 2-2. Four of the six females exposed to natural light during the mating process produced eggs sacs and offspring whereas those exposed to darkness for the same period of time produced no offspring. Statistical analysis was not conducted.

Discussion – Mating

When mating BRS, several conditions had to be taken into account and is not as simple as throwing a male and female into a container and letting "nature take its course". 1) Season – experimentation during the winter months required the surviving spiders being mated in the spring/early summer. Pairs introduced "out of season" did not mate nor was interest overly obvious. Typically, the pair would remain in their individual cardboard refuges late October to

late February rather than wander or interact. 2) Light conditions – Males and Females appeared to mate at a higher rate when exposed to natural light rather than complete darkness. However, mating occurred between pairs that had "interest", for experimental standardization the seven days in natural light versus darkness became the standard. Mated spiders were still kept in the cabinets, but the doors to the cabinets and drawers were blocked opened to allow the entrance of natural light.

The use of the cardboard refuge to transfer BRS into the mating chamber turned out to be an important factor in successful mating. In most cases, BRS web their refuge and wandering BRS will trail thin strands as they move about (Vetter, 2015). Using the webbed refuges in the cups along with the spiders rather than clean and unwebbed cardboard appeared to lessen the shock of the move and allowed the spider to remain with most of its original webbing. When transferring spiders from individual cups to mating chambers, moving the refuge along with the spider rather than introducing a new piece of cardboard to a clean chamber appeared to cause less stress on spiders.

Handling

Loss due to handling can occur when transferring spiders to different containers. BRS can and do move quickly if agitated. Early transferring attempts involved grabbing the spider by a leg, which occasionally resulted in loss of leg. Moving the refuge with the spider within was the easiest method of transferring spiders to new cups, however, if the spider was out of the refuge and moving the cardboard substrate would not move the spider, the cup would be angled downward, opening toward the new container. To "encourage" the spider to move, forceps or a small spatula was used as a prod, lightly touching the abdomen of the spider while keeping the opening tilted toward the new cup (Figure 2.14). Moving BRS in this manner resulted in fewer

legs lost and fewer deaths because of handling. Eliminating the walnut substrate allowed quicker and "cleaner" movement of BRS.

Feeding

In order to maintain a viable colony of several hundred live spiders, an acceptable diet and method of providing that diet had to be developed. Flightless *D. hydei* and *Acheta domesticus*, utilized as prey, were purchased from the Kansas State University Insect Zoo. One thousand quarter inch and one thousand eighth inch crickets arrived every two-weeks during active trial periods. Crickets were placed in Sterilite® 55 liter (58 quart) plastic tubs (Sterilite Corporation, Townsend MA) measuring 59.7 cm length x 42.9 cm width x 31.1 cm height, (23 ½" L x 16 ⅞" W x 12 ¼" H). Cricket colonies were maintained with Cricket Power Food® (Timberline Live Pet Foods, Marion IL) and water. Crickets were freeze-killed and fed to the colony spiders weekly. *Drosophila* received from the Insect Zoo colony were contained in Fabri-Kal® polystyrene 32oz., tall containers with vented lids and maintained on a diet of agar, yeast, cornmeal and molasses. *Drosophila* were maintained on the diet for about 60 days before the diet became too dry. New colonies were started by mixing and moving adult flies to the new food.

Figures Chapter 2

Figure 2.1 BRS collecting Abilene, KS, 2014

BRS collecting in a little used outbuilding, Abilene, KS, 2014

Figure 2.2 Effectiveness of headlamps

BRS collecting in another outbuilding Abilene, KS June 2015. Headlamps (circled) were necessary prior to opening the large door. Picture on left demonstrates the brightness of light when directed at viewer.

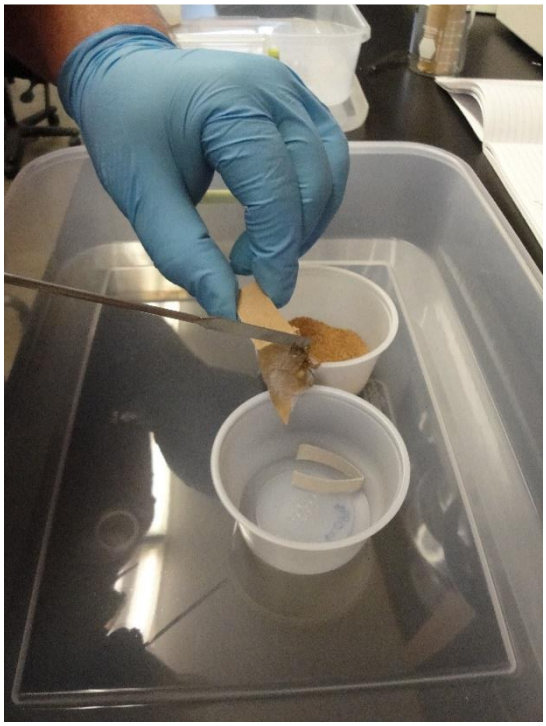
Figure 2.3 BRS on cardboard "refuge"

BRS on cardboard "refuge" cricket placed in center of "V" as prey

Figure 2.4 Crushed walnut initially used as substrate

Crushed walnut initially used as substrate with cardboard strip for "refuge"

Figure 2.5 Moving BRS using "refuge"



Moving BRS using cardboard “refuge”

Figure 2.6 Adult BRS feeding on cricket

Freeze-killed crickets provided no threat to the BRS and were accepted readily.

Figure 2.7 BRS Colony in cups and contained in cabinet

Cabinet with BRS individually contained in trays, doors were left open for natural light or closed for trials requiring darkness.

Figure 2.8 Rearing cup maintenance

Moving BRS.

Figure 2.9 BRS "Nurseries"

Female BRS remained with egg sac until hatch. Hatchlings were then moved to individual cups and fed fruit flies.

Figure 2.10 Third instar hatchling

3rd instar hatchling (center) surrounded by *Drosophila heydei*

Figure 2.11 BRS copulation

Typically, the female will remain upright and the male will inseminate by going below her, in this photo the female is on her dorsal side and the male is inseminating upright above her.

Figure 2.12 Mating Chambers prepared for BRS

Unmarked mating chambers prepared for BRS.

Figure 2.13 Moving BRS

Moving a spider by transferring its cardboard refuge instead of seizing the spider.

Figure 2.14 Moving BRS to new rearing cup

Encouraging adult BRS into new container without crushed walnut substrate

Tables Chapter 2

Table 2-1 Colony virgin females exposed to natural light

	1	2	3	4	5	6
Colony Virgins	22/24	53/62	13/35	0	0	23/54
	7 Days Natural Light L:D 12/12					

Table 2-2 Colony virgin females exposed to darkened cabinet

	1	2	3	4	5	6
Colony Virgins	0	0	0	0	0	0
	7 Days Darkened Cabinet L:D 0/24					

References Chapter 2

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Chapter 3 - Scavenging Preference – Pesticide Efficacy

- *Using weight change and mortality as indicators of biological impact:*
    - *Determine if the BRS is an opportunistic scavenger*
    - *Determine if predation or scavenging has significant impact*

- *Determine the acute (short-term) effects on BRS that scavenge on insecticide-killed prey measured by:*
    - *Weight change*
    - *Mortality*

There is no artificial diet for BRS, live prey is difficult to work with and can be a hazard to the spider (Figure 3.1). Freeze-killing crickets was a possible solution, however, prey acceptability to the BRS had to be determined. Thus, a prey choice experiment was conducted to determine if selected BRS demonstrated a preference between prey mechanically killed (decapitation or head crushing) and prey that had been freeze-killed. Crushing the head of the prey with forceps, (mechanically), resulted in crickets moving and kicking erratically opposed to freeze-killing which resulted in immobile and thus safer prey for the spiders (Figure 3.2).

Initial Preference Testing - Scavenging

Twenty BRS (Figure 3.3), five from each of four categories based on sex and origin: 1) Male, field collected/wild-caught (W/C), 2) Female, W/C, 3) Male, lab raised (L/R), 4) Female, (L/R), were randomly selected and starved for three weeks prior to the test. Prey were placed on opposite sides of a 17.1 cm diameter (6.75 inch) x 5 cm (2 inch) height (Nuconic Packaging, Vernon CA) lidded container and labeled to indicate the location of the freeze-killed prey (Figure 3.4). BRS were introduced to the preference container by inverting the rearing cup and placing it in the middle of the container. The rearing cup was left inverted with the spider contained in the center of the chamber for ~30 minutes prior to introducing prey into the chamber. Following the placement of crickets, the lab was darkened and rearing containers lifted allowing the BRS

freedom of movement within the testing chamber. Testing chamber lids were locked into place and BRS were observed using red lights from the headlamps for two hours. Prey choice and elapsed time of choice were recorded.

Results – Initial Preference Testing

Five of the twenty BRS fed during the two-hour trial. Mechanically-killed prey were fed upon by one field collected female and one lab raised female. Freeze-killed prey were fed upon by one field collected male and one lab raised female. One lab raised male fed on both prey types while a field collected male fed on a mechanically-killed cricket two hours post-prey introduction.

Discussion – Initial Preference Testing

This experiment was initiated to streamline colony feeding and shorten the amount of time required in colony maintenance. It was also beneficial in that it allowed another option for the study of scavenging. Results of this test indicate that of the BRS tested, there was no clear preference between freeze-killed and mechanically killed crickets, nor do BRS sex and origin appear to be a factor in prey choice between the two prey types. Statistical analysis was not conducted or needed as these were observations and were continually verified over the next two years BRS testing proceeded.

Pesticides

The two pesticides used throughout this study, were: 1) a synthetic pyrethroid, (Ortho® Home Defense Max) active ingredients by weight: Bifenthrin 0.05% and Zeta-Cypermethrin 0.0125%. This pesticide was chosen for the active ingredient as a comparison to Schwarting and Whitworth (2015) study in which this particular (Ortho®) pesticide was one of two used. The

synthetic pyrethroid pesticide is in a premixed, liquid spray form and has both brown recluse spider and house cricket listed specifically on the label.

In general, synthetic pyrethroids have a mode of action in insects that effects the normal function of the nerves by altering the rate of change of voltage-sensitive sodium channels (Soderlund, 2010). Bifenthrin which is categorized as a Type I synthetic pyrethroid, *"produces long trains of action potentials (burst discharges) following a single stimulus with little or no effect on resting potential"* (Soderlund, 2010). Whereas, Zeta-Cypermethrin (an isomer of Cypermethrin) is categorized as a Type II synthetic pyrethroid *"that does not induce repetitive firing but instead causes a use-dependent block of action potential coupled with depolarization of the resting potential"* (Soderlund, 2010).

2) The second pesticide used was a Spinosad, a bacterial fermentation product, used primarily in organic gardening and around residences, active ingredients: Spinosad (mixture of spinosyn A and spinosyn D) 0.001% and 99.999% "other ingredients". Captain Jack's Deadbug Brew® is labeled for lepidopteran and other arthropod pests, (not specifically labeled for crickets or brown recluse spiders). This pesticide was chosen as an alternate to synthetic pyrethroids and has a mode of action that focuses on the over stimulation of the insect central nervous system (CNS). In *Hayes' Handbook of Pesticide Toxicology*, Ujváry describes Spinosad poisoning in three phases: *"phase one are prolonged involuntary muscle contractions typically the elevation of the body and straightening of the hindlegs, in phase two uncoordinated movement occurs and fine tremors appear in the muscles and typically the insect falls on its back, in the final phase apparently due to neuromuscular fatigue, all movements and tremors cease and paralysis follows"* (Ujváry, 2010).

Determination of pesticide efficacy on BRS and prey

Plastic 5.9 L Sterilite® rectangular containers (Townsend, MA) were used as the testing/kill chambers (Figure 3.5) and measured 38.1 x 29.2 x 8.3 (length x width x height) cm (15 x 11.5 x 3.3 inches). One container was labeled and used as a control, with the interior surface sprayed with water only. Two other containers were used as kill chambers and had their interior surfaces sprayed with the designated pesticide mentioned. These interior surfaces were initially sprayed/coated with the appropriate liquids to the point of runoff and allowed to dry.

To determine that the pesticides were in fact toxic to BRS, four (two per chamber) recently fed (within 24 hours) BRS were randomly selected and individually placed in the middle of each test chamber, after the liquid sprays were allowed to dry (~60 minutes), covered with the container lid, and observed (Figure 3.6). BRS were kept in contact with the sprayed surfaces for 120 minutes then removed and placed in individually marked empty rearing cups (Fabri-Kal® 4-ounce translucent portion cups) and observed. Observations continued for the first 15 min and then every 30 min for the first two hours. Following the initial two-hour period, observations were made hourly for the next six hours and then every twelve hours for the next 96 hours. After the 96-hour observation, surviving BRS were provided a small-decapitated cricket and returned to their rearing cup.

To determine that the pesticides were also toxic to the crickets, the same procedures were evaluated with four (two per container) small (1/8 - 1/2") house crickets.

Results - Pesticide efficacy on BRS and prey

Pyrethroid (Ortho Home Defense Max®)

The pyrethroid killed both the BRS and crickets. Initially, BRS showed no ill effects due to the pyrethroid following the 120-minute exposure time. Observations of the BRS after

removal from the "treatment" chamber to an unused empty rearing cup indicated the BRS were unaffected. The spiders went into the usual "hide" position, legs pulled up in the typical *Loxosceles spp* slant legged resting appearance (Figure 3.7), and remained quiet. During the observation period, each time the rearing cup was moved the BRS moved as if alarmed, as did those not exposed to pesticide. Approximately 12-hours post-exposure, the BRS died. Both BRS were in the characteristic "death pose" (Figure 3.8) with their legs tucked under the body. Gentle prodding with forceps elicited no reaction, nor did actually picking up BRS by a leg. The "control" BRS (subjected to the water chamber), showed no ill effects of the trial and were alive at the end of the 96-hour observation period. Thus, since treating a surface with water had no effect on spiders – all treatments involving water were combined into one control treatment

All crickets exposed to the pyrethroid were moribund within fifteen minutes of contact and considered dead after prodding with forceps two hours post exposure. The crickets (exposed to the water chamber) were alive following the 96-hour observation period.

Spinosad (Captain Jack's Deadbug Brew®)

BRS and cricket reaction to the Spinosad was much slower. After the 120-minute exposure period, the BRS were still moving, appearing completely unaffected, as were the crickets. BRS and crickets were not removed from the chamber, instead were left in contact with the Spinosad for a longer period and observed every thirty minutes for additional 120-minutes. After four hours of exposure some effects were detected in the crickets, three of the ten were ventral side up and unmoving; the BRS had settled into their usual "rest" position and remained quiet which increased the surface area of the spider in contact with the Spinosad. Gentle shaking of the chamber caused the BRS to react as if alarmed. However, after eight hours their

movements appeared to slow. Crickets and BRS were left overnight in the Spinosad chamber; after eighteen hours of contact all crickets and BRS were dead.

Discussion - Pesticide efficacy on BRS and prey

Both pesticides killed BRS and crickets eventually. The Spinosad® was not nearly as quick in its ability to kill as the pyrethroid however; deaths of both can be attributed to its exposure. Although it was not labeled for either BRS or crickets it was selected to provide an alternative to those homeowners concerned with chemicals within their homes or gardens. Many consumers are concerned with the introduction of chemicals, especially in and around their homes. Investigating the efficacy of a product used typically in organic gardening as a comparison to "general use" synthetic organic pesticides allows the possibility of alternatives that are feasibly more "environmentally friendly" but still may kill some common pests that BRS may encounter.

Figures Chapter 3

Figure 3.1 BRS killed by prey (cricket)

BRS can be killed by prey – thus the importance of BRS accepting freeze-killed prey as well as live and with no ill effects on aspects of biology.

Figure 3.2 Preference testing

Lab Raised male BRS (held under cup); crickets placed to either "side" (freeze killed marked with pink tape).

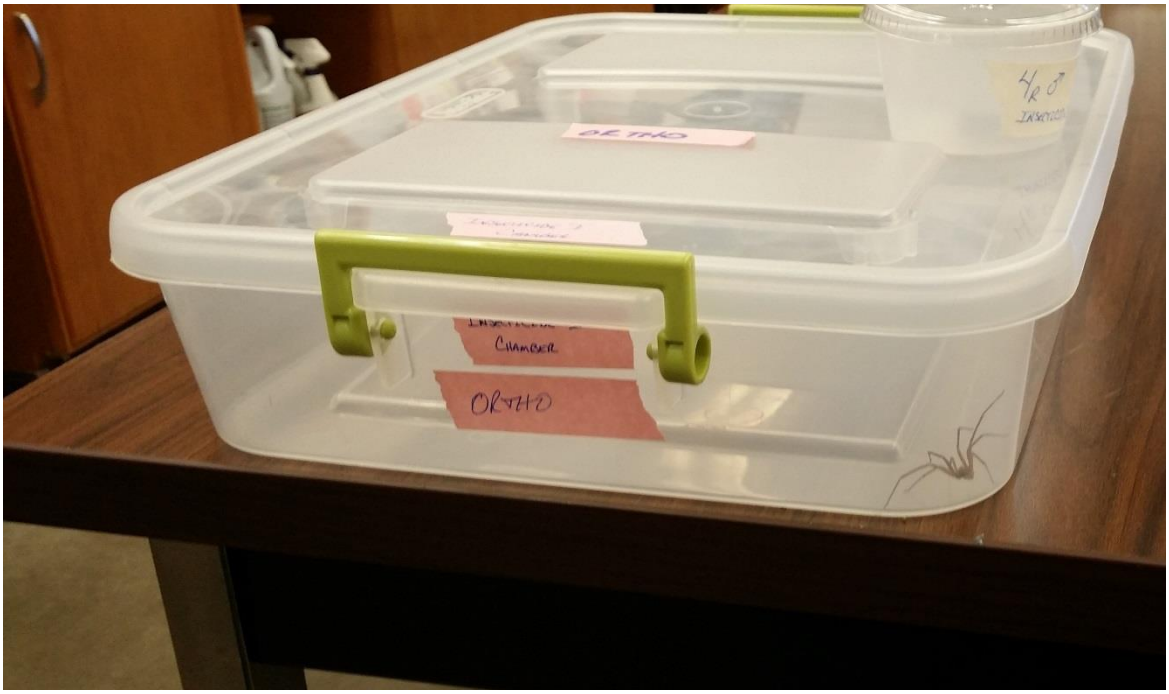
Figure 3.3 BRS placement prior to testing

Contained BRS in preparation for preference testing (prior to cricket placement)

Figure 3.4 BRS feeding on freeze-killed cricket

BRS Feeding on freeze killed prey

Figure 3.5 BRS in "kill" chamber



Sterilite® container used as “Kill Chamber”

Figure 3.6 Pesticide efficacy test

BRS in Kill Chamber testing efficacy of pyrethroid pesticide

Figure 3.7 Male BRS in resting position

Male BRS in “typical” resting position, legs pulled up, “slant legged” appearance

Figure 3.8 "Death" pose

Characteristic “death” pose with legs curled under the body

References Chapter 3

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Chapter 4 - Experiment: Effects of Prey Quality

Introduction – Scavenging versus Predation

After determining BRS would readily feed on freeze-killed prey, the question of whether prey quality declined as the prey desiccated became important. The decline of prey quality and the effect of nutritive value for scavenging BRS were explored.

Methods and Materials - Scavenging versus Predation

Using weight change and survival of BRS as measures of prey quality, 156 mature BRS (Table 4-1) were randomly divided into thirteen different treatments of twelve BRS per treatment attempting to keep the sex ratio and origin (field collected (W/C) or lab raised (L/R)) as evenly distributed as possible (Figure 4.1). Trials were conducted for eight weeks with prey introduced weekly and left with the BRS for twenty-four hours. Feeding was easily determined 1) by the death of the prey and/or 2) the deflation of the prey. An eight-week trial length was selected in an attempt to allow for predator satiation. BRS, possibly satiated after one feeding, would then feed again at some point during the intervening eight-week trial period. Eight weeks allowed BRS multiple opportunities to feed and assisted in an attempt at addressing the satiation question raised by previous research.

Treatments were kept separate by utilizing trays able to hold twelve rearing cups and marked with specific treatments and times. The trays, plastic 5.9 L Sterilite® rectangular containers were lidded, and clearly marked on both the lid and tray for each of the treatments. Each treatment had the same BRS breakdown: BRS #s 1 & 2 were L/R males, #s 3 & 4 were W/C males, #s 5 – 8 were L/R females and BRS #s 9 – 12 were W/C females.

During his study of scavenging, Sandidge (2003), conducted preference testing by feeding BRS an assortment of prey once weekly then starving them for two weeks. Cramer

(2008) found the BRS were not receptive to prey with such short time intervals and extended the starvation period to four weeks and obtained a higher rate of feeding per spider. Prior to beginning this study, our BRS were not fed for eight weeks, doubling the Cramer (2008) starvation period. When fed during the trial, the prey was left in the presence of the BRS for 24-hours. Vetter (2015) remarked that the spiders in his study would feed almost as soon as the cricket was placed in the chamber; Hite (1966) observed that BRS began to feed within two hours after prey introduction. Sandidge and Hopwood (2005) determined BRS fed readily under low light conditions. During this study, the prey were placed into the cup with BRS under normal laboratory light conditions, and then placed into a darkened laboratory cabinet in an attempt to reproduce the typical conditions for BRS occupying structures. Containers were weighed empty, then reweighed with the spider to obtain spider weight at the initiation of the trial, and recorded. Ambient temperatures were 68 – 72° F (20 - 22° C).

For predation, the live prey treatment was used as a control to compare the effect (if any) of water on prey, no treatment was applied to the crickets prior to feeding them to the BRS. The water treatment was used to determine if water used in pesticides had any effect on the prey; both the untreated and water treatment crickets were alive for testing.

For the portion of the study focused on scavenging, crickets killed by freezing were used as the control. To standardize desiccation times, crickets were allowed to thaw at room temperature for thirty-minutes before the desiccation periods were considered to begin. The thirty-minute thaw period was determined during colony maintenance; crickets removed from the freezer were allowed to thaw for thirty-minutes prior to feeding colony spiders. No more than forty-eight hours prior to prey introduction, crickets were placed into the freezer and killed by freezing at (-20° C). Ninety minutes prior to test initiation 48 crickets for the scavenging portion

of the study were removed from the freezer and allowed to warm to room temperature, i.e. thirty-minute thaw plus sixty minute desiccation in the case of the 1-hour treatment, continuing to desiccate at room temperature until the desiccation period elapsed. During this same ninety-minute period, the bottom surface of the "kill" chambers, (plastic 5.9 L Sterilite® rectangular lidded containers), for the water and pesticide treatments were sprayed to the point of run off and allowed to dry. Freeze-killed crickets desiccated for the same amount of time that live crickets were exposed to treated chambers; namely one, eight, twenty-four or seventy-two hours. Once the exposure time had elapsed, crickets were fed individually to BRS per treatment and period. All prey were introduced to the rearing containers with forceps and the lid closed. Each of the forceps used to transfer crickets to BRS containers were marked by treatment to prevent cross contamination. BRS were fed under laboratory light conditions then placed into a cabinet and kept in darkness.

The eight hour treatments were fed after the prey were exposed to the respective treatment for eight hours, and the same procedures as previously explained were followed. Each BRS was given the specific prey and the treatments returned to the cabinet. All BRS were exposed to the prey for twenty-four hours, then the prey removed from the container and the container immediately re-weighed to determine weight change. The respective treatments continued for eight weeks using the process outlined.

Results - Scavenging versus Predation

BRS Predation: Untreated

12/12 = 100% BRS survival (Table 4-3).

Overall, the predation treatment consisted of both the live and water treated prey. No treatment was applied to the crickets in the "live" treatment prior to feeding them to the BRS.

Thus, the “live” treatment was considered a control when comparing the effect of water on prey. The twelve surviving BRS in this treatment had a mean weight gain of 11.17 mg ± 9.06 (SE), (Table 4-3), an overall 26.36% gain in weight for the treatment, and no deaths. Determination of BRS feeding was by the death of the cricket and its subsequent deflation.

BRS Predation: Water exposed prey

44/48 = 91.67% BRS survival (Table 4-3)

The water exposed prey treatment had four BRS deaths: week 2, BRS #4 (24-hour) was killed and eaten by the cricket, week 3, BRS #11 (72-hour) cause undetermined; week four, BRS #10 (1-hour) cause undetermined and BRS #9 (72-hour) died from undetermined cause. Results showed an overall weight gain in all treatments (Figure 4.2) with a mean weight change of BRS fed water exposed prey of 11.61 mg ± 2.39 (SE) not depicted.

1-hour water-exposed prey

Eleven surviving BRS in the 1-hour treatment survived the eight-week trial resulting in an overall mean weight gain of 12.45 mg ± 5.34 (SE). As a treatment, the mean percentage weight change was 31.59% gain.

8-hour water-exposed prey

Twelve surviving BRS in the 8-hour treatment showed a mean weight gain of 8.92 mg ± 6.19 (SE), a mean percentage weight gain of 22.99% for the treatment.

24-hour water-exposed prey

Eleven surviving BRS in the 24-hour treatment showed a mean weight gain of 11.55 mg ± 3.46 (SE), a mean percentage weight gain of 24.58% for the treatment.

72-hour water-exposed prey

Ten surviving BRS in the 72-hour treatment showed a mean weight gain of 14 mg ± 3.53 (SE), a mean percentage weight gain of 22.67% for the treatment.

BRS Scavenging: Desiccation (Freeze-killed prey)

44/48 = 91.67% BRS survival (Figure 4.3 Table 4-3)

Overall, in the freeze-killed prey treatments there were four BRS deaths: week one, BRS #6 (72-hour), cause undetermined; week two, BRS #9 (24-hour) and BRS #12 (72-hour), both of indeterminate causes and week seven, BRS #3 (72-hour), cause undetermined. Treatment mean weight change (Table 4-3) for BRS scavenging upon desiccated prey was 2.77 mg ± 1.76 (SE).

1-hour Desiccation (Freeze-killed prey)

Twelve surviving BRS in the 1-hour treatment showed a mean weight gain of 12.58 mg ± 3.02 (SE), a mean percentage weight gain of 28.25% for the treatment.

8-hour Desiccation (Freeze-killed prey)

Twelve surviving BRS in the 8-hour treatment showed a mean weight gain of 2.75 mg ± 3.03 (SE), a mean percentage weight gain of 8% for the treatment.

24-hour Desiccation (Freeze-killed prey)

Eleven BRS in the 24-hour treatment showed a mean weight gain of 2.27 mg ± 2.65 (SE), a mean percentage weight gain of 10.28% for the treatment.

72-hour Desiccation (Freeze-killed prey)

Nine BRS in the 72-hour treatment showed a mean weight **loss** of 9.67 mg ± 1.33 (SE), a mean percentage weight **loss** of 12.72% for the treatment.

BRS Scavenging: Pesticide-killed prey (Pyrethroid)

21/48 = 43.75% BRS survival (Figure 4.4 Table 4-3)

Overall, there were twenty-seven BRS deaths:

- Week 1: six deaths in the 1-hour treatment, two deaths in the 8-hour treatment and two deaths in the 24-hour treatment.
- Week 2: one death in the 1-hour treatment, three deaths in the 8-hour treatment, two deaths in the 24-hour treatment and one death in the 72-hour treatment.
- Week 3: one death in the 1-hour treatment, one death in the 8-hour treatment, and one death in the 72-hour treatment.
- Week 5: one death in the 72-hour treatment.
- Week 6: two deaths in the 1-hour treatment, two deaths in the 8-hour treatment, and two deaths in the 24-hour treatment.

Deaths of BRS did not occur in weeks, 4, 7 or 8. Treatment mean weight change (Table 4-3) for BRS scavenging upon pesticide-killed prey was -21.43 mg ± 2.64 (SE).

1-hour Pesticide-killed prey (Pyrethroid)

2/12 = 16.7% BRS survival

Two surviving BRS in the 1-hour treatment had a mean weight loss of 28.5 mg ± 2.5 (SE), a mean percentage weight loss 40.43% for the treatment.

8-hour Pesticide-killed prey (Pyrethroid)

4/12 = 33.3% BRS survival

Four surviving BRS in the 8-hour treatment had a mean weight loss of 28.25 mg ± 7.66 (SE), a mean percentage weight loss 23.11% for the treatment.

24-hour Pesticide-killed prey (Pyrethroid)

6/12 = 50% BRS survival

Six surviving BRS in the 24-hour treatment had a mean weight loss of 26 mg ± 4.31 (SE), a mean percentage weight loss 29.54% for the treatment.

72-hour Pesticide-killed prey (Pyrethroid)

9/12 = 75% BRS survival

Nine surviving BRS in the 72-hour treatment had a mean weight loss of 13.78 mg ± 3.16 (SE), a mean percentage weight loss 16.30% for the treatment.

Table 4-2 illustrates the percentage of survivability for entire treatments. To obtain percent weight change, the mean starting weight for each treatment was obtained and subtracted from the mean end weight and the difference divided by the mean start weight. The result was then multiplied by 100 to determine a percentage, using only the BRS that survived after eight weeks.

Discussion - Scavenging versus Predation

The hypotheses are: 1) BRS, as an opportunistic feeder, derives equal benefit as a predator or a scavenger and, 2) BRS scavenging upon insecticide-killed prey has a detrimental effect on the BRS.

Once it was determined that BRS would feed on freeze-killed crickets as readily as mechanically killed, the question of declining prey quality over time became important. In other words, does desiccation have an impact on the ability of the BRS to obtain nutritive value, as measured by weight change and survivability? As discussed previously, BRS are wandering hunters and typically roam at night. In keeping with the management strategy of a general pesticide application for BRS or general pest management, pests may cross newly pesticide treated areas soon after spraying. The toxins may or may not take effect immediately, allowing the pest to move away from the treated area later to be found and fed upon by a scavenging BRS. The time elapsed from prey death to being scavenged by BRS could be anywhere from hours to days dependent upon when and where the prey died.

BRS feeding on live prey resulted in a mean weight gain of 11.17 mg ±9.06 (SE) vs a mean weight gain for the water treated prey of 11.61 mg ±2.39 (SE) which led to the decision to combine the treatment results as a predation control treatment, weight gain 11.52 mg ±2.65 (SE), and discontinue testing the untreated prey as a separate treatment. BRS scavenging on desiccated prey resulted in a mean weight gain of 2.77 mg ±1.76 (SE) vs a mean weight loss of -21.43 mg ±2.64 (SE) for BRS scavenging upon pesticide-killed prey. While the means are not considered significant, (apart from the pyrethroid treatment), significance in mean weight change was noted in the weight gain within treatments: predation (live prey) 1-hour water-exposed treatment (12 mg), and 72-hour water exposed treatment (14 mg), scavenging 1-hour freeze-killed treatment (12.5 mg). The mean weight gain overall demonstrates that BRS do benefit from predation versus scavenging.

Freeze-killed prey results indicated that scavenging is beneficial up to three time periods (1-hour, 8-hour and 24-hour) and about the same as live prey for two of the same time periods (excluding the deaths due to prey killing the BRS) 1 and 8-hour. 24-hour scavenging vs predation resulted in a greater than nine milligram difference in mean weight gain with the predation treatment out gaining the scavenging treatment. BRS scavenging on prey killed for ≥ 72-hour is not beneficial, with a mean weight loss of 9.7 mg, which suggests that scavenging on prey that has been dead longer than forty-eight hours may not provide adequate nutrition.

Based upon the increased mortality and mean weight loss of surviving BRS, results of this experiment indicate detrimental effects to BRS that feed on pesticide-killed prey. As a treatment, BRS scavenging on pesticide-killed prey resulted in 43.75% survivability with the least survivability occurring in the 1-hour pesticide-killed prey, (2 of 12 BRS survived). Significant mean weight loss occurred in all four time periods with the greatest occurring in the

prey exposed for the lower amount of time, -28.5 mg, -28 mg and -29 mg, 1-, 8-, 24-hour exposure, respectively.

These results appear to support the second hypothesis that "BRS that scavenge on pesticide-killed prey will be detrimentally effected". The support for this hypothesis can be seen in the decreased survivability and mean weight loss for all BRS fed pesticide-killed prey treatment. In opposition to the Sandidge 2003 study, BRS feeding upon insecticide killed prey, BRS died in the first week of exposure to prey killed by insecticide.

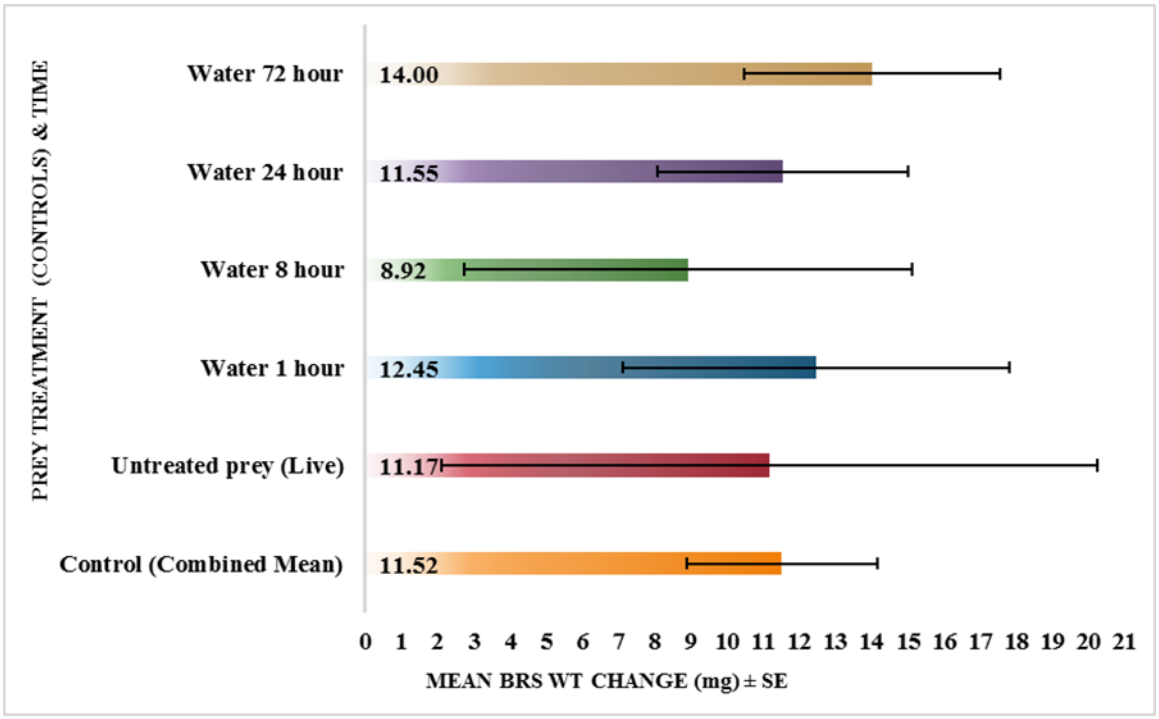
The first hypothesis is not as clearly supported i.e. "BRS as opportunistic feeders derive equal benefit as a predator or scavenger". Weight gain for the one-hour desiccation time treatment was similar to that for the live and water treated prey, but was less as desiccation times increased. More testing of scavenging and predation are required to gain a better insight.

Figures – Chapter 4

Figure 4.1 Initial experimental setup

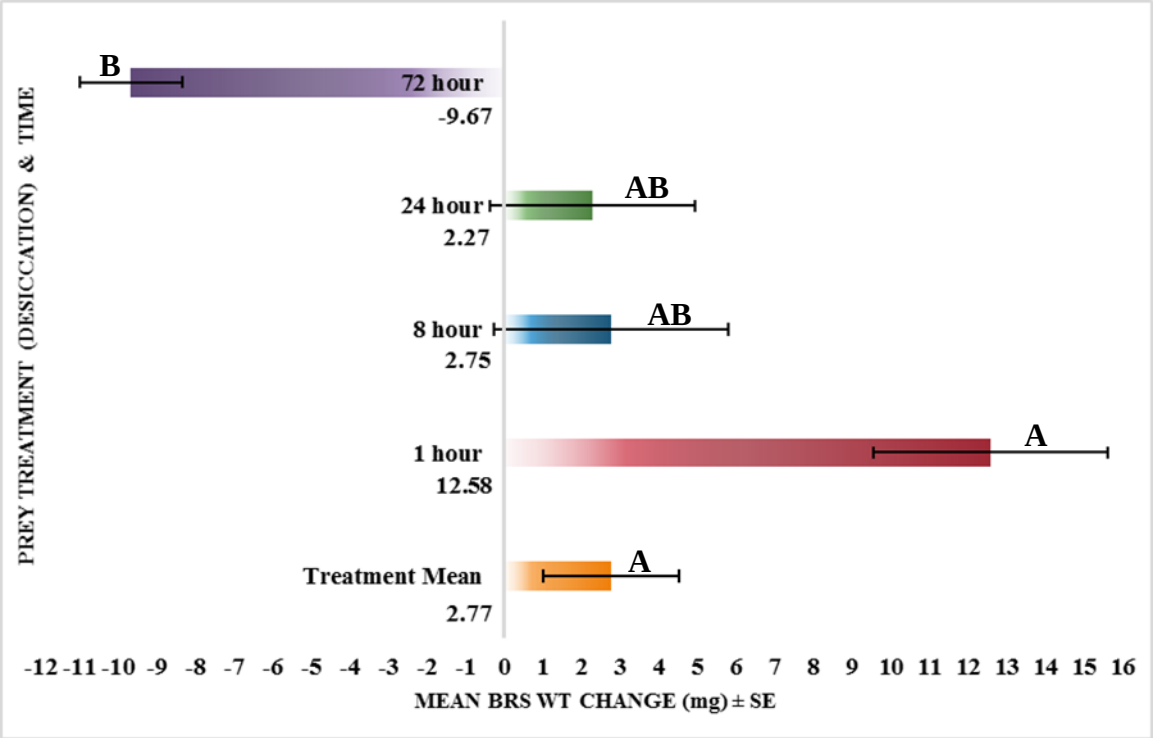
Experimental set up one replication; green letter lab reared (L/R); red letter field collected (W/C)

Figure 4.2 Predation Mean Weight Change (control)



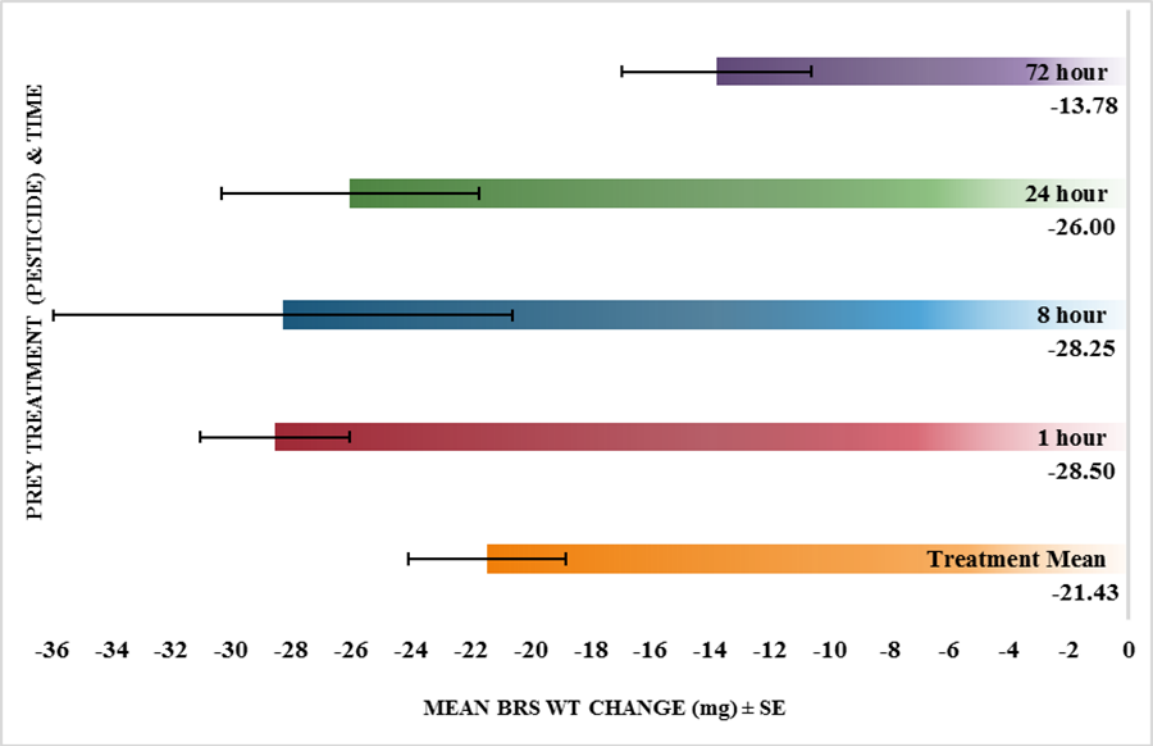
No significant differences in means $p < 0.05$

Figure 4.3 Scavenging Mean Weight Change – Desiccation time (Freeze-killed prey)



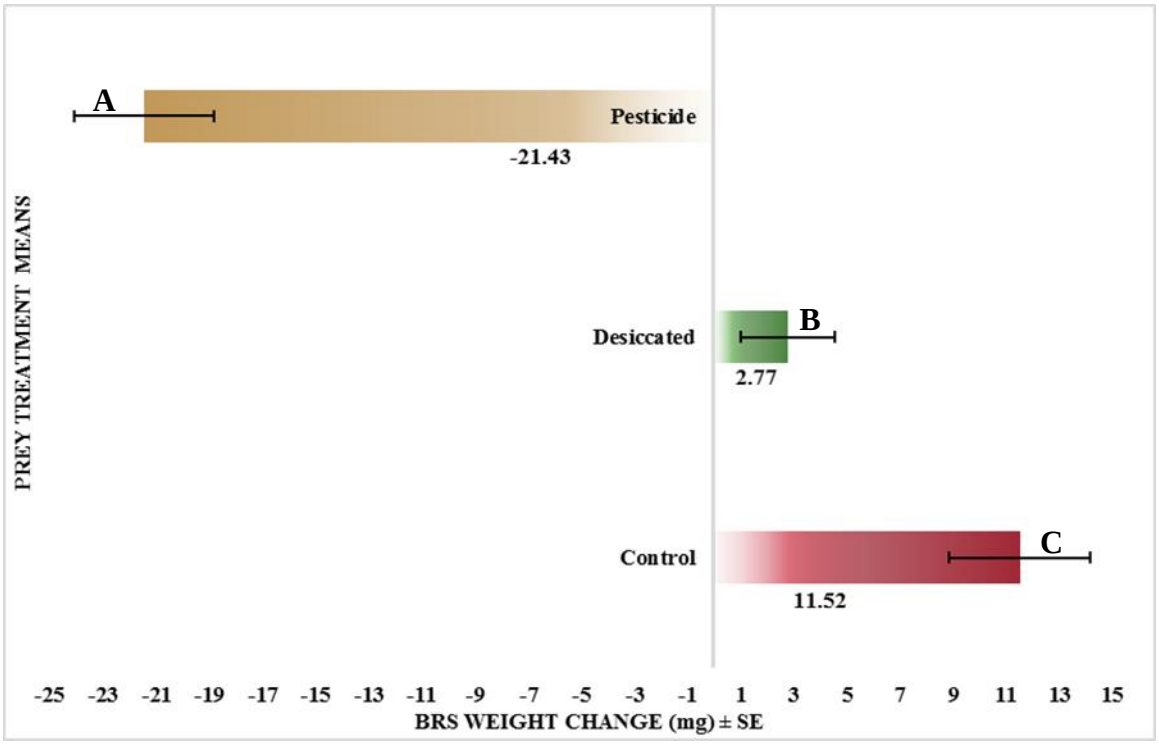
Means with the same letter do not differ significantly $p < 0.05$

Figure 4.4 Scavenging Mean Weight Change – Insecticide-killed prey



No significant differences at Alpha 0.05 P-value= 0.0804

Figure 4.5 BRS Mean Weight Change by Treatment



Means with the same letter do not differ significantly $p < 0.05$

Tables – Chapter 4

Table 4-1 BRS Count by Treatment Type and Interval

Prey Treatment	1 hour	8 hour	24 hours	72 hours	Total BRS - 156
Untreated	12				12
Water	12	12	12	12	48
Desiccated	12	12	12	12	48
Pesticide	12	12	12	12	48

Table 4-2 BRS Treatment Survivability and Mean Weight Change

Treatment	# BRS Survived	Mean Weight Change	Survivability %
Predation (Controls)	**56/60**	**11.52 mg ± 2.65 (SE)**	**93.33%**
Scavenging (Desiccation)	**44/48**	**2.77 mg ± 1.76 (SE)**	**91.67%**
Scavenging (Pesticide)	**21/48**	**-21.43 mg ± 2.64 (SE)**	**43.75%**

Table 4-3 Treatment Means, Standard Errors and Standard Deviations

	Treatment	Mean Wt Change (mg)	Standard Error	Standard Deviation
Control	Control (Combined Mean)	11.52	2.65	19.82
	Untreated prey (Live)	11.17	9.06	31.37
	Water 1 hour	12.45	5.34	17.72
	Water 8 hour	8.92	6.19	21.44
	Water 24 hour	11.55	3.46	11.48
	Water 72 hour	14.00	3.53	11.17
Desiccation	Treatment Mean	2.77	1.76	11.68
	1 hour	12.58	3.02	10.46
	8 hour	2.75	3.03	10.50
	24 hour	2.27	2.65	8.79
	72 hour	-9.67	1.33	4.00
Pyrethroid Pesticide	Treatment Mean	-21.43	2.64	12.10
	1 hour	-28.50	2.50	3.54
	8 hour	-28.25	7.66	15.33
	24 hour	-26.00	4.31	10.55
	72 hour	-13.78	3.16	9.47

References Chapter 4

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Chapter 5 - Experiment: BRS Scavenging using Pyrethroid and Spinosad Pesticide

Introduction

Management techniques for BRS typically involve the judicious use of pesticides and/or glue traps, both of which are also commonly used for other household pests. However, the use of pesticides may cause concern with some members of the public and more "environmentally friendly" pesticides may be of interest those concerned with limiting the impact of toxic chemicals on the environment.

Building upon and using the preliminary results obtained in the previous study involving prey desiccation and scavenging; three factors influenced the choice of treatment exposure and prey exposure times: 1) the desiccation study results, 2) efficacy of pesticides upon the prey and 3) practices of pest control operators (PCOs). Using weight change and survivability as indicators, the objectives of this study were to determine the effect of scavenging on BRS, using prey killed by three different methods. Our research attempted to determine if BRS repeatedly scavenging on insecticide-killed prey has an acute effect on their population

Time period determination

Prey exposure of 24-hours was selected for prey treatments. The predation trials resulted in the treatment mean weight change and 24-hour mean weight change being nearly identical. When examining scavenging on freeze-killed prey, the treatment mean was closer to the 8-hour mean, however; for scavenging on pesticide-killed prey, the 24-hour mean was closer to the overall treatment mean. Therefore, 24-hour prey treatment appeared closest to the overall treatment means in two of the three treatments and negligible in the third.

Preliminary efficacy testing of Spinosad demonstrated prey (crickets) exposure time to the insecticide required eighteen hours or longer. Whereas when exposed to the synthetic pyrethroid the crickets typically died within fifteen minutes. Due to the increased time necessary to kill the crickets with Spinosad, a prey exposure time of 24-hours was selected.

Pest control operators (PCO's) and their practices also assisted in the final determination of the 24-hour exposure of prey to treatment and subsequent exposure of BRS to the treated prey. It is not an uncommon practice for PCO's to spray a structure early in the day. Allowing some time for the pests to be exposed to the chemical and allowing time to elapse before a BRS finds the dead pest to scavenge upon; 24-hour exposure is not an unusual premise. Previous data suggested (Table 4-3) that desiccation for longer than 72-hours provided limited benefit as measured by weight gain. Therefore, prey exposed to the treatment for 24-hours and then in turn, exposed to the BRS for 24-hours allowed a window of 24-48-hour post-treatment prey exposure to the BRS. This time can easily be justified in a recently treated structure and is in keeping with the applied entomological focus of the laboratory; therefore, 24-hour prey treatment was selected as well as 24-hour prey exposure to BRS. Having a standard period for prey treatment not only allowed consistency within the experiment but also provided enough time for the crickets to show effects of the pesticides chosen.

Methods and Materials - Pyrethroid and Spinosad

Nine-hundred seventy (970) brown recluse spiders (BRS), captured from various locations around North-Central Kansas, or raised in the lab, from 2013 thru 2017 were used in this study. 240 adults and 192 juveniles (unsexed) were used in separate 8-week scavenging trials; 180 adults were used in a scavenging preference study; 358 juveniles were maintained as a colony for rearing purposes and a developmental study. As juveniles matured and their sex

determined they were transferred to one of the 8-week trials. Spiders that survived scavenging trials were mated within treatments to determine fecundity. Five replications of adults and four of juveniles with twelve BRS per prey treatment were utilized in eight-week trials. Forty-eight BRS were randomly assigned to one of four prey treatments: pyrethoid-killed, Spinosad-killed, freeze-killed or water-treated. The fifth replication differed slightly from the first four replications due to the ratio of male to female BRS tested. In the fifth replication, the ratio male to female was equal (6:6), the previous four replications, male to female ratio was approximately 4:8 (male to female) due to BRS availability, (Figure 5.1). All BRS were randomly assigned to a treatment via a random number generator. Overall, 240 adult BRS and 192 juveniles, (sixty (60) adult BRS and forty-eight (48) juveniles per treatment), were utilized.

Individual BRS containers were used for each trial, using the same Fabri-Kal® polystyrene 4oz. portion cups, with clear Fabri-Kal® lids (Kalamazoo, MI) and rectangular 2.5 x 1.5cm (~ 1 x ½ in) piece of cardboard, creased in the middle, as used when rearing the colony. The cup with cardboard and lid was weighed and the weight recorded on the cup, the BRS was then placed into the cup and reweighed, the difference in weight being the initial spider weight. The cup was numbered and a random number generator utilized to assign numbered BRS to specific treatments. To ensure uniform male and female representation in each treatment, sexes were assigned to a block of numbers and the random number generator ran with only that block of numbers. The treatments were also randomly assigned a number (1-8 for two iterations) in an attempt at more randomization. Juvenile BRS were simply assigned numbers from 1-96 and assigned to random treatments as described with the adults. Ambient temperatures were 68 – 72° F (20 - 22° C).

No more than forty-eight hours prior to prey introduction, the crickets used as the scavenging control, were killed by freezing at (-20° C). Twenty-four hours prior to test initiation the frozen crickets were removed from the freezer and allowed to thaw to room temperature. Testing chambers for the insecticide treated crickets, were sprayed with the specified treatment to the point of run off and allowed to dry. Once chambers were dry, at least twenty-four live crickets were placed into each of the chambers and lids locked into place by treatment type. Ultimately, to ensure cricket death, Spinosad was sprayed directly on the surviving crickets following the 24-hour exposure.

After twenty-four hours of exposure, crickets were provided individually to BRS per treatment. BRS were fed under laboratory light conditions then placed into a cabinet and kept in darkness. All BRS were exposed to the prey for twenty-four hours, the prey was removed from the container upon mortality and a determination of whether the BRS fed were determined each week as the prey was removed from the cup.

Once assigned to a specific treatment, BRS were placed in separate trays with lids, capable of holding a single layer of twelve rearing cups. The trays, plastic 5.9 L Sterilite® rectangular containers were clearly marked on the lid and tray for each treatment. Feeding procedure followed the model used in the previous study involving scavenging and desiccation. Each treatment was offered at a specific time/day allowing twenty-four-hour prey exposure to each BRS within treatment type. See Table 5-2 for an example schedule of a one-week period. In keeping with the protocol established in the desiccation study, trials lasted eight weeks with prey introduced to spiders for a twenty-four period once weekly. Following the eight-week trial, surviving BRS were weighed to determine weight change and paired within treatment type for mating.

Results - Scavenging using Pyrethroid and Spinosad Killed Prey

Male BRS in earlier trials (specifically the pyrethroid treatment) did not survive in adequate numbers to conduct fecundity studies for pyrethroid. In an attempt to remedy the lack of male survivors, two additional trials of pyrethroid only were conducted with an equal ratio of male: female (six and six). Unfortunately, the same results continued with the loss of males preventing pyrethroid fecundity experimentation.

Adult BRS survival and change of weight by treatment

Table 5-3 depicts the survivorship percentage and mean weight change in mg of BRS in all trials conducted.

Pyrethroid killed prey– (Male 1/22: Female 8/38) Sixty BRS were provided prey killed by the pyrethroid insecticide; of the sixty tested, nine survived the initial eight-week period, the surviving male died the next week prior to transfer to mating chamber, an eight-week survival percentage of 15%. Survivor mean weight change (loss) was -23.89 mg ± 7.27 (SE).

Spinosad killed prey – (Male 16/22: Female 23/38) Sixty BRS were provided prey killed/treated with Spinosad; of the sixty tested, thirty-nine survived the initial eight-week period, a survival percentage of 65%. Survivor mean weight change (gain) was 13.15 mg ± 2.92 (SE)

Scavenging – (Male 20/22: Female 38/38) Sixty BRS were provided prey killed by freezing; of the sixty tested, fifty-eight survived the initial eight-week period, a survival percentage of 96.67%. Survivor mean weight change (gain) was 10.15 mg ± 2.70 (SE).

Predation – (Male 22/24: Female 31/36) Sixty BRS were provided live prey; of the sixty tested, fifty-three survived the initial eight-week period, a survival percentage of 88.33%. Survivor mean weight change (gain) was 18.19 mg ± 3.09 (SE).

Juvenile BRS survival and weight change by treatment

Pyrethroid – Forty-eight BRS were provided prey killed by the pyrethroid insecticide; of the forty-eight tested, ten survived the initial eight-week period, a survival percentage of 20.83%. Survivor mean weight change (loss) was -8.40 mg ± 3.20 (SE).

Spinosad – Forty-eight BRS were provided prey killed/treated with a Spinosad insecticide; of the forty-eight tested, thirty-six survived the initial eight-week period, a survival percentage of 75%. Survivor mean weight change (gain) was 9.08 mg ± 2.23 (SE).

Scavenging – Forty-eight BRS were provided prey killed by freezing; of the forty-eight tested, forty-eight survived the initial eight-week period, a survival percentage of 100%. Survivor mean weight change (gain) was 5.33 mg ± 1.37 (SE).

Predation – Forty-eight BRS were provided live prey; of the forty-eight tested, forty-eight survived the initial eight-week period, a survival percentage of 100%. Survivor mean weight change (gain) was 24.67 mg ± 2.76 (SE).

Discussion: Scavenging using Pyrethroid and Spinosad Killed Prey

Pyrethroid insecticide

Survival was pointedly different between treatments, the pyrethroid treated prey resulted in the survival of 15% of the adults and 20.83% of the juveniles. These results appear to be in opposition to the Sandidge (2003) report in which he determined no obvious negative reaction to those BRS feeding upon insecticide-killed prey. Not only do BRS have a low level of survival when feeding upon pyrethroid-killed prey but in the terms of weight change they demonstrated considerable weight loss compared to other treatments. In adult BRS, the average weight change of the surviving BRS was a loss of 23.89 mg. BRS average weight in this study ranged from 63-

93 mg, so this equals about a loss of one-third (1/3) body weight in eight weeks. This may be considered an issue regarding nutritive value.

Spinosad insecticide

BRS scavenging on Spinosad-killed prey resulted in 65% adult survival, and 75% juvenile survival. In early trials, the Spinosad insecticide killed the prey albeit slowly. As the trials progressed it was less effective as a contact insecticide, not killing the crickets as efficiently. Spinosad is not labeled for crickets or BRS and was only used as an alternative to determine if secondary effects of treatment would occur. Weight change was another interesting result, despite the prey being treated with an insecticide, adult BRS scavenging on Spinosad-killed prey had an overall average weight gain of 13 mg where juvenile BRS had an overall average weight gain of 9 mg. The survival and weight gain suggests that BRS scavenging on Spinosad-killed prey would not be as an effective treatment for control of BRS.

Freeze-killed prey

BRS scavenging on freeze-killed prey resulted in overall adult survival of 95%, and 100% in juveniles. The three deaths occurring in the adult trials were indeterminate, potentially caused by handling or spider age. Adult spider age was difficult to determine when spiders were field collected. This leads to the possibility of testing only lab raised BRS thus definitively assessing age of spiders within treatments. Weight change for both juveniles and adults was positive; adult BRS gained on average 10 mg, whereas juvenile BRS average weight gain was 5 mg.

Predation

Adult BRS predation resulted in seven deaths and an overall survival of 88%. Two of the deaths were attributed to prey killing the spider, the other five of indeterminate causes, again

potentially handling or spider age. Juvenile BRS had 100% survival with no deaths due to prey killing the spider. Juveniles were fed smaller crickets than those fed to the adults and potentially the reason for the survival percentage, again the age of the spider did not have an effect, as all juveniles were approximately the same age. Weight change for both the adults and juveniles was positive; adult BRS had a mean weight change of 18 mg whereas juvenile BRS weight change was 25 mg.

As can be seen in Table 5-2 (Figure 5.6), survival percentages of BRS in both adult and juvenile are lower when prey is treated with an insecticide. Scavenging versus predation resulted in a lower survival for BRS as predators especially if the prey is able to fight back and potentially kill the BRS. Table 5-2 (Fig 5.5) also shows the average weight gain for both adult and juvenile is higher for predation than scavenging alone which lends strength to the argument that BRS are opportunistic scavengers but receive positive benefit as predators when weight change is the measured factor.

Figures Chapter 5

Figure 5.1 Trial of Ninety-six (96) BRS in numerical order

Ninety-six (96) BRS laid out in numerical order prior to random placement into treatments

Figure 5.2 BRS replication hierarchy

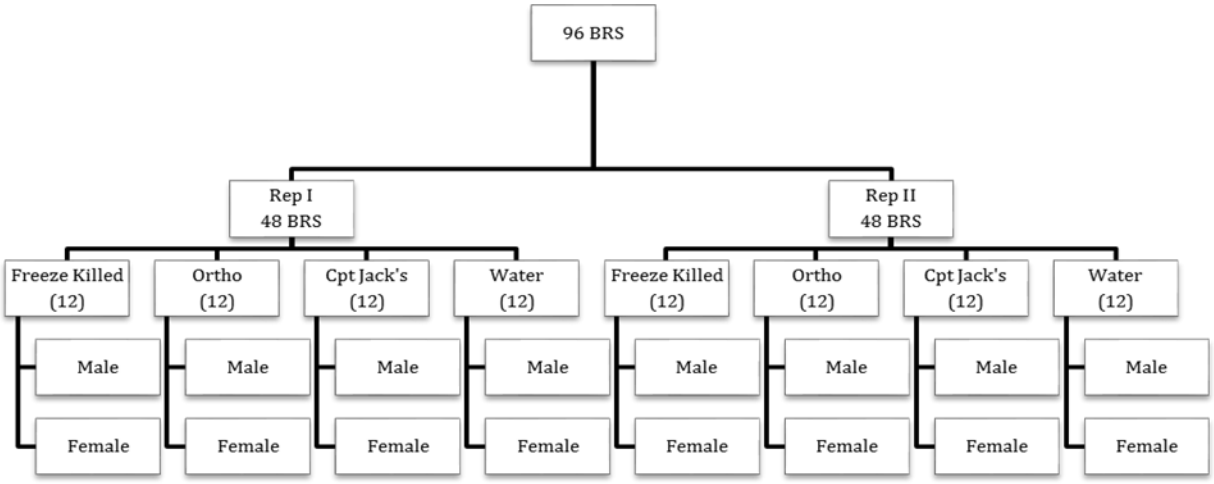
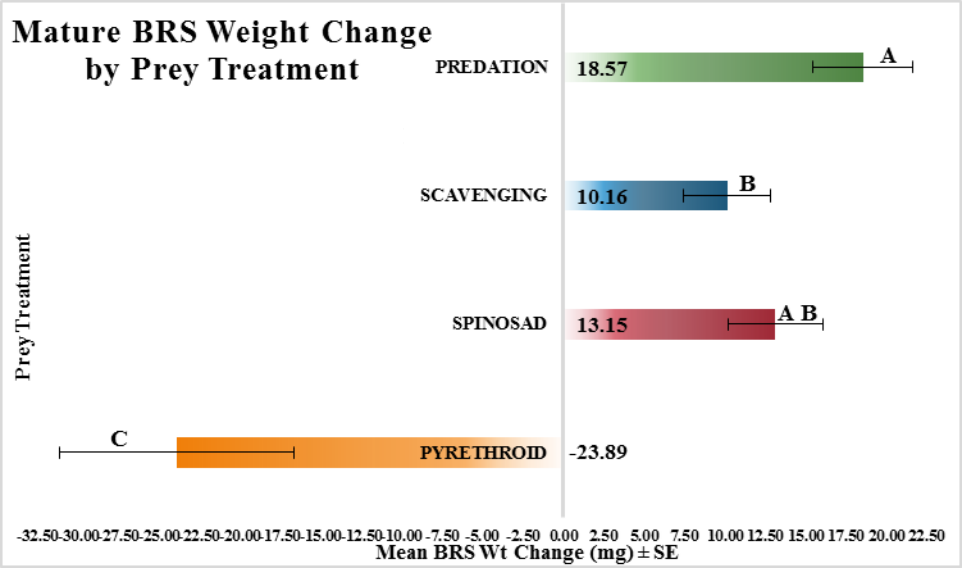
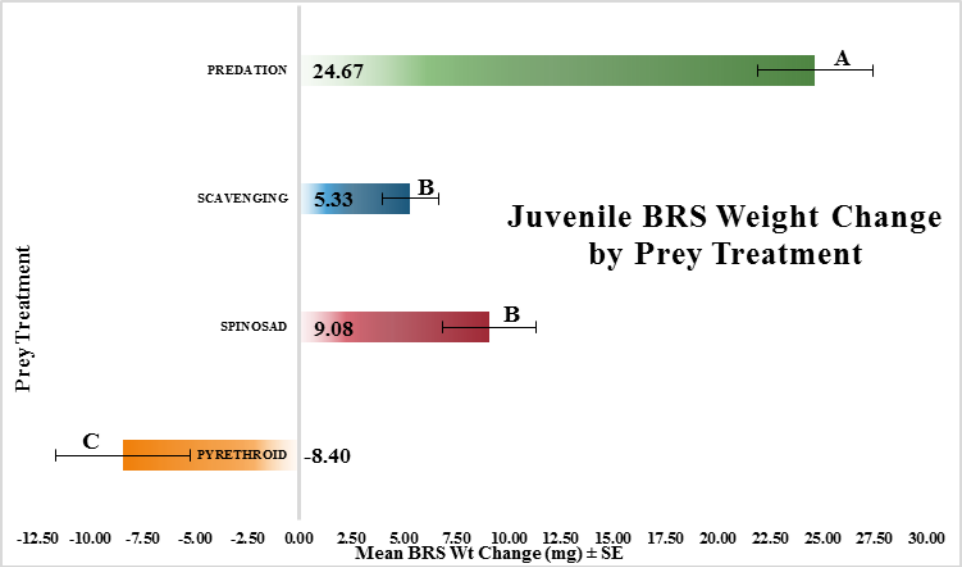


Figure 5.3 Mature BRS Weight Change by Prey Treatment



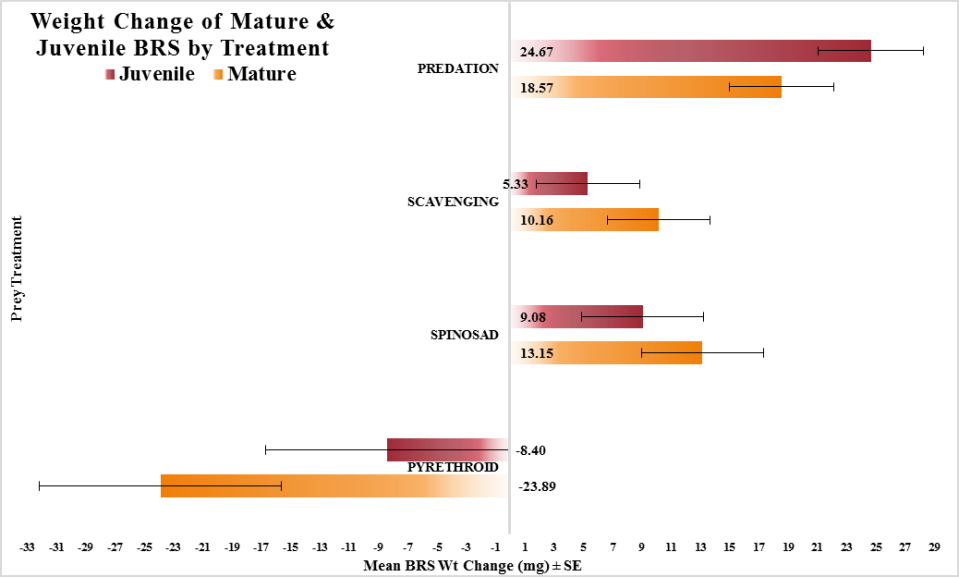
Means with the same letter do not differ significantly $p < 0.05$

Figure 5.4 Juvenile BRS Weight Change by Prey Treatment



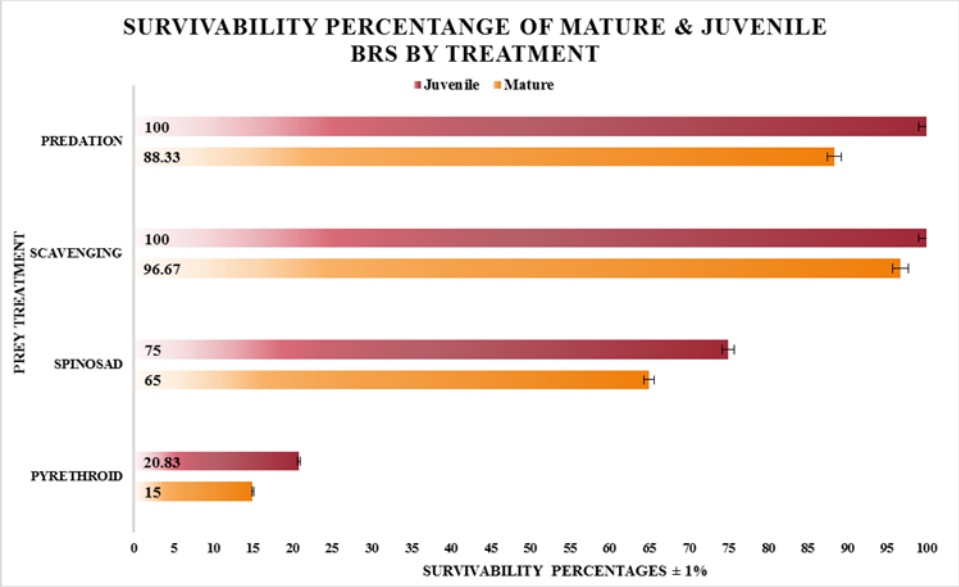
Means with the same letter do not differ significantly $p < 0.05$

Figure 5.5 Prey Treatment Comparison Mature and Juvenile BRS



Significant differences between Mature and Juvenile BRS within prey treatment types at Alpha 0.05 were not found.

Figure 5.6 Survivability Percentage Mature and Juvenile BRS by Prey Treatment



Tables Chapter 5

Table 5-1 Example one-week schedule

	Monday	Tuesday	Wednesday	Thursday	Friday
8:00	Spray Ortho 1 Chamber (Prep)	Thaw FZN 2 Prey (Prep)	BRS Treatment FZN 2 (feed BRS)	BRS Observation FZN 2 (Prey Removal)	Freeze Prey (Prep for next week)
:30	Thaw FZN 1 Prey (Prep)	BRS Treatment FZN 1 (Feed BRS)	BRS Observation FZN 1 (Prey Removal)		
9:00	Prey in Ortho 1 Chamber (Prep)	BRS Treatment Ortho 1 (Feed BRS)	BRS Observation Ortho 1 (Prey Removal)		
:30					
10:00	Spray Ortho 2 Chamber (Prep)		Spray H2O Chamber 2 (Prep)		
:30			Prey in H20 2 Chamber (Prep)		
11:00	Prey in Ortho 2 Chamber (Prep)	BRS Treatment Ortho 2 (Feed BRS)	BRS Observation Ortho 2 (Prey Removal)	BRS Treatment H2O 2 (Feed BRS)	BRS Observation H2O 2 (Prey Removal)
:30					
12:00	Spray Jacks 2 Chamber (Prep)				
:30					
13:00	Prey in Jacks 2 Chamber (Prep)	BRS Treatment Jacks 2 (Feed BRS)	BRS Observation Jacks 2 (Prey Removal)		
:30			Spray H2O 1 Chamber (Prep)		
14:00	Spray Jacks 1 Chamber		Prey in H2O 1 Chamber (Prep)	BRS Treatment H2O 1 (Feed BRS)	BRS Observation H2O 1 (Prey Removal)
:30					
15:00	Prey in Jacks 1 Chamber (Prep)	BRS Treatment Jacks 1 (Feed BRS)	BRS Observation Jacks 1 (Prey Removal)		
:30					
16:00					
:30					
Legend	Yellow = Preparation	Green = Live crickets from tub	Red = Feeding treated prey to BRS	Orange = BRS Observation & Prey Removal	

Table 5-2 Weight Change and Survival Comparison between Mature and Juvenile BRS

	Adult BRS			Juvenile BRS		
Prey Treatment	Mean Survival %	Mean wt Change (mg)	SE	Mean Survival %	Mean wt Change (mg)	SE
Pyrethroid	15%	-23.89	7.27	20.83%	-8.40	3.20
Spinosad	65%	13.15	2.92	75%	9.08	2.23
Scavenging	96.67%	10.16	2.70	100%	5.33	1.37
Predation	88.33%	18.57	3.09	100%	24.67	2.76

References Chapter 5

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Chapter 6 - Fecundity

- *Determine long-term (chronic) effects of scavenging on BRS that feed upon insecticide-killed prey measured by egg production and hatchling emergence.*

Introduction – Fecundity

Immediately following the eight-week trials, fecundity trials were conducted in an attempt to determine potential chronic effects of prey treatments upon scavenging BRS. BRS were randomly paired within treatment

Methods and materials - Fecundity

Using egg production and hatchling emergence as the measure of fecundity, one-hundred thirty-eight (138) total mating attempts were conducted by placing adult pairs together in a mating chamber for a one-week period (12:12 L:D). Following the one-week pairing the adult pairs were separated and placed into their trial cups.

Fecundity was measured by randomly pairing BRS within the same treatment regime and counting the number of hatchlings and unhatched eggs. BRS mating attempts occurred following each of the trials. With the exception of juvenile BRS, surviving spiders were mated within treatments as closely as possible. Immediate issues arose within treatment, as those fed on pyrethroid treated prey did not have the survival of those feeding on Spinosad or freeze-killed prey. Attempts to rectify the shortage of pyrethroid treated males and females were not overly successful. A shorter trial of only half the length of the eight-week trials resulted in one male and one female surviving and mated. No egg sac was produced. Following the mating experiments, spiders were exposed to natural light during season, and egg sac production increased.

Hatchling and egg counts were the result of removing the female from the cup and freeze killing the hatchlings to get an accurate count. Thus, hatchlings as a result of the trials were not used for colony enhancement.

Results - Fecundity

Of the 138 attempts at mating 64 females produced egg sacs during the entire trial period. Of the 64 females, 13 produced two egg sacs within the same season and two produced three sacs within the same season for a total egg sac production of seventy-nine (79). The fecundity portion of the study (Table 6-4) was initially hampered due to the severe lack of male survivors within the pyrethroid treatments. Other treatments had better male survivorship and could be mated within treatment.

Pyrethroid treated prey

Despite multiple attempts, there were no male survivors able to mate with surviving females within the pyrethroid treatment, thus no mating occurred in these treatments.

Scavenging - Spinosad treated prey

Sixteen (16) successful mating's from twenty-three surviving females produced eighteen egg sacs, two females produced a second egg sac in the same season.

Scavenging - Freeze-killed prey

Twenty-five (25) successful mating's from thirty-eight surviving females produced thirty-one egg sacs, five second egg sacs and one female producing a third egg sac in the same season.

Predation - Live prey

Twenty-three (23) successful mating's from thirty-one surviving females produced thirty egg sacs, six (6) second egg sacs and one female produced a third egg sac in the same season.

Overwinter egg sac production

Seven females, within the three treatments (pyrethroid treatment excluded), exposed to males in late fall, overwintered and twelve egg sacs were produced the following spring and summer, resulting in the hatching of 318 spiderlings collectively (Figure 6.7). Males were not re-introduced to the females following the late fall mating period. Three Spinosad prey treated and two predation females produced two egg sacs each and one of the predation females produced a third egg sac.

Spinosad (three females) – 131 hatched from 179 eggs in five egg sacs.

Freeze-killed prey (one female) one egg sac, thirty hatched and eight unhatched.

Live prey (three females) 106 hatched from 146 eggs in six egg sacs.

Discussion - Fecundity

An overall look at egg sac production as well as eggs hatching by treatment type for the study can be seen in Table 6-5/Figure 6.6. Looking at total egg production by treatment overall (Tables 6-6, 6-7, 6-8, Fig 6.6), predation produced slightly more eggs than that of scavenging on freeze-killed prey (predation 1242 eggs, freeze-killed 1232), and Spinosad (764). The hatch percentages were not significantly different at 95% confidence: predation 82.1%, Spinosad 80.1% while scavenging on freeze-killed prey resulted in a 79.6 % hatch rate. Tables 6-6 through 6-8 show the mean of hatched and unhatched eggs by treatment and Figure 6.6 shows a graph of the means for comparison.

Of specific interest is the mean hatch numbers of BRS. Scavenging on Spinosad-killed prey and predation had the same mean hatch rate while scavenging on freeze-killed prey was lower, but not significantly lower. Whereas, for the unhatched eggs average per egg sac, scavenging on Spinosad treated prey had the slightly greater amount with a mean of 8.4

unhatched eggs per egg sac while scavenging on freeze-killed prey was slightly less at 8.1 eggs per sac. Predation on live prey resulted in exactly one less unhatched egg per sac at 7.4 when compared to feeding on Spinosad treated prey. A general linear model (GLM) analysis in SAS version 9.4 was conducted on egg sac production. At 95% confidence, there were no significant differences.

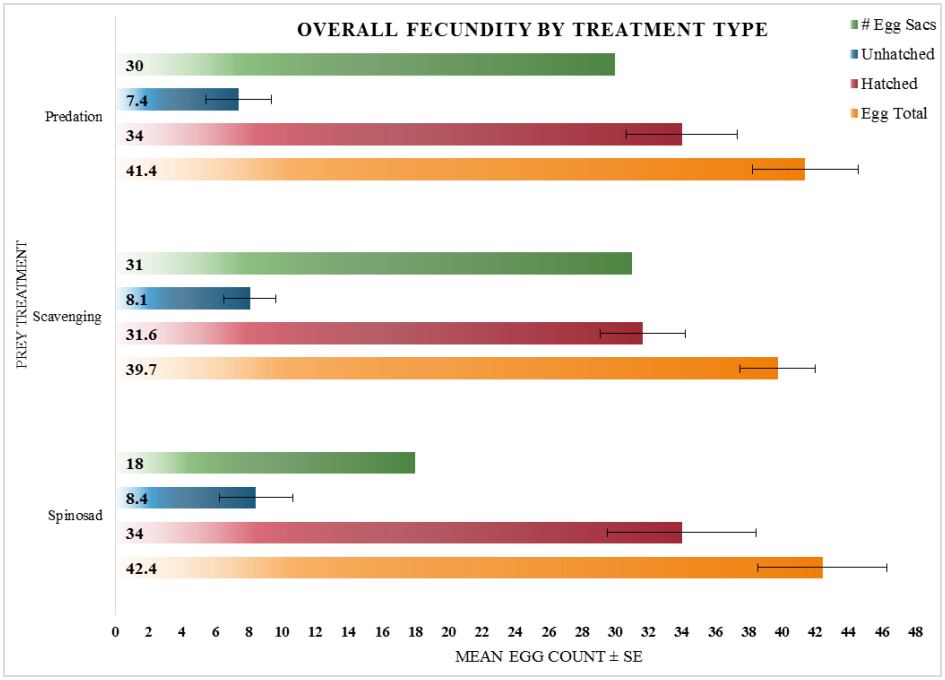
While these differences were not significant, they are of interest, despite treating the prey differently. Freeze killing, and Spinosad were scavenging opportunities for the BRS versus live untreated prey and resulted in minimal differences in egg production and subsequent hatching. Scavenging then, whether upon freeze-killed or Spinosad treated prey had minimal impact, and no significant difference, on the amount of offspring produced compared to predation.

Another interesting outcome and particular interest were the females exposed to males in the late fall. They did not produce egg sacs until the following spring, therefore termed "overwinter" in the study. Sixteen females total were exposed to males in the late fall and resulted in seven producing egg sacs the following spring some of which produced a second and even third in the same season without exposure to a male. Nine other overwinter females were monitored through the same period and did not produce egg sacs. This is of interest not only for the biological processes involved with keeping sperm viable for that length of time but their resulting egg production did not require a recent mating in order to produce offspring. Whether evolutionarily this is a benefit remains to be investigated, however hatchlings emerging earlier in the year would have an advantage when foraging having limited competition from other BRS hatchlings. Potentially however, if hatching early in the season prior to prey being available, there would be limited insect prey on which the hatchlings could feed. This overwintering and early egg sac production should be investigated more fully.

As can be seen in Table A-5, the number of eggs in the second egg sacs, highlighted in purple, and third egg sacs, highlighted in orange, generally appear to decrease with subsequent egg sacs. However, multiple egg sacs appear to be the exception rather than the rule with single egg sacs being the prevailing behavior in a season.

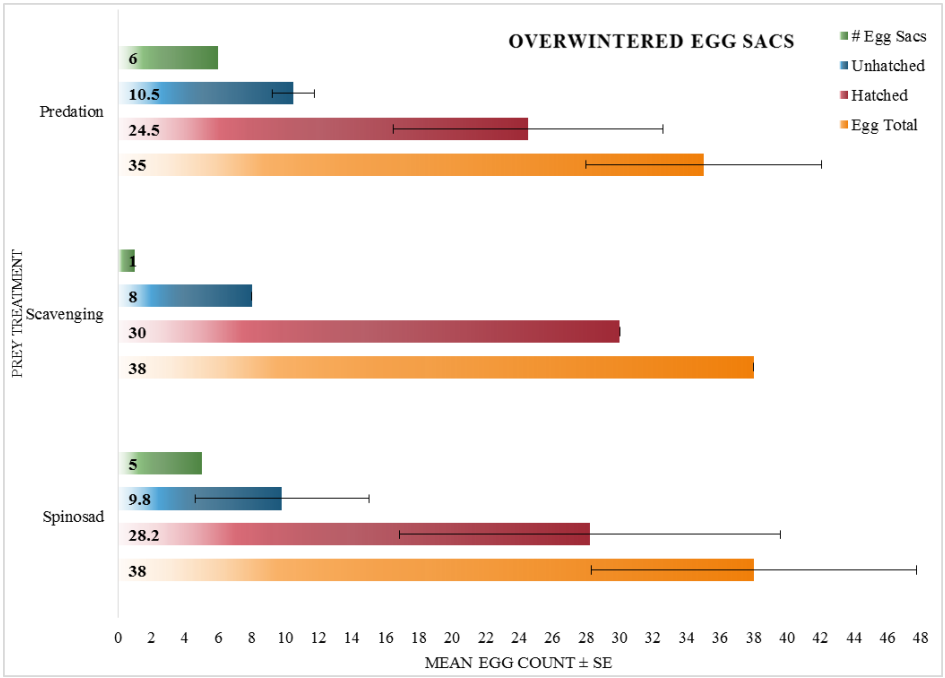
Figures Chapter 6

Figure 6.1 Mean Egg Production by Prey Treatment



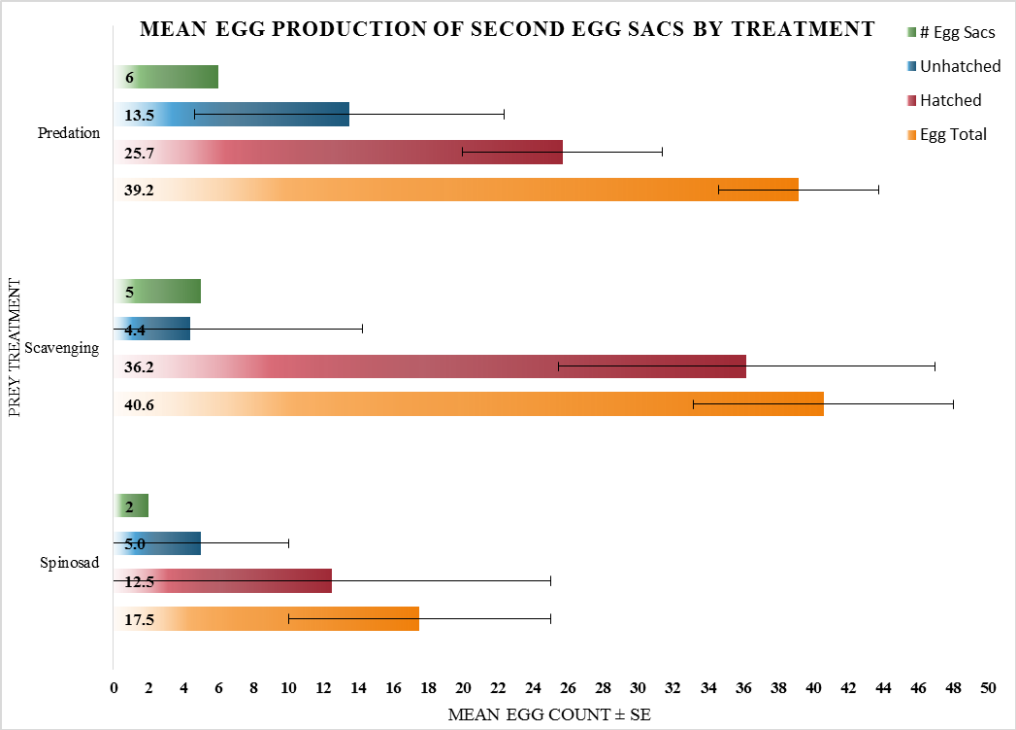
Differences between Prey Treatments were not significant at Alpha 0.05

Figure 6.2 Overwintered Egg sacs



Differences between Prey Treatments were not significant at Alpha 0.05

Figure 6.3 Mean egg production of second egg sacs



Differences between Prey Treatments were not significant at Alpha 0.05

Tables Chapter 6

Table 6-1 Overwinter Egg Sac production

Late Fall Mating (Overwinter)				
TRT	Hatched	Unhatched	TRT Total	Egg Sacs
Spino	141	49	190	5
FZN	30	8	38	1
Live	147	63	210	6
Total	318	120	438	12

Table 6-2 BRS Mated by Prey Treatment (includes Overwinter Numbers)

Prey Treatment	Females	Egg sac	ES hatched	Hatch	Unhatched	Egg Sac Hatch%
Live	23	30	28	1020	222	93.3%
Freeze-killed	25	31	30	981	251	96.8%
Spinosad	16	18	17	612	152	94.4%

Table 6-3 Scavenging on Spinosad-killed Prey Results

Spinosad	Hatched Eggs	Unhatched Eggs	Egg Totals	Hatch%	Unhatch%
Total	612	152	764	80.1%	19.9%
Mean	34	8.4	42.4		
Range	0-76	0-29	69 (10-79)		
STD Error	4.47	2.21	3.87		

Table 6-4 Scavenging on Freeze-killed Prey Results

Freeze-killed	Hatched Eggs	Unhatched Eggs	Egg Totals	Hatch%	Unhatch%
Total	981	251	1232	79.6%	20.4%
Mean	31.65	8.1	39.74		
Range	0-61	0-23	40 (22-62)		
STD Error	2.54	1.198	2.26		

Table 6-5 Predation (Live Prey) Results

Live Prey	Hatched Eggs	Unhatched Eggs	Egg Totals	Hatch%	Unhatch%
Total	1020	222	1242	82.1	17.9
Mean	34	7.4	41.4		
Range	0-71	0-57	65 (14-79)		
STD Error	3.34	1.97	3.15		

References Chapter 6

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Chapter 7 - Discussion/Study Analysis

BRS scavenging on pesticide-killed prey was studied to determine if scavenging on prey killed by pesticides had an acute effect on the population of BRS, or chronic effect by reducing other aspects of biological fitness, i.e. fertility/fecundity. The goals were to determine the effect of scavenging on selected aspects of BRS biology using prey killed by three different methods (synthetic pyrethroid, Spinosad, and freezing). Additionally, we examined the effect scavenging had on fecundity through egg production and hatchling emergence, using prey killed by the same methods.

Selection of Pesticides

The pesticides used were a synthetic pyrethroid and a Spinosad. The pyrethroid was chosen for the active ingredient as a comparison to previous studies and had both brown recluse spider and house cricket listed specifically on the label. The Spinosad was chosen as an alternative to synthetic pyrethroid chemical insecticides and was labeled for lepidopteran and other arthropod pests, (not specifically labeled for crickets or brown recluse spiders). Many consumers are concerned with the application of chemicals, especially in and around their homes. Investigating the efficacy of a product used in organic gardening as a comparison to "general use" synthetic organic pesticides allows the possibility of alternatives that are feasibly more "environmentally friendly" but still may kill some common pests that BRS may encounter.

Hypotheses

Restating the original hypotheses: 1) BRS, as an opportunistic feeder, derive as good or greater benefit as a predator than as a scavenger and, 2) BRS scavenging upon insecticide-killed prey has a detrimental effect on the BRS.

Acute effects of Insecticide Toxicity

From an acute (short-term) effects standpoint, the data in this study supports both hypotheses. Acute effects, measured by mortality and weight change, show predation had greater benefit than scavenging, specifically when BRS scavenge upon prey killed by pesticide, during the eight-week trials. Scavenging on pyrethroid-killed prey significantly increased the mortality of BRS when compared to scavenging on other types of treated prey. Following exposure to the pyrethroid pesticide both the prey (cricket) and BRS died as would be expected for a pesticide labeled for both. However, during the eight-week trials an added benefit of BRS scavenging upon synthetic pyrethroid-killed prey was those BRS surviving the trial lost on average nearly 1/3rd of their body weight. Potentially, surviving BRS simply did not feed, thus a repellency effect may have occurred within the treated prey. Spinosad-killed prey was not nearly as effective in killing BRS or the crickets used as prey and overall, BRS gained body weight. This too is to be expected as Spinosad is labeled predominately for lepidopteran pests and typically used in gardening rather than as a method of control in structures.

Chronic effects of Insecticide Toxicity

An indicator of chronic (long-term) effects was measured in fecundity. BRS males and females were placed together for mating following the eight weeks of treatment to determine the effect on reproduction. For pyrethroid-killed prey, there were no chronic effects because no male spiders survived long enough to mate with the few surviving females. In terms of fecundity, this can be considered an effective means of lowering a BRS population within a structure. The original hypotheses were not supported with the other treatment types. Scavenging on Spinosad-killed prey averaged 34 hatched and 8.4 unhatched eggs per egg sac whereas scavenging on freeze-killed prey averaged 31.7 hatched and 8.1 unhatched eggs per sac, a nonsignificant

difference. Predation (feeding on live prey) resulted in an average of 34 hatched and 7.4 unhatched eggs per sac, again not significantly different. It must be noted that predation may have had a better hatch percentage if not due to researcher error. At least twice during the different trials, a second unseen egg sac was frozen before being allowed to hatch. Because of this researcher error and the small sample size, a higher egg hatch percentage may have occurred.

Conclusion

The objectives of this study centered on the effects of scavenging vs. predation on BRS biology, ultimately to give insight on better management techniques for control of the BRS. Providing BRS with pesticide-killed prey, prey killed by freezing and live prey allowed the study of specific benefits of the particular treatment types. Trials were conducted for eight weeks with prey introduced for 24 hours weekly. Eight-week trials were selected in an attempt to allow for predator satiation and allowed BRS multiple opportunities to feed. BRS, possibly satiated after one feeding, would then feed again at some point during the trial. The biological measures were weight change, mortality, and the ability to reproduce (fecundity).

As previously stated by Vetter (2011), the ability to survive by scavenging does not appear to be unique to BRS, however, the effects that scavenging has on the biology of BRS has not been fully explored. BRS scavenging on the insecticide-killed prey (synthetic pyrethroid) only survived 15-19% of the time, per this study, resulting in a BRS control percentage of 81-85%. Sandidge (2003) described the BRS as an "opportunistic feeder rather than obligate predator or obligate scavenger…prefer[ring] dead over live prey", the current research showed BRS would scavenge on dead prey as well as readily feed upon live prey. While not completely refuting all aspects of the Sandidge (2003) study, the current study demonstrates BRS feeding

habits are not solely predation or scavenging and opportunistic may well be a fitting description of feeding habits.

Management techniques for BRS control typically involve the judicious use of pesticides and/or glue traps. As seen with Schwarting and Whitworth (2015), if the efficacy of a pesticide relies on the BRS contacting a specific treated substrate for a certain length of time, the "chances" of killing any number of BRS may not be good. The knowledge of BRS having opportunistic feeding habits can assist in the management of BRS in structures. Glue traps can be baited with dead insects in an attempt to attract wandering/scavenging BRS. Furthermore, if the contact pesticide kills a pest that the BRS later scavenges upon and results in the death of the BRS, then the homeowner is in a "win/win" situation in which they rid themselves of a pest and the BRS as well as demonstrated in the current study. A significant amount of BRS (both male and female) should be detrimentally affected (81-85%) when scavenging upon prey killed by a synthetic pyrethroid. Thus, insecticides when used in concert with other IPM measures such as: placement of sticky traps, filling in gaps to the outside and cleaning/organizing cluttered areas, should prove effective in lowering BRS populations within structures as well as other unwanted pests in treated structures.

In general, spiders are predators and BRS are not an exception. As related to weight change (gain), predation does appear to be slightly better biologically compared to scavenging alone for both juvenile and adult BRS. In the current study, predation resulted in a much higher weight gain overall than scavenging on freeze-killed prey. Predation however, is not without danger to the spiders; the size of prey can affect BRS mortality if the venom does not incapacitate the prey and the prey kills the BRS.

In terms of weight change, prey quality was another variable found to effect BRS biologically. BRS feeding upon freeze-killed prey, which were allowed to remain at room temperature for only 24 hours prior to introduction, exhibited a much greater survival rate and weight gain compared to those fed prey that had desiccated for 48 hours and longer. It came as no surprise that shorter desiccation times allowed for better prey quality, however determining the "tipping point" or the time that nutritive value was lessened allowed the determination of a "standard" for prey exposure/desiccation used in follow on experiments. BRS in this study did not benefit biologically through weight change when prey was left for greater than 48 hours prior to being scavenged upon. This leaves a small window of time for scavenging spiders to find and feed upon dead insects in structures! This lack of nutritive benefit received from desiccated prey coupled with the scavenging on a prey killed with a synthetic pyrethroid could be a factor in lessening BRS populations within treated structures.

When comparing predation to scavenging on pesticide-killed prey there are significant differences between the two. Sandidge (2003) observed BRS feeding upon insecticide-killed prey with "no obvious negative effect". In the current study, the survivability of BRS feeding on pesticide-killed prey that was labeled for BRS, resulted in significantly increased mortality for BRS (81-85% mortality) and ultimately resulted in the inability of trial spiders to reproduce due to lack of male spiders. It should be noted that those surviving BRS lost nearly 1/3rd of their body weight, on average, during the trial. This result, coupled with a loss of reproductive adults should ultimately lessen a population of BRS in treated structures.

Fecundity did not appear to be affected by treatment. With the exception of pyrethoid-killed prey being unable to reproduce due to the lack of male spiders, the other three treatments did not appear to have much differentiation. A possible explanation of the relatively poor

fecundity results could be due to trial timing. Typically, BRS are active April to October and not all trials were conducted during the BRS peak "active times". Thus, trials ended when BRS were out of "season" and may have been part of the initial difficulty of obtaining mating results. Trials that ended during BRS "active times" did result in mating and obtaining some fecundity results. Following the "out of season" trials, spiders were kept separate and mating attempts were conducted early the following spring, consequently any treatment effect may have been metabolized/lost by the time the mating actually occurred.

General observations

BRS are typically reclusive and did not show any aggression when captured, collected or handled, preferring it seems to run away rather than bite. Throughout this study, hundreds of BRS were collected, handled, and transferred from container to container without a single bite. On several occasions spiders would escape a container and run up a hand or arm in an attempt to escape but not once was anyone bitten while attempting to re-capture.

Interestingly, some BRS in late fall appear to have a lighter colored abdomen when going into the semi-dormant state. This observation occurred several times over the course of the study. Whether it is an indicator of BRS going into the overwinter resting state or simply a color change due to the type of prey consumed has not been elucidated.

Another interesting observation was that of maternal care. Female BRS would remain on or around their egg sac until hatchling emergence. On two separate occasions we filmed the female pulling and tugging upon the egg sac in what appeared to be activity designed to assist the hatchlings emerge from the egg sac. This activity of the female ceased once hatchlings started to emerge from the egg sac. While not proof in itself of maternal care by the female BRS, the observed behavior does support other researchers in their observations regarding maternal

care in BRS. Subsequent attempts at capturing on film other females demonstrating the behavior were unsuccessful.

Overall, despite the concern of a medically important bite, the brown recluse spider is not an animal of which to be overly fearful. Knowledge of habitat, clear identification and situational awareness can go a long way in the prevention of harmful interactions with this spider. In homes, two very simple steps can assist in preventing a suitable habitat for the BRS: 1) the use of cardboard boxes for storage containers should be avoided and 2) storage areas should be cleaned regularly. Preventing a place for BRS to reside will greatly assist in lessening the chance of a spider human interaction.

Finally, despite the misgivings of some to use pesticides in homes, they appear to be beneficial in reducing BRS populations within properly treated structures. When using any pesticide, the label recommendation should always be followed. It cannot be emphasized enough however, that pesticides alone will not eliminate a BRS population in structures and other pest management techniques must be utilized. Limit access to the structure by eliminating places of ingress and egress, fill cracks or holes in the foundation/walls that allow pests to enter. Limiting the food source by preventing pests from entering and treating the structure with the label rate amount of pesticide prevents the BRS from feeding. As seen in this study, should the BRS feed on pests killed by pesticides labeled for BRS the chance of killing the BRS is greatly increased (81-85%). Ultimately, in properly treated structures, the spider population will eventually decrease and the opportunities for BRS/human interaction will lessen tremendously.

References Chapter 7

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Appendix A - SAS 9.4 Output for each Chapter

Table A-1 Chapter 4 Weight Change (mg) of BRS by Treatment and Time

SAS 9.4 Data Output

The GLM Procedure

Weight change (mg) of BRS by treatment and time

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	15	36801.98060	2453.46537	9.70	<.0001
Error	226	57179.04419	253.00462		
Corrected Total	241	93981.02479			

R-Square	Coeff Var	Root MSE	Wt Change (mg) Mean
0.391589	607.1421	15.90612	2.619835

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	15	36801.98060	2453.46537	9.70	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	15	36801.98060	2453.46537	9.70	<.0001

t Tests (LSD) for Wt Change (mg)

Alpha	0.05
Error Degrees of Freedom	226
Error Mean Square	253.0046
Critical Value of t	1.97052

Differences of Treatment Least Squares Means								
Comparisons significant at the 0.05 level are indicated by ***.								
Treatment	**Treatment**	**Difference Between Means**	**Standard Error**	**t Value**	**Pr > |t|**	**Lower**	**Upper**	
FZN1hr	FZN24hr	10.3106	6.6396	1.55	0.1218	-2.7728	23.394	
FZN1hr	FZN72hr	22.25	7.0139	3.17	0.0017	8.4289	36.0711	***
FZN1hr	FZN8hr	9.8333	6.4936	1.51	0.1313	-2.9625	22.6292	
FZN1hr	Ortho1hr	41.0833	12.1485	3.38	0.0008	17.1445	65.0221	***

FZN1hr	Pesticide	34.0119	5.756	5.91	<.0001	22.6696	45.3542	***
Treatment	**Treatment**	**Difference Between Means**	**Standard Error**	**t Value**	**Pr > |t|**	**Lower**	**Upper**	
FZN1hr	Predation	1.0655	5.0598	0.21	0.8334	-8.905	11.0359	
FZN1hr	PredW1hr	0.1288	6.6396	0.02	0.9845	-12.9546	13.2122	
FZN1hr	Scavenging	9.8106	5.1801	1.89	0.0595	-0.3969	20.0182	
FZN24hr	FZN72hr	11.9394	7.1493	1.67	0.0963	-2.1484	26.0271	
FZN24hr	FZN8hr	-0.4773	6.6396	-0.07	0.9428	-13.5607	12.6061	
FZN24hr	Ortho24hr	28.2727	8.0727	3.5	0.0006	12.3654	44.18	***
FZN24hr	Pesticide	23.7013	5.9202	4	<.0001	12.0355	35.3671	***
FZN24hr	Predation	-9.2451	5.2458	-1.76	0.0794	-19.5821	1.0918	
FZN24hr	PredLive	-8.8939	6.6396	-1.34	0.1817	-21.9774	4.1895	
FZN24hr	PredW24hr	-9.2727	6.7824	-1.37	0.1729	-22.6375	4.0921	
FZN24hr	Scavenging	-0.5	5.362	-0.09	0.9258	-11.0658	10.0658	
FZN72hr	FZN8hr	-12.4167	7.0139	-1.77	0.078	-26.2377	1.4044	
FZN72hr	Ortho72hr	4.1111	7.4982	0.55	0.584	-10.6642	18.8865	
FZN72hr	Pesticide	11.7619	6.3371	1.86	0.0648	-0.7256	24.2494	
FZN72hr	Predation	-21.1845	5.7122	-3.71	0.0003	-32.4406	-9.9285	***
FZN72hr	PredLive	-20.8333	7.0139	-2.97	0.0033	-34.6544	-7.0123	***
FZN72hr	PredW72hr	-23.6667	7.3084	-3.24	0.0014	-38.0679	-9.2654	***
FZN72hr	Scavenging	-12.4394	5.8191	-2.14	0.0336	-23.906	-0.9728	***
FZN8hr	Ortho8hr	31	9.1834	3.38	0.0009	12.904	49.096	***
FZN8hr	Pesticide	24.1786	5.756	4.2	<.0001	12.8363	35.5209	***
FZN8hr	Predation	-8.7679	5.0598	-1.73	0.0845	-18.7383	1.2026	
FZN8hr	PredLive	-8.4167	6.4936	-1.3	0.1962	-21.2125	4.3792	
FZN8hr	PredW8hr	-6.1667	6.4936	-0.95	0.3433	-18.9625	6.6292	
FZN8hr	Scavenging	-0.02273	5.1801	0	0.9965	-10.2303	10.1848	
Ortho1hr	Ortho24hr	-2.5	12.9873	-0.19	0.8475	-28.0917	23.0917	
Ortho1hr	Ortho72hr	-14.7222	12.4344	-1.18	0.2377	-39.2244	9.7799	
Ortho1hr	Ortho8hr	-0.25	13.7751	-0.02	0.9855	-27.3941	26.8941	
Ortho1hr	Pesticide	-7.0714	11.7707	-0.6	0.5486	-30.2658	16.123	
Ortho1hr	Predation	-40.0179	11.4464	-3.5	0.0006	-62.5732	-17.4625	***
Ortho1hr	PredLive	-39.6667	12.1485	-3.27	0.0013	-63.6055	-15.7279	***
Ortho1hr	PredW1hr	-40.9545	12.2271	-3.35	0.0009	-65.0483	-16.8608	***
Ortho1hr	Scavenging	-31.2727	11.5001	-2.72	0.007	-53.9339	-8.6116	***
Ortho24hr	Ortho72hr	-12.2222	8.3833	-1.46	0.1462	-28.7416	4.2971	
Ortho24hr	Ortho8hr	2.25	10.2674	0.22	0.8267	-17.982	22.482	
Ortho24hr	Pesticide	-4.5714	7.3631	-0.62	0.5353	-19.0805	9.9377	
Ortho24hr	Predation	-37.5179	6.8327	-5.49	<.0001	-50.9817	-24.054	***
Ortho24hr	PredLive	-37.1667	7.9531	-4.67	<.0001	-52.8383	-21.495	***

Ortho24hr	PredW24hr	-37.5455	8.0727	-4.65	<.0001	-53.4528	-21.6381	***
Treatment	**Treatment**	**Difference Between Means**	**Standard Error**	**t Value**	**Pr > |t|**	**Lower**	**Upper**	
Ortho24hr	Scavenging	-28.7727	6.9222	-4.16	<.0001	-42.4131	-15.1323	***
Ortho72hr	Ortho8hr	14.4722	9.5584	1.51	0.1314	-4.3627	33.3072	
Ortho72hr	Pesticide	7.6508	6.3371	1.21	0.2286	-4.8367	20.1382	
Ortho72hr	Predation	-25.2956	5.7122	-4.43	<.0001	-36.5517	-14.0396	***
Ortho72hr	PredLive	-24.9444	7.0139	-3.56	0.0005	-38.7655	-11.1234	***
Ortho72hr	PredW72hr	-27.7778	7.3084	-3.8	0.0002	-42.179	-13.3765	***
Ortho72hr	Scavenging	-16.5505	5.8191	-2.84	0.0049	-28.0171	-5.0839	***
Ortho8hr	Pesticide	-6.8214	8.6775	-0.79	0.4326	-23.9206	10.2777	
Ortho8hr	Predation	-39.7679	8.2322	-4.83	<.0001	-55.9895	-23.5462	***
Ortho8hr	PredLive	-39.4167	9.1834	-4.29	<.0001	-57.5127	-21.3206	***
Ortho8hr	PredW8hr	-37.1667	9.1834	-4.05	<.0001	-55.2627	-19.0706	***
Ortho8hr	Scavenging	-31.0227	8.3067	-3.73	0.0002	-47.3912	-14.6542	***
Pesticide	Predation	-32.9464	4.0701	-8.09	<.0001	-40.9666	-24.9262	***
Pesticide	PredLive	-32.5952	5.756	-5.66	<.0001	-43.9375	-21.2529	***
Pesticide	PredW1hr	-33.8831	5.9202	-5.72	<.0001	-45.5489	-22.2174	***
Pesticide	PredW24hr	-32.974	5.9202	-5.57	<.0001	-44.6398	-21.3083	***
Pesticide	PredW72hr	-35.4286	6.1113	-5.8	<.0001	-47.471	-23.3861	***
Pesticide	PredW8hr	-30.3452	5.756	-5.27	<.0001	-41.6875	-19.0029	***
Pesticide	Scavenging	-24.2013	4.2188	-5.74	<.0001	-32.5144	-15.8882	***
Predation	Scavenging	8.7451	3.2044	2.73	0.0069	2.4308	15.0594	***
PredLive	Predation	-0.3512	5.0598	-0.07	0.9447	-10.3216	9.6192	
PredLive	PredW1hr	-1.2879	6.6396	-0.19	0.8464	-14.3713	11.7955	
PredLive	PredW24hr	-0.3788	6.6396	-0.06	0.9546	-13.4622	12.7046	
PredLive	PredW72hr	-2.8333	6.8106	-0.42	0.6778	-16.2537	10.5871	
PredLive	PredW8hr	2.25	6.4936	0.35	0.7293	-10.5458	15.0458	
PredLive	Scavenging	8.3939	5.1801	1.62	0.1065	-1.8136	18.6015	
PredW1hr	Predation	0.9367	5.2458	0.18	0.8584	-9.4002	11.2736	
PredW1hr	PredW24hr	0.9091	6.7824	0.13	0.8935	-12.4557	14.2739	
PredW1hr	PredW72hr	-1.5455	6.9499	-0.22	0.8242	-15.2403	12.1494	
PredW1hr	PredW8hr	3.5379	6.6396	0.53	0.5947	-9.5455	16.6213	
PredW1hr	Scavenging	9.6818	5.362	1.81	0.0723	-0.884	20.2476	
PredW24hr	Predation	0.0276	5.2458	0.01	0.9958	-10.3093	10.3645	
PredW24hr	PredW72hr	-2.4545	6.9499	-0.35	0.7243	-16.1494	11.2403	
PredW24hr	PredW8hr	2.6288	6.6396	0.4	0.6925	-10.4546	15.7122	
PredW24hr	Scavenging	8.7727	5.362	1.64	0.1032	-1.7931	19.3385	
PredW72hr	Predation	2.4821	5.4606	0.45	0.6499	-8.2781	13.2424	
PredW72hr	PredW8hr	5.0833	6.8106	0.75	0.4562	-8.3371	18.5037	

PredW72hr	Scavenging	11.2273	5.5723	2.01	0.0451	0.247	22.2076	***
Treatment	**Treatment**	**Difference Between Means**	**Standard Error**	**t Value**	**Pr > |t|**	**Lower**	**Upper**	
PredW8hr	Predation	-2.6012	5.0598	-0.51	0.6077	-12.5716	7.3692	
PredW8hr	Scavenging	6.1439	5.1801	1.19	0.2368	-4.0636	16.3515	

Table A-2 Chapter 5 Differences of Treatment*Stage Least Squares Means

Differences of Treatment*Stage Least Squares Means (Alpha 0.05)							
Treatment	Stage	Treatment	Stage	Standard Error	DF	t Value	Pr > |t|
Predation	Juvenile	Predation	Mature	3.5963	293	1.70	0.0909
Pyrethroid	Juvenile	Pyrethroid	Mature	8.2929	293	1.87	0.0628
Scavenging	Juvenile	Scavenging	Mature	3.5218	293	-1.37	0.1718
Spinosad	Juvenile	Spinosad	Mature	4.1716	293	-0.98	0.3300

Table A-3 Chapter 5 Weight Change Mature BRS

The GLM Procedure

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	3	14117.80181	4705.93394	10.92	<.0001
Error	155	66822.58813	431.11347		
Corrected Total	158	80940.38994			

R-Square	**Coeff Var**	**Root MSE**	**WtChange Mean**
0.174422	176.4490	20.76327	11.76730

Source	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
Treatment	3	14117.80181	4705.93394	10.92	<.0001

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Treatment	3	14117.80181	4705.93394	10.92	<.0001

t Tests (LSD) for WtChange

Alpha	0.05

Error Degrees of Freedom	155
Error Mean Square	431.1135
Critical Value of t	1.97539

Comparisons significant at the 0.05 level are indicated by *.**						
Treatment Comparison	**Difference Between Means**	**95% Confidence Limits**		**t Value**	**Pr > |t|**	
Predation - Spinosad	5.412	-3.241	14.065	1.24	0.2185	
Predation - Scavenging	8.411	0.617	16.205	2.13	0.0346	***
Predation - Pyrethroid	42.455	27.668	57.242	5.67	<.0001	***
Scavenging - Spinosad	-2.999	-11.492	5.495	-0.70	0.4866	
Pyrethroid - Spinosad	-37.043	-52.210	-21.875	-4.82	<.0001	***
Pyrethroid - Scavenging	-34.044	-48.738	-19.350	-4.58	<.0001	***

Table A-4 Chapter 5 Weight Change Juvenile BRS

The GLM Procedure

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	3	14300.71493	4766.90498	22.98	<.0001
Error	138	28625.95979	207.43449		
Corrected Total	141	42926.67472			

R-Square	**Coeff Var**	**Root MSE**	**WtChange Mean**
0.333143	121.5264	14.40259	11.85141

Source	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
Treatment	3	14300.71493	4766.90498	22.98	<.0001

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Treatment	3	14300.71493	4766.90498	22.98	<.0001

t Tests (LSD) for Juvenile BRS Weight Change

Alpha	0.05
Error Degrees of Freedom	138
Error Mean Square	207.4345
Critical Value of t	1.97730

Comparisons significant at the 0.05 level are indicated by *.**						
Treatment Comparison	**Difference Between Means**	**95% Confidence Limits**		**t Value**	**Pr > |t|**	
Predation - Spinosad	15.583	9.304	21.862	4.91	<.0001	***
Predation - Scavenging	19.335	13.522	25.149	6.58	<.0001	***
Predation - Pyrethroid	33.067	23.167	42.966	6.60	<.0001	***
Scavenging - Spinosad	-3.752	-10.031	2.527	-1.18	0.2394	
Pyrethroid - Spinosad	-17.483	-27.663	-7.303	-3.40	0.0009	***
Pyrethroid - Scavenging	-13.731	-23.631	-3.832	-2.74	0.0069	***

Table A-5 chapter 6 Successful mating results (raw data)

Spinosad 18 Egg Sacs			16 Female
BRS #	S. Hatched	S. Unhatched	S. Total Eggs
58*	12	29	41
	0	10	10
35	38	9	47
16	42	0	42
48	39	0	39
7	47	9	56
87*	66	1	67
	25	0	25
17	33	0	33
63	37	14	51
82	29	2	31
3	35	23	58
5	76	3	79
54	41	0	41
2	43	0	43
86	8	13	21
43	13	22	35
72	28	17	45

Overwintered
* 2nd Egg Sac
** 3rd Egg sac

Frozen 31 Egg Sacs			25 Female
BRS #	F. Hatched	F. Unhatched	F. Total Eggs
89	30	8	38
22	5	22	27
55	10	13	23
34	25	0	25
14	23	0	23
96	13	19	32
33	30	23	53
80	41	3	44
62**	23	0	23
	61	0	61
	34	0	34
25	20	7	27
38	37	0	37
11	38	0	38
27	25	0	25
15*	58	4	62
	52	0	52
1*	41	0	41
	42	0	42
20	43	18	61
68	33	13	46
73*	34	3	37
	26	0	26
10	41	0	41
	0	22	22
9	44	9	53
66	17	22	39
49	28	13	41
29	37	19	56
56	41	14	55
52	29	19	48

Live/H20 30 Egg Sacs			23 Female
BRS #	L. Hatched	L. Unhatched	L. Total Eggs
51	22	11	33
85**	59	7	66
	25	7	32
	0	15	15
95	37	0	37
21	16	0	16
59	24	3	27
40	14	0	14
93	66	2	68
39	30	1	31
67	23	10	33
71	22	2	24
4	53	9	62
23*	13	12	25
	28	11	39
26*	23	21	44
	29	0	29
42	40	0	40
61	71	8	79
8*	63	11	74
	29	1	30
37*	42	7	49
	43	5	48
12	33	8	41
19	51	0	51
	0	57	57
46	56	0	56
77	28	0	28
50	33	9	42
57	47	5	52

Table A-6 Chapter 6 BRS Overall Fecundity SAS 9.4 Output Students t Test

The GLM Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	47342.95230	5917.86904	28.73	<.0001
Error	228	46957.03082	205.95189		
Corrected Total	236	94299.98312			

R-Square	Coeff Var	Root MSE	Eggs Mean
0.502046	52.51996	14.35102	27.32489

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	8	47342.95230	5917.86904	28.73	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	8	47342.95230	5917.86904	28.73	<.0001

t Tests (LSD) for Eggs
Fecundity Overall

Alpha	0.05
Error Degrees of Freedom	228
Error Mean Square	205.9519
Critical Value of t	1.97042

Comparisons significant at the 0.05 level are indicated by *.**						
Treatment Comparison	**Difference Between Means**	**95% Confidence Limits**		**t Value**	**Pr > |t**	*******
PredEggTotal - SpinEggTotal	-1.044	-9.475	7.386	-0.24	0.8074	
PredEggTotal - ScavEggTotal	1.658	-5.584	8.900	0.45	0.6523	
ScavEggTotal - SpinEggTotal	-2.703	-11.082	5.677	-0.64	0.5258	
PredHatched - SpinHatched	0.000	-8.431	8.431	0	1	
PredHatched - ScavHatched	2.355	-4.887	9.597	0.64	0.5224	
ScavHatched - SpinHatched	-2.355	-10.734	6.025	-0.55	0.5803	

Comparisons significant at the 0.05 level are indicated by ***.						
Treatment Comparison	**Difference Between Means**	**95% Confidence Limits**		**t Value**	**Pr > |t**	*******
ScavUnhatch - SpinUnhatch	-0.348	-8.727	8.032	-0.08	0.9349	
PredUnhatch - SpinUnhatch	-1.044	-9.475	7.386	-0.24	0.8074	
PredUnhatch - ScavUnhatch	-0.697	-7.939	6.545	-0.19	0.8498	

Table A-7 Chapter 6 Overwintered Females SAS 9.4 Output Students t Test

The GLM Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	4381.40000	547.67500	1.74	0.1344
Error	27	8498.60000	314.76296		
Corrected Total	35	12880.00000			

R-Square	Coeff Var	Root MSE	Eggs Mean
0.340171	72.91052	17.74156	24.33333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	8	4381.400000	547.675000	1.74	0.1344

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	8	4381.400000	547.675000	1.74	0.1344

t Tests (LSD) for Eggs
Overwintered Females

Alpha	0.05
Error Degrees of Freedom	27
Error Mean Square	314.763
Critical Value of t	2.05183

Comparisons significant at the 0.05 level are indicated by ***.						
Treatment Comparison	**Difference Between Means**	**95% Confidence Limits**		**t Value**	**Pr > |t|**	*******
ScavEggTotal - SpinEggTotal	0.00	-39.88	39.88	0	1	
PredEggTotal - SpinEggTotal	-3.00	-25.04	19.04	-0.28	0.7822	
PredEggTotal - ScavEggTotal	-3.00	-42.32	36.32	-0.16	0.8768	
ScavHatched - SpinHatched	1.80	-38.08	41.68	0.09	0.9269	
PredHatched - ScavHatched	-5.50	-44.82	33.82	-0.29	0.7763	
PredHatched - SpinHatched	-3.70	-25.74	18.34	-0.34	0.7332	
PredUnhatch - SpinUnhatch	0.70	-21.34	22.74	0.07	0.9485	
PredUnhatch - ScavUnhatch	2.50	-36.82	41.82	0.13	0.8972	
ScavUnhatch - SpinUnhatch	-1.80	-41.68	38.08	-0.09	0.9269	

Table A-8 Chapter 6 Second Egg Sacs SAS 9.4 Output Students t Test

The GLM Procedure

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	8	7169.56923	896.19615	3.26	0.0087
Error	30	8255.86667	275.19556		
Corrected Total	38	15425.43590			

R-Square	**Coeff Var**	**Root MSE**	**Eggs Mean**
0.464789	68.39025	16.58902	24.25641

Source	**DF**	**Type I SS**	**Mean Square**	**F Value**	**Pr > F**
Treatment	8	7169.569231	896.196154	3.26	0.0087

Source	**DF**	**Type III SS**	**Mean Square**	**F Value**	**Pr > F**
Treatment	8	7169.569231	896.196154	3.26	0.0087

t Tests (LSD) for Eggs
Second Egg Sac comparisons

Alpha	0.05
Error Degrees of Freedom	30
Error Mean Square	275.1956
Critical Value of t	2.04227

Comparisons significant at the 0.05 level are indicated by *.**						
Treatment Comparison	**Difference Between Means**	**95% Confidence Limits**		**t Value**	**Pr > |t|**	*******
ScavEggTotal - SpinEggTotal	23.100	-5.245	51.445	1.66	0.1065	
PredEggTotal - ScavEggTotal	-1.433	-21.948	19.082	-0.14	0.8875	
PredEggTotal - SpinEggTotal	21.667	-5.996	49.329	1.6	0.1202	
ScavHatched - SpinHatched	23.700	-4.645	52.045	1.71	0.098	
PredHatched - ScavHatched	-10.533	-31.048	9.982	-1.05	0.3027	
PredHatched - SpinHatched	13.167	-14.496	40.829	0.97	0.3388	
PredUnhatch - SpinUnhatch	8.500	-19.162	36.162	0.63	0.5351	
PredUnhatch - ScavUnhatch	9.100	-11.415	29.615	0.91	0.3722	
ScavUnhatch - SpinUnhatch	-0.600	-28.945	27.745	-0.04	0.9658	