DISSERTATION

SPIDERS AS POTENTIAL APHID PREDATORS IN EASTERN COLORADO AGROECOSYSTEMS

Submitted by
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ABSTRACT

SPIDERS AS POTENTIAL APHID PREDATORS IN EASTERN COLORADO AGROECOSYSTEMS

Spiders are indigenous, ubiquitous natural enemies that have been associated with reduced pest densities and may be particularly useful in reducing aphid densities. Therefore, it is critical to determine the spider fauna within these agroecosystems, spiders that may be key biological control agents for conservation, and determine if alternative cropping systems can enhance or maintain these particular spider species.

The inclusion of sustainable agricultural systems is an important component of integrated pest management. The faunal composition of spiders in eastern Colorado agroecosystems was described and analyzed to determine whether a crop-intensified system resulted in greater spider density and biodiversity than a conventional system. Three sites in eastern Colorado-Akron, Briggsdale, and Lamar-were studied. From 2002-2007, 11,207 spiders from 17 families and 119 species were collected from pitfall, vacuum, and lookdown sampling techniques. Crop intensification had little effect on spider density or biodiversity. Spider mean densities/activity densities and biodiversity were low for all years and sites, with the exception of 2005 and 2006. At all sites, the fauna was dominated by hunting spiders in the Lycosidae and Gnaphosidae families

(72%), which differs from the dominance of web-building spiders in western European agroecosystems.

Before establishing whether predators can contribute to the biological control of a pest, it is important to determine the availability of the pest for prey. *Diuraphis noxia* is an important economic pest in wheat agroecosystems in Colorado. Thus, the falling rate of *D. noxia* from wheat infested at 1x and 10x aphid infestation levels and resistant and susceptible varieties was measured. Falling rates ranged from 0.7% to 69.5% in Fort Collins, CO, and from 1.4% to 59.5% in Akron, CO. The falling rate of *D. noxia* was more influenced by plant growth stage than aphid densities, with the highest falling rate occurring prior to wheat senescence. Resistant wheat plants did not have increased aphid falling rates. The falling rate of *D. noxia* was highest at lower aphid densities, thus epigeal predator consumption of *D. noxia* can occur at lower aphid densities. Nevertheless, the falling rate of *D. noxia* clearly indicates that these prey can represent an important food source for ground predators.

It is the conservation of key species and not necessarily the conservation of predators per se that is important for effective biological control. Therefore, it is critical to identify which predators are consuming pests in the field. Species-specific primers and the polymerase chain reaction were used to determine if two dominant spiders, *Tetragnatha laboriosa* and *Pardosa sternalis*, were consuming *D. noxia* DNA in the field. A partial 1146 bp sequence from the mitochondrial cytochrome oxidase I (COI) gene was used and aligned with other non-target sequences to create two primer pairs that amplified a 227 bp fragment of *D. noxia* DNA. A total of 64 and 71 *T. laboriosa* and *P. sternalis*,

respectively, were collected from within three *D. noxia* infestation levels-0x, 1x, and 10x-in Fort Collins, CO, from May-July at the following wheat stages: boot, inflorescence, anthesis, milk, and dough. Of the spiders collected in the field, 32% and 48% of *T. laboriosa* and *P. sternalis* tested positive for *D. noxia* DNA. Additionally, 92% of *T. laboriosa* were collected at the 1x or 10x *D. noxia* infestation levels combined, which indicated that *T. laboriosa* responded to increased *D. noxia* densities. *Pardosa sternalis*, however, was more evenly distributed within aphid infestation levels.

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CHAPTER 1- INTRODUCTION

The percentage of natural ecosystems converted to agriculture has increased substantially for decades within the United States (Matson et al. 1997). Agricultural systems are characterized by frequent disturbances such as sowing, tillage, pesticide and herbicide applications, and crop harvest. These disruptions present challenges for natural enemies, particularly by causing mortality or forcing emigration (Stinner and House 1990, Haugton et al. 2001, Thorbek and Bilde 2004). The adoption of sustainable agricultural practices could help to maintain predator diversity within agroecosystems and alleviate pest pressure (Pimentel 1961, Matson et al. 1997, Tilman 2001).

The Pest-*Diuraphis noxia* (Kurdjumov)

Biology

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), in its native range utilizes both anholocyclic and holocyclic life cycles (Kiriac et al. 1990). The anholocyclic life cycle is parthenogenetic, an asexual form of reproduction where fertilization and embryo development both occur in the absence of males. The holocyclic cycle includes the sexual stages, which occur in the fall and produce overwintering eggs. In the United States, *D. noxia* utilizes an anholocyclic cycle (Kiriac et al. 1990), and

males are yet to be reported (Burd et al. 1998). *Diuraphis noxia* also exhibits telescoping generations, where aphids give birth to viviparae that are pregnant with successive generations of viviparae. *Diuraphis noxia* produces both winged and wingless forms with the winged populations produced in accordance with declining host quality (Baugh and Phillips 1991). Additionally, in western Canada and the United States, *D. noxia* can maintain overwintering populations when soil temperatures are between 0ºC and -5ºC or higher (Butts 1992, Butts and Schaalje 1997). Eight biotypes of *D. noxia* have been discovered in the United States with five unique to Colorado (Puterka et al. 1992, Shufran et al. 1997, Haley et al. 2004, Burd et al. 2006, Weiland et al. 2008). A biotype is a distinctive genetic population of aphids that differs in how it damages a resistant plant (Puterka and Peters 1990).

Life History

Diuraphis noxia is a common pest of wheat, *Triticum aestivum* L. (Poales: Poaceae), and other susceptible small grains in all major wheat growing countries except Australia (Elliott et al. 1998). *Diuraphis noxia* is native to the southern USSR, Iran, Afghanistan, and countries that border the Mediterranean Sea (Hewitt et al. 1984) and was introduced to the United States in Texas in 1986 (Stoetzel 1987). It has since spread throughout the western United States (Hein et al. 1990).

Although *D. noxia* is one of the most economically important pests of wheat in the United States (Burd et al. 1998), other aphids also cause economic losses in small-grain crops. The bird cherry oat aphid, *Rhopalosiphum padi* L. (Hemiptera: Aphididae), and the greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) are prevalent in early fall and winter while *D. noxia* tends to dominate wheat fields from early spring until

harvest in dryland wheat agroecosystems in western Texas (Michels and Behle 1989). Densities of *D. noxia* tend to be greatest between the jointing stage of wheat (Zadoks 30) (based on Zadoks scale, a widely used cereal development scale in agriculture (Zadoks et al. 1974) and wheat heading (Zadoks 50) (Girma et al. 1990). Fall *D. noxia* infestations in wheat reduce yield while spring infestations reduce the number of seeds per plant, seed weight, and dry weight of the wheat (Archer et al. 1998).

Damage

The damage to wheat plants by *D. noxia* is distinct from other cereal aphids. A protein elicitor produced by *D. noxia* induces susceptible symptoms in plants (Lapitan et al. 2007). Susceptible symptoms include leaves that fail to unfurl, purple or white streaking, plant stunting, and prostrate growth (Bush et al. 1989, Archer et al. 1998, Burd and Burton 1992, Walters et al. 1984, Hewitt et al. 1984). Disturbances to the osmoregulatory processes of the wheat also occur with increased *D. noxia* densities (Riedell 1989). Winter wheat appears to be most sensitive vegetatively and reproductively to *D. noxia* when plants are infested after vernalization (Gray 1990). However, yield losses from wheat can occur from fall, spring or both infestations (Girma 1993). Infestation by *D. noxia* can further harm the wheat plant by reducing cold-hardiness, predisposing the plant to winterkill, and reducing yield (Thomas and Butts 1990, Girma et al. 1993). If *D. noxia* infestations occur earlier (i.e., before stem elongation, Zadoks 30), wheat can typically recover (Kriel et al. 1986, Butts et al. 1997).

Diuraphis noxia is well adapted to feeding on its wheat host and surviving the summer on non-cultivated grasses (Armstrong et al. 1991). It utilizes approximately 40 grass host plants (Poales: Poaceae) (Kindler et al. 1993). *Diuraphis noxia* colonizes

these alternate hosts, in addition to volunteer wheat and barley, between wheat harvest and fall planting (Kindler and Springer 1989, Feng et al. 1992, Archer and Bynum 1993). In South Africa, after the wheat emerges, *D. noxia* colonizes the wheat from nearby volunteer wheat or other *Bromus* spp. (Poales: Poaecae) (Hewitt et al. 1984, Kriel at al. 1986). The migration of *D. noxia* from grasses on volunteer wheat can occur around a month after the wheat has emerged (Kriel et al. 1986). With the ability to survive on off-season grasses and at colder temperatures, and to disperse efficiently, *D. noxia* can sustain densities during and between wheat growing seasons.

Management

Because of its successful survival traits and the extensive damage it can cause, management of *D. noxia* has been challenging. Management techniques have included chemical, cultural, and biological controls and host plant resistance. Control of *D. noxia* historically has relied on pesticides. For chemical control to be economically effective, treatment should be timed according to pest-specific economic injury levels. Since wheat typically has a low profit margin, insecticide treatments can reduce profits by approximately 20% (Peairs 1998).

The incorporation of plant resistance into wheat varieties has become a major focus of *D. noxia* management. Until recently, planting resistant wheat varieties helped to avoid chemical applications. Furthermore, wheat varieties possessing the gene *Dn4* showed promise for management of biotype Russian wheat aphid 1 (RWA1) (Randolph et al. 2003), and wheat cultivars containing this gene were recommended for *D. noxia* management as they successfully maintained densities below economic thresholds.

However, management has been complicated in the United States due to the discovery of a new biotype of *D. noxia* in 2003, biotype RWA2 (Haley et al. 2004). Biotype RWA2 emerged based on its virulence to cultivars containing the genes *Dn4* and *Dny*, which also confer resistance to biotype RWA1 (Haley et al. 2004, Collins et al. 2005, Jyoti and Michaud 2005, Qureshi et al. 2006, Peng et al. 2007). Several biotypes have since been discovered (Burd et al. 2006, Weiland et al. 2008), which has currently precipitated the need for other tactics to manage *D. noxia*.

An additional component of *D. noxia* management is the use of cultural controls. Cultural control is defined as the manipulation of the cropping ecosystem through the modification of farming practices to discourage target pests or encourage the presence of natural enemies (Peairs 1998). Some of these techniques include modified crop biodiversity, crop intensification, sanitation, grazing, fertilization, irrigation, row spacing, crop intensification, and planting dates. Sanitation consists of removing volunteer wheat plants from adjacent fields and controlling weeds, residue, and other hosts of *D. noxia*. *Diuraphis noxia* infestations have increased in areas where volunteer wheat host plants are present (Hewitt et al. 1984, Halbert et al. 1988). Grazing with cattle has reduced *D. noxia* densities by 75% in southeastern Colorado (Walker and Peairs 1998). Proper fertilization, reduction of drought stress through irrigation, early planting, and narrow row spacing have been beneficial for reducing *D. noxia* densities (Peairs 1998). Thus, the inclusion of cultural techniques can be economical for *D. noxia* management.

Biological control also can be an important component of the integrated pest management of *D. noxia*. An extensive classical biological control effort was conducted in the United States for *D. noxia* management. Around 85,000 predator and parasitoid

individuals were collected from their native ranges and shipped to the United States for release, including *Aphelinus* spp. parasitoids (Hymenoptera: Aphelinidae) and parasitoids within the subfamily Aphidiinae (Hymenoptera: Braconidae) that parasitize *D. noxia* (Pike et al. 1997, Hopper et al. 1998). Further, it has been estimated that over 2.5 million natural enemies have been released in the state of Colorado by several organizations (Prokrym et al. 1998), which include over 90% hymenopteran parasitoids, a small percentage of ladybeetles (Coleoptera: Coccinellidae), and dipteran predators. Despite this substantial release, only four hymenopteran species were recovered or established (Prokrym et al. 1998). A mass-release study utilizing *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) and *Chrysoperla rufilabris* (Burmeister) (Neuroptera: Chrysopidae) was implemented with exclusion cages, and exclusion did not reduce *D. noxia* densities or improve wheat yields (Randolph et al. 2002). Augmentation has not been an effective contribution to *D. noxia* management.

Conservation biological control has been suggested as an important component of *D. noxia* pest management. In France, peak densities of *D. noxia* were measured as 40-100 times lower than in the United States, which may be partially attributable to the presence of indigenous predators and parasitoids (Chen and Hopper 1997). The absence of indigenous natural enemies resulted in an 11-fold increase of *D. noxia* in wheat fields in northeastern Colorado (Mohamed et al. 2000). Similarly, recent results from an exclusion study appear to show that the natural enemy complex reduces field populations of *D. noxia* in Colorado (Peairs et al., unpublished data).

Biological control

Biological control can be utilized as an effective pest management alternative for pesticides. There are three basic biological control strategies-classical, inundative, and conservation. Classical biological control involves the introduction and establishment of exotic natural enemies to decrease exotic pest densities below levels that are injurious to crops (Elliott et al. 1996). When classical biological control has been successful, it may be due to the efficient dispersal and reproductive capabilities of these predators (Van Lenteren et al. 2003). However, some biological control agents often have non-target effects and may displace native organisms (Howarth 1991, Hadfield et al. 1993, Elliott et al. 1996). Inundative biological control involves a mass release of either native or exotic natural enemies to control pest populations. Establishment with inundative biological control has not always been successful due to undesirable conditions for the introduced predator, emigration, timing and method of release, and predation/cannibalism (Collier and VanSteenwyk 2004, Crowder 2007). Conservation biological control is the implementation of techniques that enhance or maintain indigenous natural enemy populations (Barbosa 2003). Native natural enemies are an important component of integrated pest management. For example, the exclusion of indigenous generalist predators from cropping systems, such as carabid and staphylinid beetles (Coleoptera: Carabidae and Staphylinidae) and spiders (Araneae) has been associated with an increase in aphids in wheat fields in the United Kingdom (Holland and Thomas 1997) and in northeastern Colorado (Mohamed et al. 2000). Furthermore, generalist predators (i.e. predators that feed on a variety of prey) can be enhanced or sustained with conservation biological control techniques, significantly reducing pest densities by 73% and increasing

yield or reduced crop damage in 71% of reviewed biological control studies (Symondson et al. 2002). Conservation biological control has received further attention because little disruption to the ecosystem occurs with its implementation, and many techniques used to conserve natural enemies are practical from an agronomic standpoint (Peterson and Westfall 2004).

Conservation biological control techniques

From an agronomic perspective, the physical, chemical, and biological composition of the soil can be negatively impacted by conventional tillage (Hendrix et al. 1986, Stinner and House 1990, Symondson et al. 1996, Baguette and Hance 1997, Krooss and Schaefer 1998, Kladivko 2001). Conservation biological control techniques within agroecosystems might include conservation tillage or no tillage, reduced mechanical disturbances, mulching, strip-cropping, reduced pesticide use, increased structural or vegetational diversity, and crop diversification (Riechert and Bishop 1990, Stinner and House 1990, McNett and Rypstra 2000, Samu 2003, Schmidt et al. 2004, Thorbek and Bilde 2004). No tillage or reduced/conservation tillage mechanisms can positively impact agronomic properties and arthropod communities by reducing mechanical input to the system. Conservation or reduced tillage produces minimal disturbance during planting which can increase the activity density of predators (Blumberg and Crossley 1983, Heimbach and Garbe 1996, Schmidt et al. 2005). Conservation tillage has been associated with reduced pest densities. Wheat and sorghum in the Great Plains with reduced or no tillage had lower greenbug densities than areas with conventional tillage (Burton and Krenzer 1985, Burton et al. 1987).

Mechanical disturbances in agroecosystems include planting, cultivation, weed

control, and crop harvest. These disturbances can affect the natural enemies within the system. Spiders demonstrated reduced densities and direct mortality following mechanical weed control, grass cutting, soil loosening, plowing, and conventional tillage (Everts et al. 1989, Thorbek and Bilde 2004).

Mulching within an agroecosystem can provide refuges for predators (Riechert and Bishop 1990, Schmidt et al. 2004) and relieve intraguild predation (Finke and Denno 2003), which occurs when predators within the same guild feed on one another (Polis and Holt 1992). Spider densities increased in response to the addition of mulch and mulch and buckwheat treatments in vegetable gardens in the US (Riechert and Bishop 1990). The addition of thatch to a *Spartina* marsh system provided refuges for *Pardosa* (Araneae: Lycosidae) and *Grammonota* (Araneae: Linyphiidae) spiders and *Tytthus* (Hemiptera: Miridae) mirid bugs (Finke and Denno 2006), reducing intraguild predation and enhancing primary productivity within the marshes.

Pesticides are used to manage crop pests and weeds. The reduction of herbicide and insecticide inputs can benefit natural enemy populations both indirectly and directly. Insecticide applications reduced linyphiid spider populations by 56% (Thomas and Jepson 1997). Herbicides, however, mostly have indirect effects on spider densities by reducing vegetation and, hence, web-attachment sites (Baines et al. 1998, Haughton et al. 2001). The loss of plant structural complexity and biodiversity disturbs spider habitat. When a high rate of glyphosate was applied, web-building spider densities were reduced (Everts et al. 1989, Haugton et al. 1999).

An increase in vegetational diversity can influence natural enemy populations (Risch et al. 1983, Andow 1991, Riechert 1999, Oberg 2007). Additional vegetation can provide

a variety of additional prey (Feber et al. 1998). A meta-analysis of invertebrate natural enemies in complex habitats found that spiders, in particular, preferred structurally enhanced habitats (Langellotto and Denno 2004), which also has been demonstrated with modeling (Topping and Sunderland 1994; Topping 1997, 1999). One management technique to structurally enhance habitats is the use of strip-harvesting, which allows a portion of the crop to remain uncut or unharvested adjacent to a recently harvested area, providing a refuge for natural enemies. For instance, spider density increased over 50% in strip-harvested portions of an alfalfa/grass meadow cropping system (Samu 2003), and predator densities increased in grass-sown banks in wheat fields (Thomas et al. 1991). Sunderland and Samu (2000) reviewed literature on the effect of agricultural diversification on spider populations, and 63% of these studies reported an increase in spider densities in reponse to habitat diversification.

The addition of non-crop vegetation or more diverse crops to an agricultural landscape can facilitate predator dispersal, provide alternative prey, and encourage predator residence (Sunderland and Samu 2000, Schmidt and Tschartnke 2005, Schmidt et al. 2005, Tschartnke et al. 2008). For example, parasitism with the rape pollen beetle *Meligethes aeneus* (Fabricius) (Coleoptera: Nitidulidae) increased while damage of the rape crop decreased as the agroecosystem landscape increased in complexity (Thies and Tscharntke 1999). Similarly, spider diversity increased when additional non-crop habitat surrounded several winter wheat organic and conventional fields (Schmidt et al. 2005). Linyphiid spider densities increased within winter wheat agroecosystems surrounded by non-crop habitat (Schmidt and Tscharntke 2005).

Crop intensification in a dryland agriculture is a production system and conservation biological control technique that consists of more crops and a shorter overall fallow period (Farahani et al. 1998). By introducing a summer crop such as corn, millet, sorghum, or sunflower in late spring, the fallow period can be reduced from 14 months to 10 months. Consequently, this may be beneficial for natural enemies, as crop rotations can be synchronized so other prey sources and habitat for predators are consistently available (Altieri 1994). Additionally, crop intensification offers several agronomic benefits, such as improved water use efficiency (Peterson et al. 1996), improved soil aggregate stability (Shaver et al. 2003), an increase in soil carbon sequestration (Sherrod et al. 2003), and increased grain yield (Dhuyvetter 1996, Peterson and Westfall 2004). These agronomic advantages are attractive to farmers, and this creates further interest in understanding the natural enemy complex in crop-intensified systems.

Spiders as biological control agents

Spiders are prime candidates for biological control within agroecosystems. They are indigenous, generalist predators that can function as biological control agents within agroecosystems (Moulder and Reichle 1972, Nyffeler and Benz 1987, Riechert and Bishop 1990, Young and Edwards 1990, Kajak et al. 1991, Kajak 1997). Spiders can colonize fields early, feed on alternative prey until pest populations arrive, and target pests before they reach peak densities (Settle et al. 1996, Landis and Van der Werf 1997, Chang and Kareiva 1999, Symondson et al. 2002). Additionally, they have low metabolic rates that enables them to survive periods of starvation (Greenstone and Bennett 1980).

Spiders also may indirectly aid in pest management. The spider families Linyphiidae, Dictynidae, Theridiidae, and Agelenidae consistently keep their webs standing, and their webs can contribute to additional pest mortality (Nentwig 1987, Sunderland et al. 1986). Grasshoppers exhibited reduced feeding on grass with the mere presence of the spider *Pisurina mira* (Walckenaer) (Araneae: Pisauridae) in spite of its chelicerae being glued together to prevent predation (Schmitz et al. 1997). Similarly, the presence of spiders deterred Japanese beetles, *Popilliae japonica* (Newman) (Coleoptera: Scarabaeidae), from feeding on soybean leaves while simultaneously preventing the loss of plant biomass (Hlivko and Rypstra 2003).

Spiders have effectively reduced pest populations in agroecosystems (Riechert 1999, Johnson et al. 2000, Whitehouse and Lawrence 2001). Predation is most effective when spiders are present early in the growing season when the predator to prey ratio is high (Edwards et al. 1979, Ekbom and Wiktelius 1985, Chiverton 1986, Birkhofer et al. 2008). Halaj and Wise (2001) performed a meta-analysis of terrestrial food webs in agricultural systems and discovered that arthropod predators exhibited strong top-down effects on plants. Spider populations have reduced larval populations of the moth *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) and decreased populations of Cicadellidae (Hemiptera), Thripidae (Thysanoptera), as well as decreased populations of Aphididae (Hemiptera) in southern Bavaria (Lang et al. 1999). Riechert and Bishop (1990) found prey densities and plant damage to be lower in garden test systems with augmented spider densities. *Araneus quadratus* Clerck (Araneae: Araneidae) indirectly reduced plant damage by preying on grasshoppers (Andrzejewska et al. 1967). Sea grape leaf damage was significantly reduced when spiders preyed upon gall midges (Spiller and

Schoener 1990). Spiders fed on herbivores, increasing the dry biomass of the plant species *Solidago rugosa* Mill. (Asterales: Asteraceae) (Schmitz 2003). The spider *Anyphaena celer* (Hentz) (Araneae: Anyphaenidae) increased mortality of the herbivore *Stephanitis pyrioides* (Scott) (Hemiptera: Tingidae) (Shrewsbury and Raupp 2006). In soybean, herbivore damage was reduced where spiders were added (Rypstra and Carter 1995). Additionally, composted plots had less soybean leaf and pod damage when more spiders were present, and non-crop plants had reduced damage when *Argiope trifasciata* (Forskål) (Araneae: Araneidae) had eaten more leaf-chewing insects (Rypstra and Marshall 2005). Spider predation on pests has been further demonstrated in several other agroecosystem experiments (Riechert and Lockley 1984).

Spiders may be especially useful for reducing aphid densities (Sunderland et al. 1986, Collins et al. 2002). Based on a review of common spiders in agroecosystems, aphids (Hemiptera: Aphididae) represented approximately 14% of the prey captured by spiders (Nyffeler et al. 1994). Linyphiid spiders (Araneae: Linyphiidae) killed 31 *Sitobion avenae* (F.) (Hemiptera: Aphididae) m^2 day^{-1} in winter wheat (Sunderland et al. 1986). In Europe, experimentally manipulated increases in ground predator densities resulted in aphid reduction in maize (Lang et al. 1999), barley (Chiverton 1986, Ekbom et al. 1992, Ostman et al. 2003), and wheat (Edwards et al. 1979, Chiverton 1986, Mansour and Heimbach 1993, Collins et al. 2002, Lang 2003, Schmidt et al. 2004, von Berg et al. 2009). In addition, spider populations also have decreased damage by the greenbug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae) (Muniappan and Chada 1970, Mansour et al. 1981).

Predator biodiversity and biological control theory

The effect of increased biodiversity on the biological control of pests can range from positive to negative and is case-dependent. Therefore, it is important to have an assessment of the biodiversity of predators within an agroecosystem. An increase in predator biodiversity is not necessarily advantageous for pest management. Following the principles of the "redundancy hypothesis", species that share functional guilds can disrupt pest regulation through increased competition, potential cannibalism, or intraguild predation (Rosenheim et al. 1993, 1995; Sunderland and Vickerman 1980). Cannibalism often can be substantial, which was found among lycosid spiderlings (Wagner and Wise 1996). When densities of two intraguild predators, the lycosid spider, *Pardosa littoralis* Emerton (Araneae: Lycosidae), and the mirid bug, *Tytthus vagus* Knight (Hemiptera: Miridae), increased, densities of the pest planthopper *Prokelisia* actually increased in salt marshes (Finke and Denno 2003). Similarly, when the predators *T. vagus*, *Grammonota trivitatta* Banks (Araneae: Linyphiidae) increased, *P. littoralis*, and *Hogna modesta* (Thorell) (Araneae: Lycosidae), *Prokelisia* densities increased due to intraguild interference (Finke and Denno 2004).

Conversely, an increase in biodiversity can benefit pest management. Pest suppression has been successful when several spider species were present simultaneously (Riechert 1999). A meta-analysis showed that prey suppression by arthropods generally strengthens when natural enemy biodiversity increases (Cardinale et al. 2006). Specifically, increased predator diversity can be effective for pest suppression through resource partioning or the "species complementarity model", which suggests that species utilize resources in various ways and the combination of these species has a greater effect

on pest control than any species alone (Finke and Snyder 2008). For example, three predator species, a damsel bug, *Nabis* sp. (Hempitera: Nabidae), a parasitic wasp, *Aphidius ervi* Haliday (Hymenoptera: Braconidae), and a lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), had a synergistic effect on the suppression of the pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Hemiptera) (Cardinale et al. 2003). Similarly, the combination of flying predators and parasitoids worked synergistically to reduce densities of the cereal aphids *S. avenae*, *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae), and *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) (Schmidt et al. 2003). The combination of the predators *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae) and *Harpalus pennsylvanicus* Dej. (Coleoptera: Carabidae) increased herbivore suppression more than each species alone (Losey and Denno 1998). Carabid beetles (Coleoptera: Carabidae), lycosid spiders (Araneae: Lycosidae), and linyphiid spiders (Araneae: Linyphiidae) worked synergistically in wheat fields in Bavaria to reduce aphid densities (Lang 2003). Furthermore, other principles suggest benefits of increased biodiversity. For instance, the "diversity-stability hypothesis" states that, as the local diversity of organisms strengthens, the community stability of organisms increases (Pimentel 1961). The "insurance hypothesis of biodiversity" also states that increased species may functionally overlap under certain conditions, but changing environmental conditions may allow for complementarity among species. An increase in predator biodiversity and subsequent suppression of pest populations has been suggested with the "enemies hypothesis" (Root 1973). In summary, an increase in the biodiversity of predators can be advantageous in many instances or detrimental in other instances, which may be highly dependent on the natural enemy complex involved.

Molecular techniques to study predator-prey interactions

To make appropriate assessments of the effectiveness of biological control, it is important to measure the consumption of prey by predators in their natural conditions. Because spiders feed on pre-digested prey, analyses of predator-prey interactions are difficult. Indirect methods, such as addition/exclusion caged experiments, can be implemented to test the effects of predator presence/absence on prey populations. Additionally, direct observations can be performed. However, several confounding variables exist, such as time of feeding, dense vegetation and leaf litter presence, and disturbance to the study system (Symondson 2002). Dissection for visible prey remains is another technique that can be implemented with many taxa, although some predators, such as spiders, have no discernible trace of prey within their guts. Because of the challenges faced with examining predator-prey dynamics, gut-content analysis through either serological or molecular methodology is an efficient way to measure consumption with minimal disturbance to the study system.

To achieve high specificity in predator-prey observations when targeting pest DNA, serological techniques, such as the use of monoclonal antibodies, are ideal (Sheppard and Harwood 2005). Monoclonal antibodies have been used to detect predator-prey interactions with several hundred linyphiid spiders and the pest aphid *Sitobion avenae* (F.) (Harwood et al. 2004). Similarly, monoclonal antibodies were used to detect *S. avenae* within the guts of predaceous coccinellid beetles (Gao et al. 2009). Order-level specificity was performed with linyphiid spiders and dipteran prey (Harwood et al. 2007a). Despite the level of specificity and the ability to screen several predators for one

target prey, the development of monoclonal antibodies is time-consuming and often prohibitively expensive.

Because of the challenges with monoclonal antibody development, the use of DNA-based techniques may be more practical for studying food web ecology. Since many predators, such as spiders, consume pre-digested food and further digestion of prey DNA ensues, it is necessary to amplify very small amounts of degraded and fragmented DNA. Species-specific or group-specific primers are commonly used with PCR and can be created either through sequences retrieved from the GenBank database, a collection of all available DNA sequences, or through the use of general primers, amplification of these primers through PCR, and subsequent sequencing of these products. The sequences can then be aligned, compared with other non-target species, and prey-specific primers can be created. DNA was not properly amplified in past predator-prey studies because primers were typically created from single-copy genes (Zaidi et al. 1999). As a result, multiple copy genes, such as ribosomal or mitochondrial genes, have since been targeted. Most studies now concentrate on amplifying fragments from within these genes (Chen et al. 2000, Agustí et al. 2003a,b; de Leon et al. 2006, Harwood et al. 2007, Kuusk et al. 2008, Monzo et al. 2010). The number of possible prey species present in the field can range from 1 to 40 species (Harper et al. 2005), making the use of species-specific primers exhaustive when multiple prey species are of interest for understanding food web dynamics.

Several field studies have tested invertebrate predation in the field through PCR. Linyphiid spiders were collected from the field in Wellesbourne, UK, and tested for the presence of three common species of Collembola using primers created from the

cytochrome oxidase I (COI) mitochondrial gene, and spiders preferentially consumed the least dominant species of Collembola (Agusti et al. 2003a). Species-specific primers and PCR were used to detect *Aphis glycines* Matsumura (Hemiptera: Aphididae) from the guts of *Orius insidiosus* (Say) (Hempitera: Anthocoridae), and 32% of the predators tested positive, with most of this predation occurring early in the season when aphid densities were low (Harwood et al. 2007b). With these same species-specific primers, *O. insidiosus* was tested for the presence of *A. glycines* DNA from within the guts of both immature and adult life stages, and results showed a greater proportion of immatures tested positive for *A. glycines* in the gut (Harwood et al. 2009). When screening *Poecilus* (Coleoptera: Carabidae), Geophilidae (Geophilomorpha), and Lithobiidae (Lithobiomorpha) predators, 18.6%, 4.1%, and 4.4%, respectively, screened positive for the presence of garden chafer *Phyllopertha horticola* L. (Coleoptera: Scarabaeidae) DNA (Juen and Traugott 2007). Using both ELISA and species-specific primers with PCR, 15.5% of 1229 arthropod predators tested positive for the presence of the glassy winged sharpshooter *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) DNA (Lundgren et al. 2009). Species-specific primers for *R. padi* from the cytochrome oxidase II gene (COII) were previously created (Chen et al. 2000) and further tested from the guts of field-collected *Pardosa* spiders (Araneae: Lycosidae) (Kuusk et al. 2008). Presence of the Mediterranean fruit fly DNA, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), was tested from within the guts of *Pardosa cribata* Simon (Araneae: Lycosidae) using primers created from the internal transcribed spacer 1 (ITS1) ribosomal gene, and 5% of field-collected predators tested positive for the fly DNA (Monzó et al. 2010).

This chapter provided a description of the pest, its management, biological control, biodiversity, the role of spiders in agroecosystems, and a description of molecular approaches to determine food web dynamics. The overall goals of this dissertation were to describe the eastern Colorado spider fauna, determine if crop-intensified systems affect spider biodiversity and density, to determine if aphids represent a valid prey source for epigeal predators, and to determine if two dominant spider species prey on *D. noxia* in the field. Results from these studies will provide further understanding as to whether spiders might have a role in the biological control of pests in Colorado agroecosystems. The following hypotheses were tested:

- Spider density and biodiversity will be higher in crop-intensified agricultural systems compared with conventional systems. This is because the intensified rotation will have increased structural diversity, will provide more consistent habitat for both predators and prey, and will provide food for predators during and after wheat harvest.
- A *D. noxia*-resistant line will have a higher aphid falling rate than its paired susceptible sister line. This is because the resistant line should have flatter leaf architecture, making it difficult for aphids to reside within the leaf.
- *Tetragnatha laboriosa* (Araneae: Tetragnathidae) and *Pardosa sternalis* (Araneae: Lycosidae) will consume *D. noxia* in the field and represent important predators of *D. noxia* in wheat agroecosystems. These spider species are dominant in northern Colorado agroecosystems and are likely to feed on aphids.

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CHAPTER 2-EFFECTS OF CROP INTENSIFICATION ON THE FAUNISTIC COMPOSITION OF SPIDERS IN EASTERN COLORADO AGROECOSYSTEMS

Abstract

Crop intensification in a dryland agriculture production system includes more crops and fewer fallows per unit time. Dryland crop intensification offers several agronomic benefits and can also provide refugia and alternative prey for natural enemies during agricultural disturbances. This study examined whether a crop-intensified system resulted in greater spider density and biodiversity than a conventional system by testing the following hypothesis: An intensified crop rotation will have greater spider density and biodiversity than a conventional rotation. Three sites in eastern Colorado-Akron, Briggsdale, and Lamar-were the study sites. Spiders were sampled from 2002-2007 using pitfall, vacuum, and lookdown sampling. Data were analyzed as a randomized complete block design. Biodiversity analyses were performed with the Shannon index, rarefaction, and species accumulation curves. Crop intensification had little effect on spider density or biodiversity. Spider mean activity densities and biodiversity were low for all years and sites, with the exception of 2005 and 2006. These years may have higher densities and biodiversity due to increased precipitation and weed growth. Spider activity densities were higher from April-July in almost all years and sites, suggesting that the spiders' phenology coincided with frequent agricultural disturbances, such as

crop harvest. A total of 11,207 spiders in 17 families and 119 species were collected from all sites from 2002-2007. The number of spiders collected from Akron, Briggsdale, and Lamar were 3255, 3381, and 4571, respectively. For all sites, Lycosidae and Gnaphosidae were the dominant families, representing over 72% of the fauna collected. Other families commonly represented were Thomisidae, Philodromidae, Linyphiidae, and Salticidae. Cumulatively from 2002-2007, 77, 78, and 67 species were collected in Akron, Briggsdale, Lamar, respectively. The dominant species were the following at each site: Akron-*Schizocosa mccooki*, *Gnaphosa clara*, and *Drassyllus nannellus*; Briggsdale-*G. clara*, *S. mccooki*, and *Haplodrassus chamberlini*; Lamar-*Gnaphosa saxosa*, *S. mccooki*, and *Hogna coloradensis*. Species accumulation curves and the high percentage of singletons within the collection were indicative of undersampling at all sites. Therefore, biodiversity might have been underestimated. The density of spiders in eastern Colorado dryland agroecosystems differs from densities reported in Western Europe. The spider biodiversity in this study was dominated by spiders in the hunting guild, which differs from the over 90% Linyphiidae-dominated wheat fields in Western Europe. Moreover, this has implications for the biological control potential of spiders in Colorado agroecosystems, i.e., the dominance of hunting spiders may preclude efficient biological control of pests.

Introduction

In agroecosystems, the establishment of an environment that augments densities of indigenous predators can be important for the effective conservation biological control of pests (Cardinale et al. 2003, Snyder et al. 2006). Crop management practices can directly

influence predator density as many agricultural systems incorporate a consistent cycle of disturbances, including tillage, planting, harvest, and crop rotation (Luff 1987). With monocultures, growers often till their fields and eliminate any vegetation that is present (Nyffeler and Benz 1979), which can result in increased pest outbreaks (Pimentel 1961). Conventional farming can negatively affect the density and biodiversity of indigenous fauna (Benton et al. 2003). Arthropod biodiversity and biomass can increase in heterogeneous agricultural landscapes, coinciding with the availability of refuges, improved environments, and reduced competition (Ryszkowski et al. 1993, Sunderland and Samu 2000). Thus, often pest populations are lower in more heterogeneous landscapes (Altieri 1994).

An adjacent undisturbed non-agricultural area can be ideal for enhancing predator densities (Landis et al. 2000, Tscharntke et al. 2008) and promoting effective pest management (Tscharntke et al. 2005, 2008; Bianchi et al. 2006, Gavesh-Regev et al. 2008). However, assigning land to permanently undisturbed habitats is rarely a viable option for farmers (Tscharntke et al. 2008, Landis et al. 2000). Consequently, it is vital to explore whether more economically practical changes to current agricultural practices can be used to enhance predator establishment.

Dryland agriculture in the Great Plains has been dominated by the wheat-fallow rotation. The fallow in the Great Plains area represents an opportunity to store soil water and increase the chances for a successful crop (Peterson et al. 1996). Additionally, water storage is maximized during the fallow period if fields are weed free (Peterson and Westfall 2004). Hence, the fallow fields remain barren of vegetation, which can create an unsuitable residence for natural enemies and limit the amount of prey available. The

addition of crops to this rotation may be a practical conservation biological control technique to enhance landscape heterogeneity and, thus, increase natural enemy densities. For example, crop intensification in a dryland agriculture system includes more crops and fewer fallows per unit time (Farahani et al. 1998). Following this system, the fallow period is reduced from 14 months to approximately 10 months, by incorporating a summer crop such as corn, millet, sorghum, or sunflower in late spring following the 10-month fallow period.

Dryland crop intensification offers several agronomic benefits. For example, crop intensification has resulted in a 28% increase in water use efficiency in several eastern Colorado sites when compared with wheat-fallow (Peterson et al. 1996). Additionally, it has resulted in decreased soil bulk density (Shaver et al. 2002) and increased soil aggregation and soil aggregate stability (Shaver et al. 2003). Coupled with no-tillage practices, crop intensification can increase soil carbon sequestration (Sherrod et al. 2003). Intensification also reduces the economic reliance on one crop. Adding a summer crop also increased annualized grain yield in dryland agroecosystems (Dhuyvetter 1996, Peterson and Westfall 2004). Potential yield increases and economic profitability from crop intensification promotes adoption by growers.

From a natural enemy perspective, crop intensification may offer a variety of crops at different phases, which can provide refugia during agricultural disturbances. Crops can be synchronized so they senesce at different times throughout the year, providing alternative prey sources and habitat for predators (Altieri 1994). Additionally, the early arrival of natural enemies into a crop has resulted in effective biological control, due to

effective predator to prey ratios (Chiverton 1986, Holland and Thomas 1997, Landis and van der Werf 1997, Birkhofer et al. 2008).

Spiders are dominant natural enemies in agroecosystems, and their life histories can coincide with the predicted seasonality and disturbances associated with agricultural habitats. In the temperate zone, most spiders live for only a year, and life cycles have been described for just a few species (Foelix 1996). For spiders in central Europe, reproduction mainly occurs in May with spiderlings hatching in the summer (Tretzel, 1954). Such species have been defined as "agrobionts" (Luczak 1979). Agrobionts can participate in "cyclic colonization", which involves dispersal to fields during crop activity and dispersal out of fields into undisturbed habitats for post-harvest overwintering (Wissinger 1997). For cyclic colonization to effectively maintain natural enemy densities, it is important for reproduction to be timed appropriately with disturbances (i.e., crop harvest, cultivation) (Samu and Szinetar 2002). Furthermore, spiders need to reproduce early in the crop growing season and prior to planned disturbances to persist in agricultural habitats (Thorbek et al. 2004).

To exploit conservation biological control, it is also important to have an assessment of the biodiversity within the agroecosystem. An increase in predator biodiversity through an increased assemblage of spider species can reduce pest densities effectively (Losey and Denno 1998, Riechert 1999, Schmidt et al. 2003, Snyder et al. 2006, Straub and Snyder 2008). This can be explained by the niche complementarity hypothesis, i.e., a single pest introduces a variety of feeding niches exploitable by several predator species. For example, in an orchard study in western France, several spider species presented

different prey-capturing strategies and increased overall pest suppression (Marc and Canard 1997).

The influence of dryland crop intensification in the Great Plains on the density and biodiversity of spiders is poorly understood. From a temporal perspective, the current crop and the crop that follows in the rotation may be important for spider populations. Spiders may be more likely to remain in the system when a summer crop is introduced into the rotation and the fallow period is reduced, introducing an important refuge between crop harvest and other disturbances. From an ecological standpoint, increased structural diversity by the addition of more crops to the rotation could increase the biodiversity of the predator fauna (Landis et al. 2000). Because spiders are dominant predators in agroecosystems, it is important to address whether changes in crop practices, such as crop intensification, can affect spider biodiversity and density. This study tested the following hypothesis: An intensified crop rotation will have greater spider density and biodiversity than a conventional rotation. This hypothesis is supported by the expectation that the intensified rotation will (1) have increased structural diversity; (2) will provide more consistent habitat for both predators and prey; and (3) will provide food for predators during and after wheat harvest.

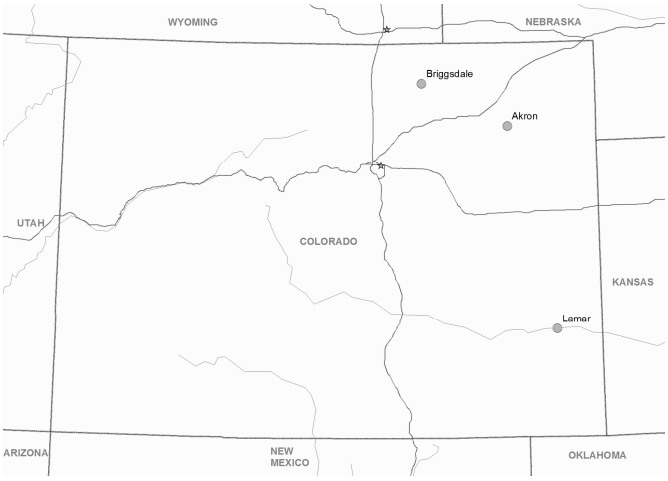
Materials and Methods

Field Sites

The eastern Colorado dryland agroecosystem study sites were: Akron, located in Washington Co. (40.16°N, 103.21°W; elevation=1420 m); Briggsdale, located in Weld Co. (40.60°N, 104.34°W; elevation=1475 m); and Lamar, located in Prowers Co.

(38.09°N, 102.62°W; 1104 m) (Figure 2.1). These sites are semi-arid, receiving an average of 350-400 mm of annual precipitation.

FIGURE 2.1. FIELD SITE LOCATIONS IN AKRON, BRIGGSDALE, AND LAMAR, CO, 2002-2007.



The study sites were part of a dryland agroecosystem study comparing four rotations. Spiders were collected from 8 plots in a conventional winter wheat/fallow rotation, and 12 plots in a winter wheat/summer crop/fallow rotation (Figure 2.2). The summer crop varied with location and plot size (Table 2.1). There were five treatments for this study (Table 2.2). All crop phases of both rotations were present in each replicate each year. Additionally, sunflower was sampled at each location from a different rotation to provide additional data for local biodiversity assessments.

TABLE 2.1. DESCRIPTION OF FIELD SITES, PLOT SIZES, AND ROTATIONS AT AKRON, BRIGGSDALE, AND LAMAR, CO, 2002-2007.

Site	Annual Precipitation[1] (mm)	Plot length	Conventional Rotation	Crop-Intensified Rotation
Akron	405	27.4 m x 54.9 m	Winter wheat-fallow	Winter wheat-corn-fallow[2]
Briggsdale	350	27.4 m x 125.0 m	Winter wheat-fallow	Winter wheat-millet-fallow
Lamar	375	30.5 m x 97.5 m	Winter wheat-fallow	Winter wheat-sorghum-fallow[3]

[1]1961-1990 mean.
[2]Summer crop changed to millet in 2005-2006, 2006-2007 crop year.
[3]Summer crop changed from grain sorghum to field sorghum in 2005-2006, 2006-2007 crop year.

TABLE 2.2. TREATMENTS FOR THE CONVENTIONAL AND CROP-INTENSIFIED ROTATIONS AT AKRON, BRIGGSDALE, AND LAMAR, CO, 2002-2007.

Treatment	Rotation	Crop
1	Conventional	Wheat
2	Conventional	Fallow
3	Crop-intensified	Wheat
4	Crop-intensified	Millet, Corn or Sorghum
5	Crop-intensified	Fallow

Site Management

All sites were dryland, receiving no supplemental irrigation. At all field sites, Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphidiidae), densities, residue amounts, spiders, and soil properties were measured. Limited data for carabid beetles (Miller 2008) and other insects were also measured from 2002-2006 with pitfall samples.

Agronomic inputs

Wheat

Winter wheat, *Triticum aestivum* L. (Poales: Poaceae), was planted in the fall from 2001 through 2006. Well-adapted cultivars were chosen for each site. Wheat plots were split. From 2001-2004, half of each wheat plot was planted with a cultivar susceptible to Russian wheat aphid biotype RWA1, and the other half was planted with a cultivar resistant to biotype RWA1. Beginning in 2005, only biotype RWA1 resistant cultivars were planted at the study sites. In Akron, the herbicide glyphosate was applied prior to

planting in all wheat plots. In Briggsdale and Lamar, glyphosate was applied to all wheat plots prior to planting, during post-emergence of wheat in the spring, and again to the wheat stubble following harvest in mid-late summer (Peterson et al. 2004). Table 2.3 lists the wheat cultivars, planting dates and rates, and fertilizer applications for all sites from 2002-2007.

TABLE 2.3. WHEAT CULTIVARS, PLANTING DATE, SEEDING RATE, AND FERTILIZER APPLICATION DATES FOR AKRON, BRIGGSDALE, AND LAMAR, CO, 2001-2007.

Crop Year	Site	Cultivars[1]	Planting Date	Seeding Rate	Fertilizer[2]
2001-2002	Akron	TAM107 (S)/Prairie Red (R)	29 Sep 2001	60 lbs/A	N, 32 lbs/A
	Briggsdale	Yuma (S)/Yumar (R)	20 Sep 2001	60 lbs/A	N, 49 lbs/A
	Lamar	TAM107 (S)/Prairie Red (R)	16 Sep 2001	40 lbs/A	N, 6 lbs/A
2002-2003	Akron	TAM107 (S)/Prairie Red (R)	29 Sep 2002	60 lbs/A	N, 32 lbs/A
	Briggsdale	Yuma (S)/Yumar (R)	19 Sep 2002	60lbs/A	N, 49 lbs/A
	Lamar	TAM107 (S)/Prairie Red (R)	7 Sep 2003	45 lbs/A	N, 6 lbs/A
2003-2004	Akron	TAM107 (S)/Prairie Red (R)	5 Oct 20031	60 lbs/A	N, 61 lbs/A
	Briggsdale	Akron (S)/Ankor (R)	17 Sep 2003	43 lbs/A	N, 48 lbs/A
	Lamar	TAM107 (S)/Prairie Red (R)	7 Sep 2003	45 lbs/A	N, 6 lbs/A
2004-2005	Akron	TAM107 (S)/Prairie Red (R)	12 Oct 2004	60 lbs/A	N, 62 lbs/A
	Briggsdale	Akron (S)/Ankor (R)	20 Sep 2004	60 lbs/A	N, 39 lbs/A
	Lamar	Akron (S)/Ankor (R)	9 Sep 2004	45 lbs/A	N, 6 lbs/A
2005-2006	Akron	Prairie Red (R)	2 Oct 2005	76 lbs/A	N, 62 lbs/A
	Briggsdale	Hatcher (R)	26 Sep 2005	67 lbs/A	N, 40 lbs/A
	Lamar	Stanton (R)/Jagalene	15 Sep 2005[3]	45 lbs/A	N, 21 lbs/A
2006-2007	Akron	Ankor (R)	1 Oct 2006	60 lbs/A	N, 60 lbs/A
	Briggsdale	Hatcher (R)	26 Sep 2006	60 lbs/A	N, 40 lbs/A
	Lamar	Hatcher (R)/Jagalene	14 Sep 2006	45 lbs/A	N, 21 lbs/A

[1]R=wheat variety resistant to biotype RWA1, S=wheat variety susceptible to biotype RWA1.
[2]N=Nitrogen.
[3]Jagalene replanted on 28 Oct 2005 due to poor emergence**.**

Fallow

At Akron, the fallow plots in the conventional rotation were tilled once with a tandem disc in the spring and swept twice prior to planting. Fallow plots were not tilled at Briggsdale. Herbicides were applied monthly during the spring and summer to the fallow plots (Peterson et al. 2004). At Lamar, the fallow in the crop-intensified rotation was swept once during mid-summer.

Summer crops

Locally-adapted summer crop varieties were planted from May to July from 2002-2007 (Table 2.4). At all sites, glyphosate was applied pre-plant, and another herbicide was applied post crop emergence (Peterson et al. 2004). Sorghum was not planted in 2002 in Lamar due to drought conditions. Sunflower was planted in Akron and Briggsdale in 2002-2004.

TABLE 2.4. CROPS, PLANTING DATES, SEEDING RATES, AND FERTILIZER APPLICATIONS FOR SUMMER CROPS AT AKRON, BRIGGSDALE, AND LAMAR, CO, 2001-2007.

Crop Year	Site	Crop	Variety	Planting Date	Planting Rate	Fertilizer
2001-2002	Akron	Corn	Dekalb DK520RR	20 May 2002	17.2 K seeds/A	N, 90 lbs/A; P, 15 lbs/A
	Akron	Sunflower	Triumph 765	10 June 2002	17.2 K seeds/A	N, 56 lbs/A
	Briggsdale	Millet	Huntsman	15 June 2002	15 lbs/A	None
	Briggsdale	Sunflower	Mycogen SF187	7 June 2002	15 K seeds/A	None
	Lamar	Sorghum[1]	NA	NA	NA	NA
2002-2003	Akron	Corn	DK46-28RR	20 May 2003	14.0K seeds/A	N, 72 lbs/A
	Akron	Sunflower	Triumph 765	10 June 2002	16.6K seeds/A	N, 47 lbs/A

Crop Year	Site	Crop	Variety	Planting Date	Planting Rate	Fertilizer
	Briggsdale	Millet	Golden German	21 June 2003	15lbs/A	None
	Briggsdale	Sunflower	Mycogen SF187	21 June 2003	15lbs/A	None
	Lamar	Sorghum	DeKalb DK636-00	28 May 2003	24 K seeds/A	N, 60 lbs/A
2003-2004	Akron	Corn	DK46-28RR	23 May 2004	14 K seeds/A	N, 72 lbs/A
	Akron	Sunflower	Triumph 765	29 May 2004	17.5 K seeds/A	N, 69 lbs/A
	Briggsdale	Millet	Huntsman	2 June 2004	15 lbs/A	N, 48 lbs/A
	Briggsdale	Sunflower	Mycogen SF187	27 May 2004	13K seeds/A	N, 69 lbs/A
	Lamar	Sorghum	DeKalb DK636-00	18 May 2004	24 K seeds/A	N, 73 lbs/A
2004-2005	Akron	Corn	DK40-08RR/YG	20 May 2005	14 K seeds/A	N, 65 lbs/A
	Akron	Sunflower	Triumph 565	9 June 2005	17.5 K seeds/A	N, 69 lbs/A
	Briggsdale	Millet	Golden German, and Grazex	14 May 2005	14 lbs/A	N, 30 lbs/A
	Lamar	Sorghum	Northrup KS310	6 June 2005	34.7 K seeds/A	N, 7.5 lbs/A
2005-2006	Akron	Millet	Huntsman	19 June 2006	15 lbs/A	N, 40 lbs/A
	Briggsdale	Millet	Huntsman	13 July 2006	15 lbs/A	N, 40 lbs/A
	Lamar	Sorghum	Sucrosorgo 405	20 June 2006	105 K seeds/A	None
2006-2007	Akron	Millet	Huntsman	2 July 2007	15 lbs/A	N, 15 lbs/A
	Briggsdale	Millet	Huntsman	21 June 2006	15 lbs/A	N, 40 lbs/A
	Lamar	Sorghum	Canex BMR 208	2 July 2007	15 lbs/A	N, 15 lbs/A

[1]Sorghum did not produce grain due to drought conditions.

Spider Sampling

Three sampling methods were combined to maximize the number of spider species collected. Pitfall traps alone do not provide an accurate representation of local faunal composition (Halsall and Wratten 1988) but instead measure activity density only (Greenslade 1964, Topping and Sunderland 1992, Sunderland et al. 1995). Some families like Salticidae remain in silken retreats on crops when the weather is unfavorable (Canard 1981) and, thus, require a more active sampling technique like vacuum sampling.

Sampling was conducted from 2002-2007 using the following methods: pitfall (April-October, 2002-2006), vacuum (May-August, 2006-2007), and lookdown sampling (May-August, 2004-2006). Lookdown sampling was used only for biodiversity analyses because there was significant variation between collectors, regardless of their level of experience. Therefore, results from lookdown sampling were not included in statistical analyses. Pitfall samples were not collected in Lamar in September, 2004, due to a collector accident and in May, 2006, due to excessive rain. Vacuum samples were not collected in Lamar in May and June, 2007, due to rainfall.

Pitfall sampling

Two pitfall traps (10 cm in diameter) were placed within the center of each plot approximately 5m from one another. The traps consisted of a 2-L bottle with the top half cut and inverted to form a 5 cm funnel at the mouth of the trap with the bottom half holding a collection cup (Miller 2000). A solder gun was used to burn holes at the bottom of the bottle for drainage. A 500 mL plastic collection cup was placed in the

bottom of the trap and filled with approximately 165 mL of a 70:30 mixture of propylene glycol: water, which served as a killing agent for any ground-active arthropods. The 2-L bottles were placed in the ground such that the lip of the 2-L bottle was flush with the surface.

Pitfall trap contents were collected seven days after they were charged in the field during the months of April through October at all three sites from 2002-2006. Traps were temporarily removed prior to field operations and replaced afterwards. The traps were covered with a 15 x 20 cm ceramic tile when not in use, and new traps were used each year.

When collecting the traps, the 500 mL plastic cup was removed. The contents were poured through a strainer lined with a paper towel. After the propylene glycol-water mixture drained, the insects and spiders on the paper towel were carefully placed within a sealed plastic bag. The bags were transferred to the laboratory in a cooler and placed in the freezer for subsequent identification.

Suction sampling/hand search

Ground spiders are captured most efficiently by hand search, while spiders present in the foliage are collected most efficiently with a D-vac suction sampler, a sampler that was designed to specifically capture arthropods (Sunderland and Topping 1995). For this study, a modified Stihl BG 55 leaf vacuum (Stihl HomeScaper Series; 417 cfm) was used in lieu of a D-vac due to affordability and ease of transport. Vacuum samples were taken monthly at all sites from May through August in 2006 and 2007. Two areas were chosen at random within each plot (Southwood 1978), and a circular toothed sheet-metal frame with an area of 0.55 m^2 was placed over the area to be sampled. The frame served as a

barrier to prevent the spiders from escaping. The vacuum was placed within the framed area and operated for five seconds for each area covered by the vacuum aperture until the entire framed area had been sampled. Following the suction sampling, the framed area was collected by hand until no remaining spiders were found. The contents of the suction sleeve from the vacuum were sifted for spiders. All spiders captured were emptied into 4.4-L bags. These bags were sealed, placed on ice, and transferred back to the laboratory for subsequent processing and identification. Upon return to the lab, the bags were emptied into trays and searched for spiders. Spiders were suctioned with an aspirator and placed individually into labeled vials with 75% ethanol.

Look down sampling

Lookdown sampling was conducted from May through August, 2004-2006. This technique involved the collection of spiders found below knee level in the crop or at the soil surface. Collections were made between the hours of 20:00-24:00 for 30 min per plot within 3 repetitions, using headlamps as a light source. Spiders were hand-collected and placed in 125 mL Nalgene cups filled with 75% ethanol and transferred to the laboratory for identification.

Spider Identification

Spiders collected from all techniques were placed in individual vials filled with 75% ethanol and labeled appropriately. Adult spiders were identified to genus under a microscope at 40-60x with an identification manual for North American spider genera (Ubick et al. 2005) and subsequently identified to species using species-specific literature from the Denver Museum of Nature and Science. Immature spiders were not identified

past the family level. All spiders from the family Salticidae were identified by Dr. Fran X. Haas. Similarly, Dr. Michael Draney (University of Wisconsin) identified all Linyphiidae. Several individuals of each species were sent to specialists for species-level verification. Spiders from the family Dictynidae were sent to Dr. Robb Bennett, Thomisidae and Philodromidae were sent to Dr. Charles Dondale, and representatives of the Theridiidae family were sent to Dr. Herbert Levi. Voucher specimens are deposited at the Denver Museum of Nature and Science in Denver, CO.

Aphid Sampling

Aphids were sampled within the wheat during various wheat stages at all three sites from 2002-2007. The density of *D. noxia* was estimated by collecting 400 tillers at random from each wheat plot at several times each year. Tillers were placed in coolers, transported to Colorado State's Agricultural, Research, Development and Education Center (ARDEC), and placed in Berlese funnels for 24h to extract the aphids into the alcohol for subsequent counting under a dissecting microscope.

Analyses

Spider density

Crop rotations were randomly assigned to plots within blocks at each site (Peterson et al. 2004). Data were analyzed as a randomized complete block design with date as a repeated measure. The effects of treatment, date, and their interaction were analyzed with the response variable of spider density for both pitfall and vacuum samples. Sites and years were analyzed separately because sites differed climatically, and years were

substantially different from one another. Pitfall trap and vacuum samples were averaged by dividing the total catch by two to avoid pseudoreplication (Hulbert 1984). Analyses were performed with the total of immature and adult spiders. Repeated measures models with autoregressive errors and unequal variances across dates were evaluated and used when justified by AIC values (Burnham and Anderson 2002). Statistical computations were performed using the "Mixed" procedure in SAS (SAS Institute 2008) with the REML estimation method and the Kenward-Roger approximation for degrees of freedom (Kenward 1997). Spider densities were square-root transformed ($x + 0.5$) to homogenize the variances. If any fixed effects within the model were significant ($P \leq 0.05$), least squares means were separated and compared with t-tests. Untransformed means are presented in tables and figures. Since the means are pooled, the standard errors are the same and, therefore, will not be included in the results or tables presented.

Additionally, spider densities for pitfalls for 2002-2006 were compared for months prior to wheat harvest (April-July) and post wheat harvest (August-October), averaged over treatments, using the "contrast" statement in SAS (SAS Institute 2008). The purpose of these contrasts was to determine if spiders were coordinating their peak densities with the active wheat growing season.

It was also of interest to compare spider densities between the conventional and crop-intensified rotations. Because there were an unequal number of treatments within each rotation, this rotational comparison was performed using the "contrast" statement in SAS (SAS Institute 2008). When a treatment by date interaction did not occur, the contrast was averaged over dates, and when there was a treatment by date interaction, the contrast was computed separately for each date.

Spider biodiversity

Biodiversity analyses were performed with adult spiders as only adults can be identified to species with accuracy. Species accumulation curves, or plots of the total number of species represented as a function of the number of individuals sampled (Colwell and Coddington 1994), were calculated for all sites to address whether sampling effort was adequate for species determination. If a sampling inventory is complete, then the number of species should form an asymptote over time as the number of individuals increases. This rarely is achieved with a smaller number of individuals or a smaller sampling inventory, thus the need for species richness estimators (Colwell et al. 2004). The species richness estimators Chao I (Chao 1987) and ACE I (Chazdon et al. 1998, Chao et al. 2000) were calculated with Estimate S (Colwell 2005) to estimate the true number of species present at each site. The number of individuals collected varies between samples, creating another complication when assessing species biodiversity. Rarefaction curves standardize the number of individuals between samples, thus allowing differences between samples or sites to be compared accurately (Gotelli and Colwell 2001). Rarefaction curves were calculated using the Cole Rarefaction function of Estimate S (Coleman 1981, Coleman et al. 1982) and were used to compare differences among treatments at each site, averaged over months and years.

The Shannon Index

The Shannon Index can be used to estimate the biodiversity of organisms within a system (Krebs 1989) and can be calculated with the following formula:

$$H = -\sum_{i=1}^{S_T} p_i \log_e p_i$$

where p_i = the proportion of individuals in the *i*th species and S_T = the total number of species. The Shannon index was calculated for each repetition and treatment, averaged over months, for each year and site. Shannon index values were low per treatment each month, which precipitated the need to pool index values by repetition each year. The H index values were converted to the actual number of species (e^H) rather than using the logarithmic values derived from the index calculations to facilitate interpretation of the data (Ricklefs 2007). Higher values of "H" indicate greater biodiversity. Statistical computations using the calculated Shannon indices were performed using the "Mixed" procedure in SAS (SAS Institute 2008) with the REML estimation method and the Kenward-Roger approximation for degrees of freedom (Kenward 1997). If any fixed effects within the model were significant ($P \leq 0.05$), least squares means were separated and compared with t-tests.

Similar to the density contrasts, spider diversity between the conventional and crop-intensified rotations was performed using contrasts of treatments with the calculated Shannon index values per year, averaged over months.

Results

Spider density

Yearly density summaries, family distribution, and species collected

A total of 11,207 spiders in 17 families and 119 species were collected. The number of spiders collected from Akron, Briggsdale, and Lamar were 3255, 3381, and 4571, respectively (Table 2.5). Of this, 14.9% were female, 26.7% male, and 58.4% immature. Spider densities were greatest in 2005 and 2006.

TABLE 2.5. SPIDERS COLLECTED AT AKRON, BRIGGSDALE, AND LAMAR, CO, 2002-2007. [1,2]

	Year					
Site	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
Akron	312	369	525	899	1013	137
Briggsdale	371	284	436	1045	1077	168
Lamar	320	292	438	1384	1846	291

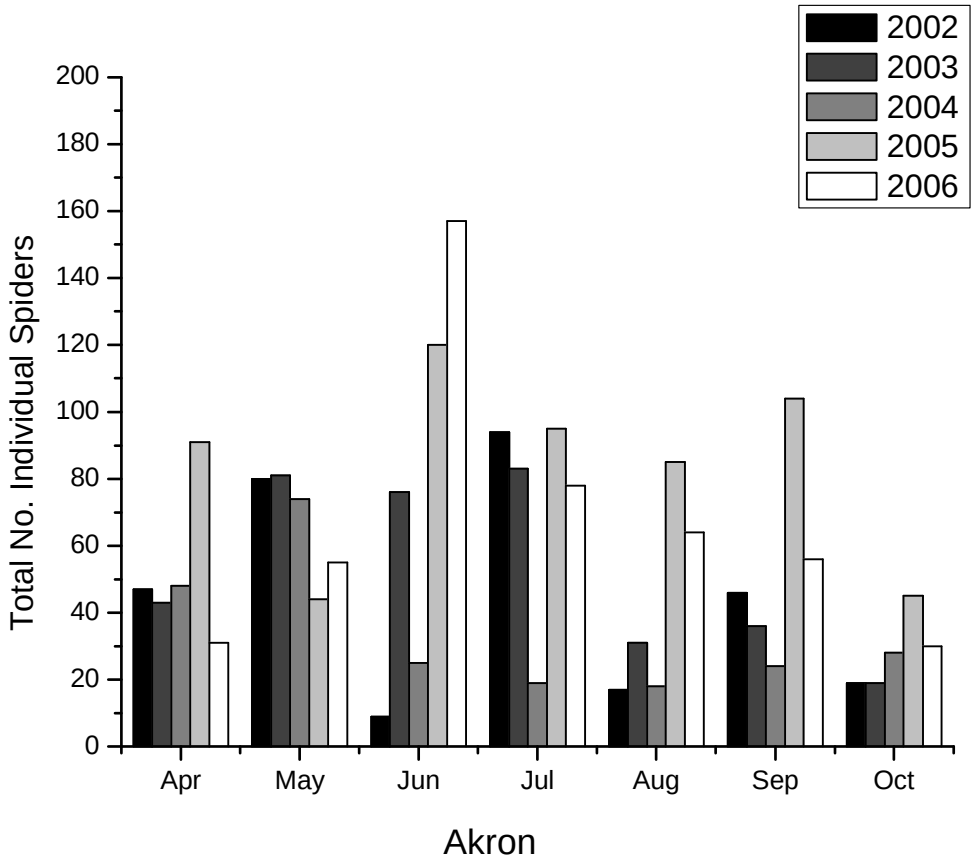
[1] From pitfall, vacuum, and lookdown sampling techniques.
[2] No Sep. pitfalls, Lamar 2004 and May 2006; no Jun-Aug lookdown, Lamar 2004; no vacuum samples, Lamar, May-June, 2007.

Pitfall activity densities by site and year

Akron

With the exception of 2005, spider activity densities gradually increased from April-July and subsequently declined thereafter (Figure 2.2). In 2002, few spiders were present in June. Densities declined again in August-October. Similarly, in 2003, spider densities increased and declined in August-October. In 2004, activity densities were very low for June-October. In 2005, activity densities were high in all months except for a slight decline in May and October. The highest activity densities for each month were maintained in 2005, except for June where 2006 had higher total activity densities. In May 2006, Akron received 10.2 cm of precipitation, which could be correlated with peak spider densities in June. Spider activity densities were higher in June, August, and September in 2005 and 2006.

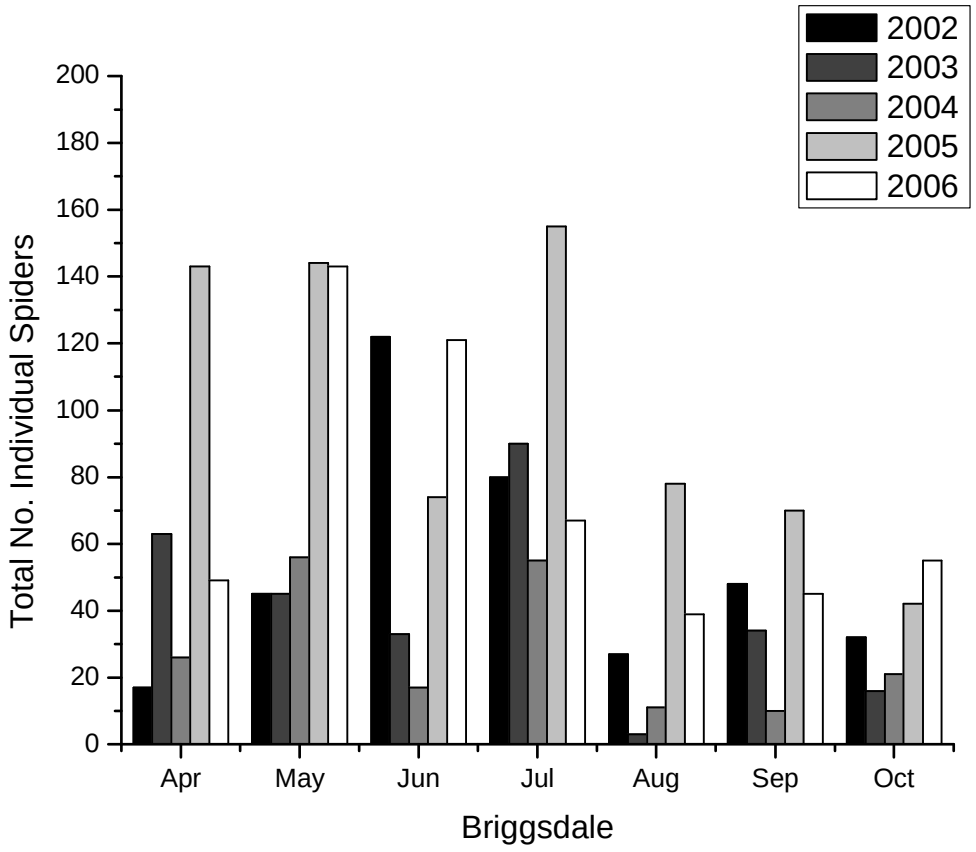
FIGURE 2.2. TOTAL SPIDER ACTIVITY DENSITIES FROM PITFALL SAMPLES AT AKRON, CO, APRIL-OCTOBER, 2002-2006.



Briggsdale

Similar to Akron, spider activity densities peaked in April-July and declined from August-October (Figure 2.3). In 2005, the increased activity densities were maintained for each month except June and October, where the highest spider densities occurred in 2006.

FIGURE 2.3. SPIDER ACTIVITY DENSITIES FROM PITFALL SAMPLES AT BRIGGSDALE, CO, APRIL-OCTOBER, 2002-2006.

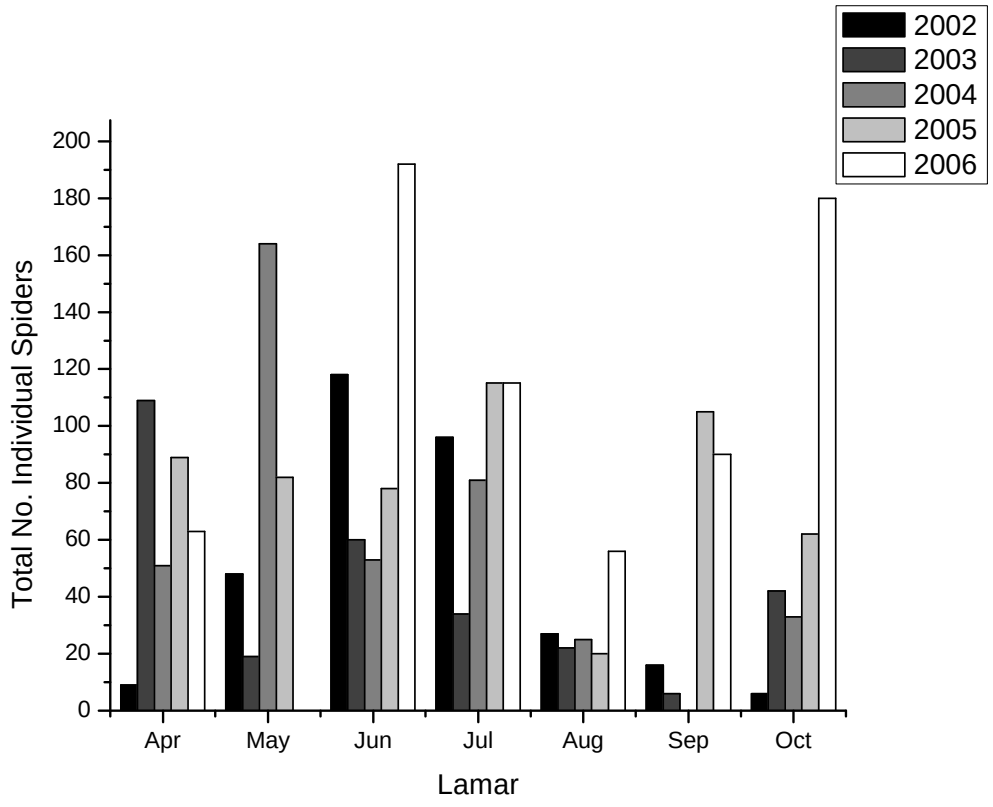


Lamar

Resembling the other two sites, peak activity densities occurred from April-July (Figure 2.4). However, activity densities rose from September-October in 2005 and also rose from August-October in 2006. There was a large peak in spider activity density in May 2004, June and October (actual sampling month was November) 2006. Above-average precipitation was also received at these times (10.2 cm of precipitation in May 2004 and June 2006 and 30.5 cm of precipitation from July-September 2006). Aside

from April 2003 and May 2004, total spider activity densities were low for 2003 and 2004 seasons.

FIGURE 2.4. SPIDER ACTIVITY DENSITIES FROM PITFALL SAMPLES AT LAMAR, CO, APRIL-OCTOBER, 2002-2006. [1]



[1]Spiders were sampled in November instead of October for 2006; no May 2006 samples.

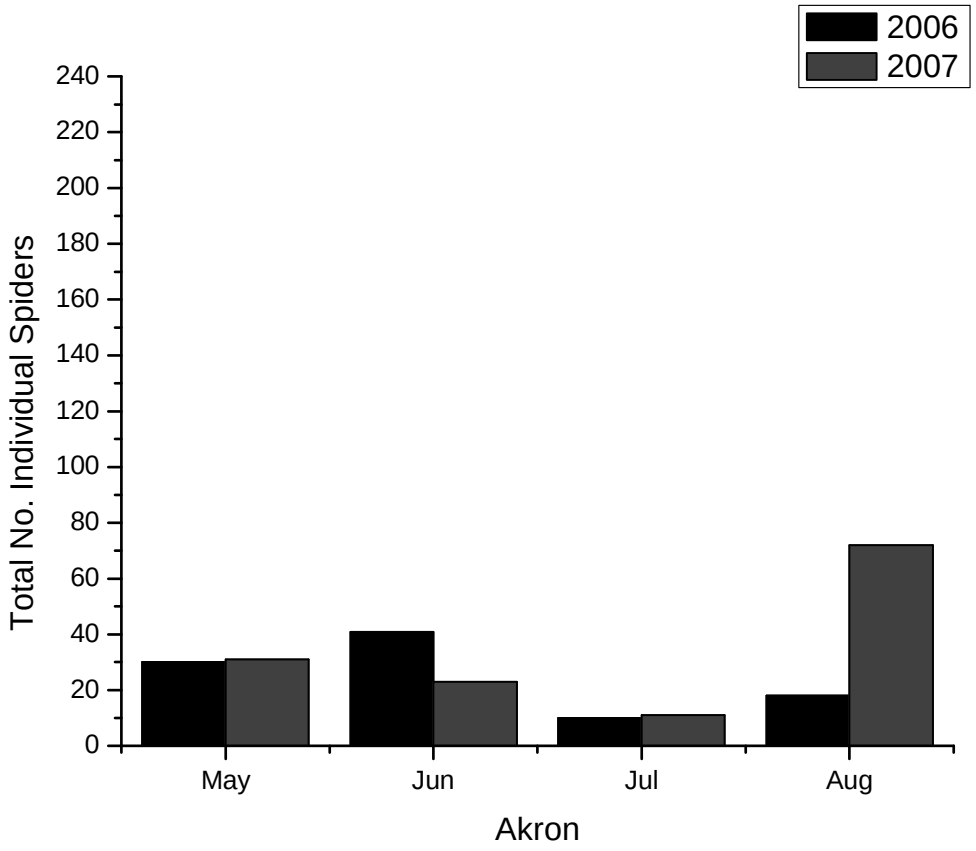
Vacuum densities by site and year

In general, mostly immature spiders (87.3%) were captured with vacuum sampling.

Akron

Spiders densities were highest in June 2006 and August 2007(Figure 2.5). May and July densities were similar between years.

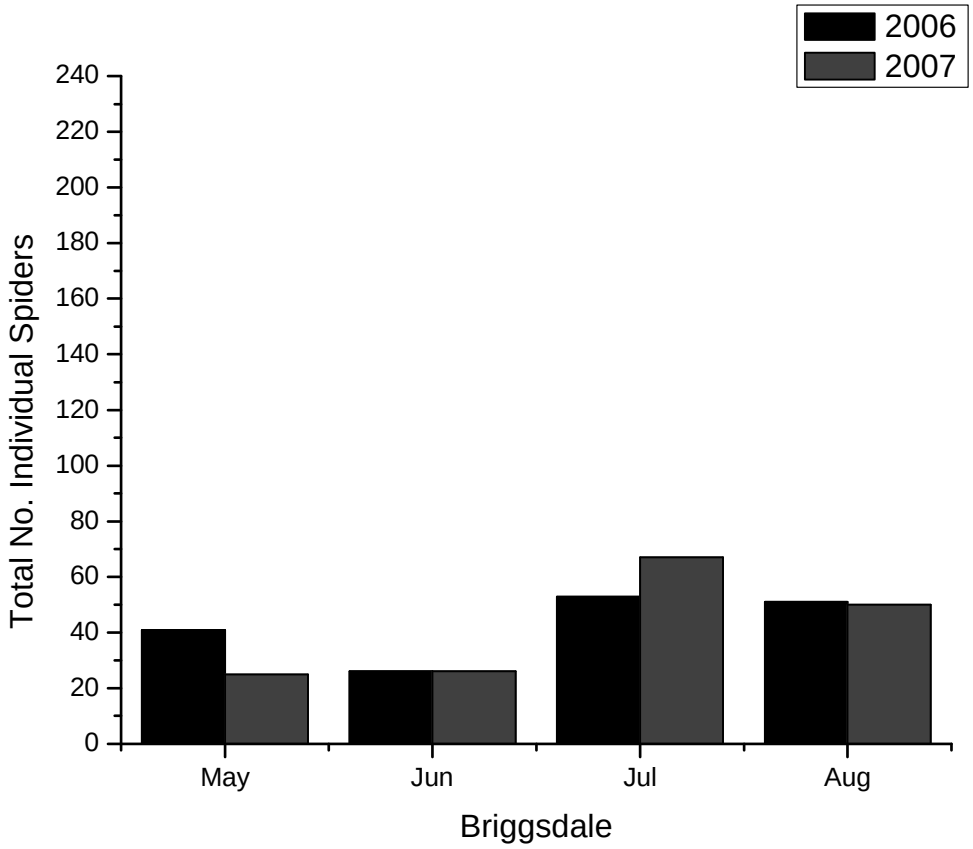
FIGURE 2.5. SPIDER DENSITIES FROM VACUUM SAMPLES AT AKRON, CO, MAY-AUGUST, 2006-2007.



Briggsdale

Densities were highest in July-August for 2006 and 2007 (Figure 2.6). Densities were twice as high in May 2006 than in May 2007. Densities were slightly higher in July 2007 compared with July 2006. June and August maintained similar densities between years.

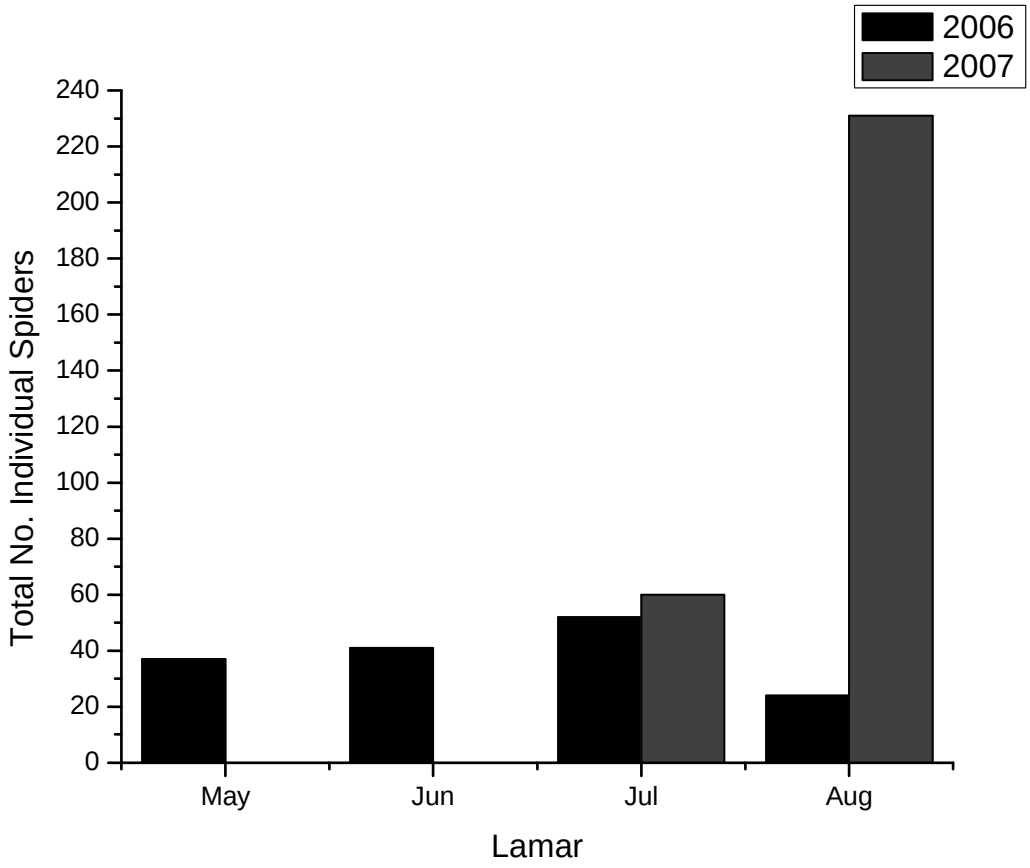
FIGURE 2.6. SPIDER DENSITIES FROM VACUUM SAMPLES AT BRIGGSDALE, CO, MAY-AUGUST, 2006-2007.



Lamar

Spider densities were low. A high density of spiders was collected in August 2007 (Figure 2.7). May and June were not sampled in 2007 due to excessive rain.

FIGURE 2.7. SPIDER DENSITIES FROM VACUUM SAMPLES AT LAMAR, CO, MAY-AUGUST, 2006-2007. [1]



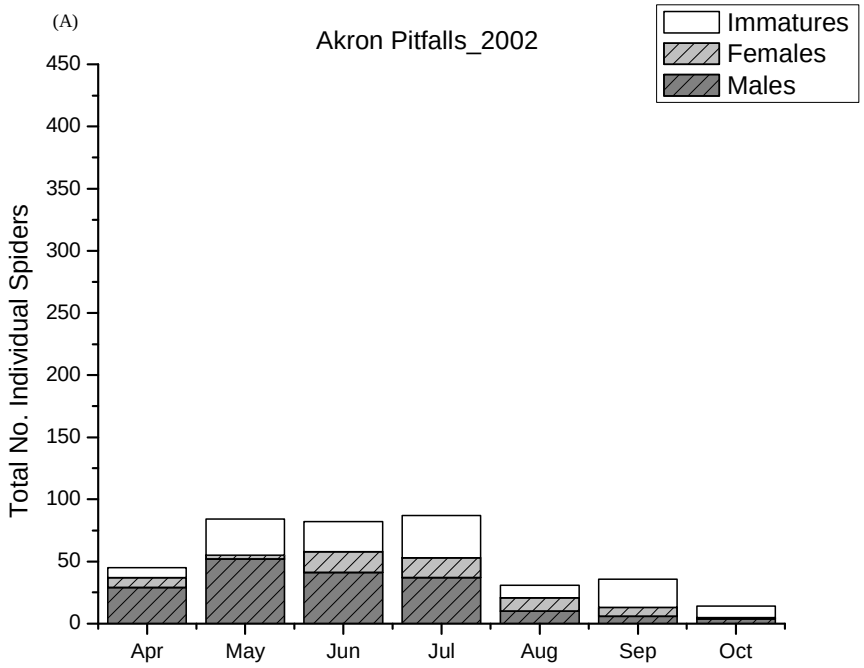
[1]No samples July and August 2007.

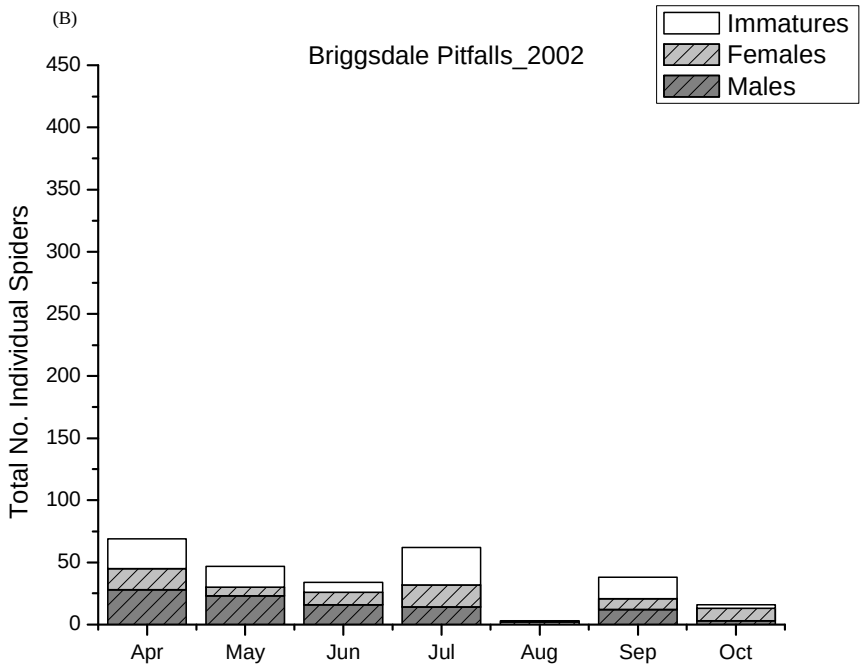
Activity densities pre versus post wheat harvest

Male, female, and immature spider individuals collected per month in pitfall samples are displayed in Figures 2.8a-o. The adult male and female activity densities were highest during April through July, and activity densities declined thereafter. In Lamar 2006, adults demonstrated a peak of spider activity-densities in April through July; however, there was a second peak in the number of adults present in pitfalls from October through November (Figure 2.8o). In Akron 2006, immatures were present in May

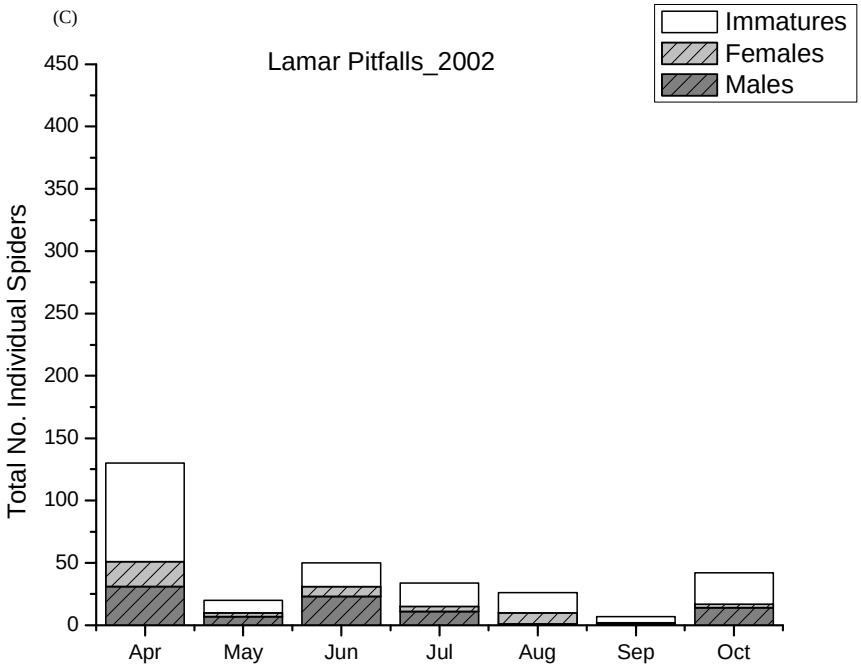
through August (Figure 2.8m). Immatures were present consistently throughout the year at all sites.

FIGURE 2.8. A-O. IMMATURE, FEMALE, AND MALE SPIDERS CAPTURED IN PITFALLS AT AKRON, BRIGGSDALE, AND LAMAR, CO, 2002-2006.

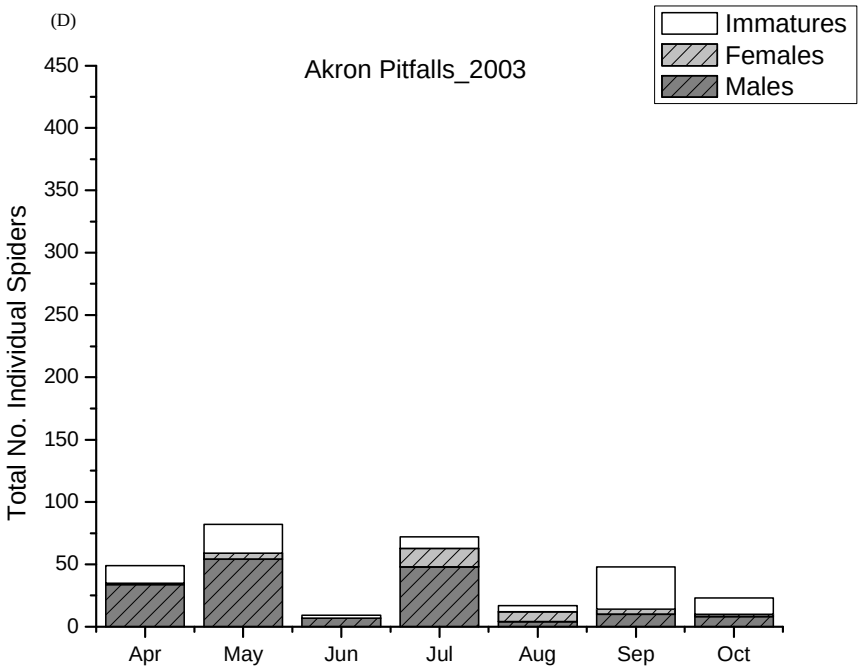




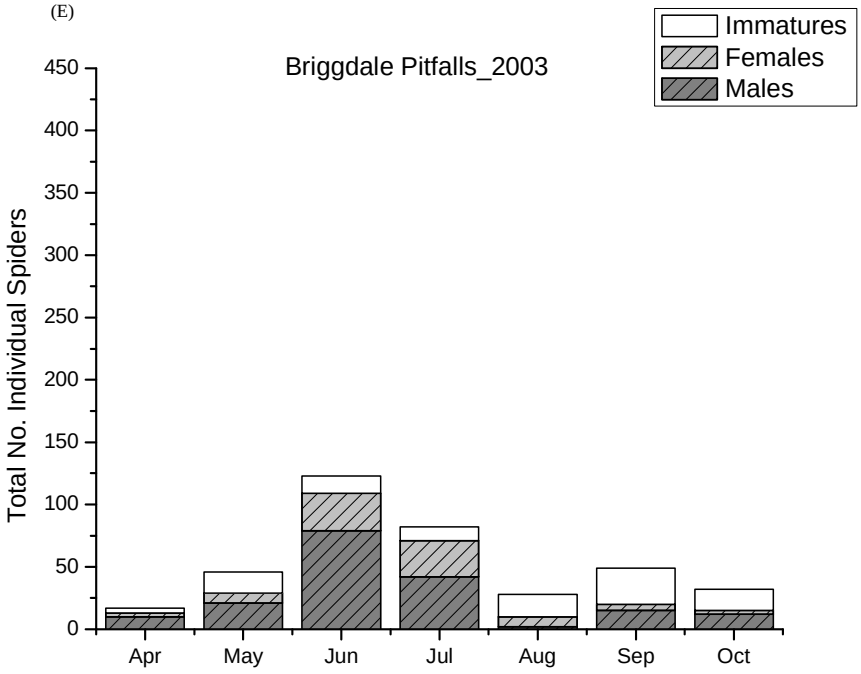
(C)


Lamar Pitfalls_2002
Immatures
Females
Males
Total No. Individual Spiders
0
50
100
150
200
250
300
350
400
450
Apr
May
Jun
Jul
Aug
Sep
Oct

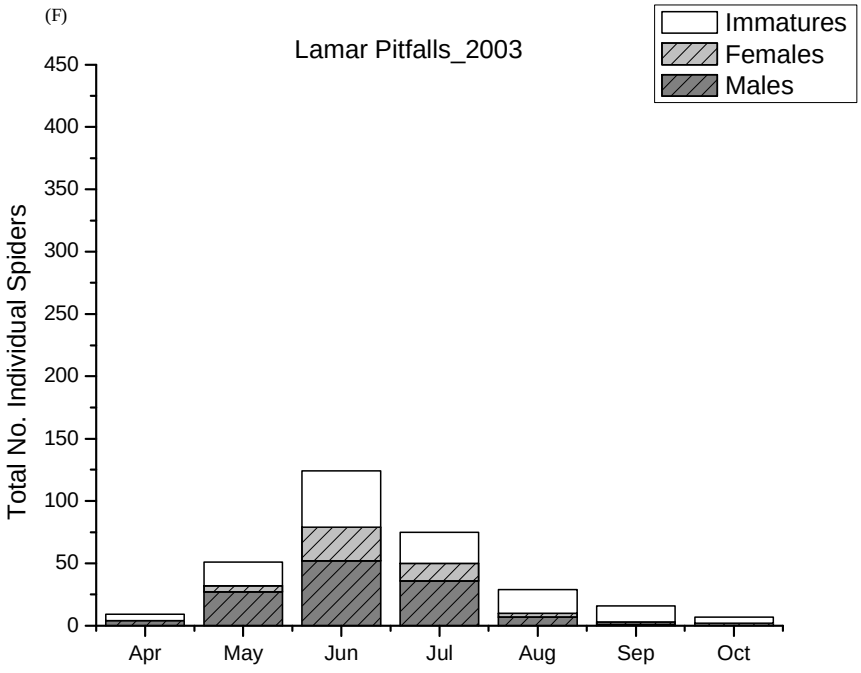
(D)


Akron Pitfalls_2003
Immatures
Females
Males
Total No. Individual Spiders
0
50
100
150
200
250
300
350
400
450
Apr
May
Jun
Jul
Aug
Sep
Oct

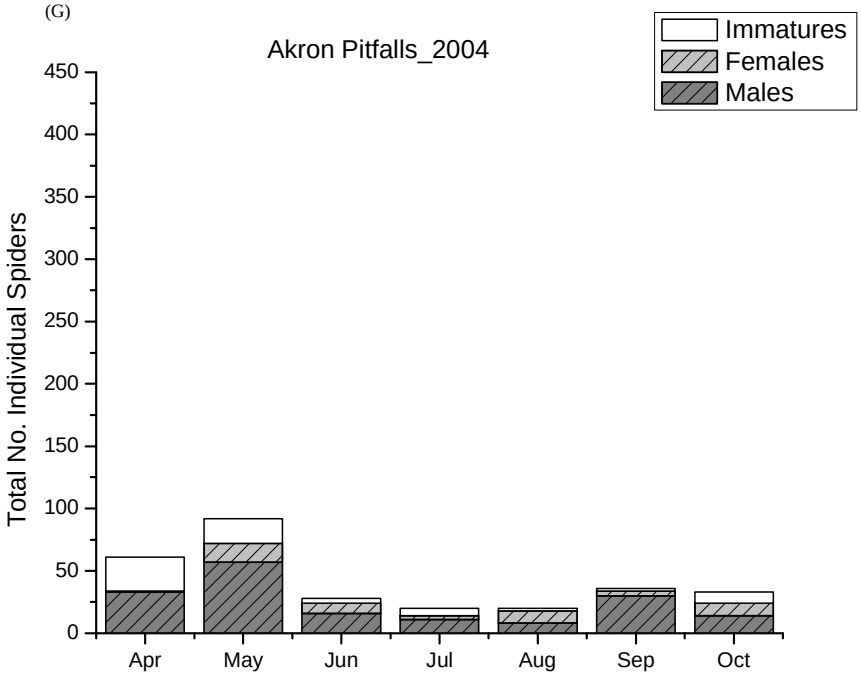
(E)


Briggdale Pitfalls_2003
Immatures
Females
Males
Total No. Individual Spiders
0
50
100
150
200
250
300
350
400
450
Apr
May
Jun
Jul
Aug
Sep
Oct

(F)


Lamar Pitfalls_2003
Immatures
Females
Males
Total No. Individual Spiders
0
50
100
150
200
250
300
350
400
450
Apr
May
Jun
Jul
Aug
Sep
Oct

(G)


Akron Pitfalls_2004
Immatures
Females
Males
Total No. Individual Spiders
0
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100
150
200
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300
350
400
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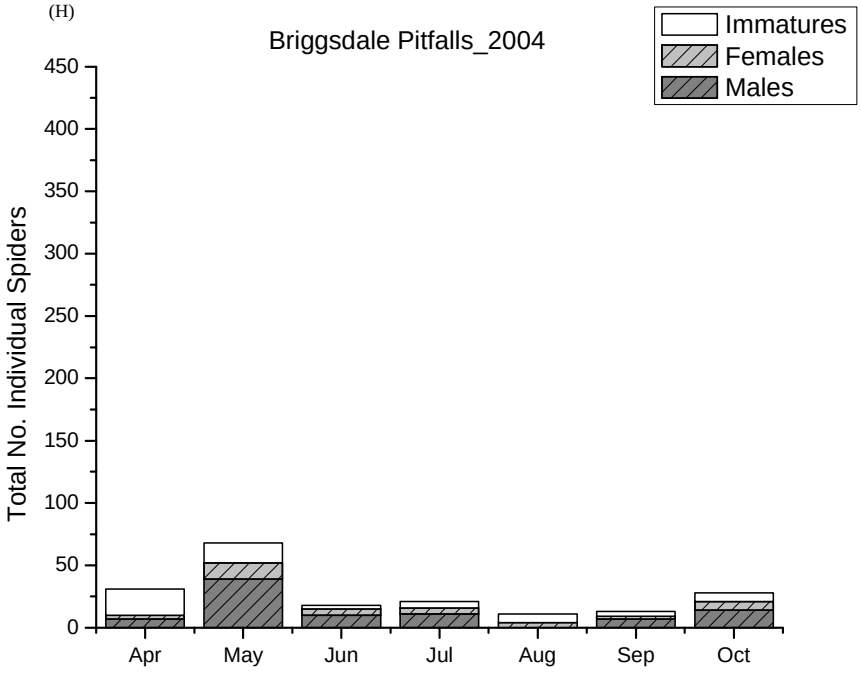
Apr
May
Jun
Jul
Aug
Sep
Oct

(H)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Immatures
Females
Males
Total No. Individual Spiders
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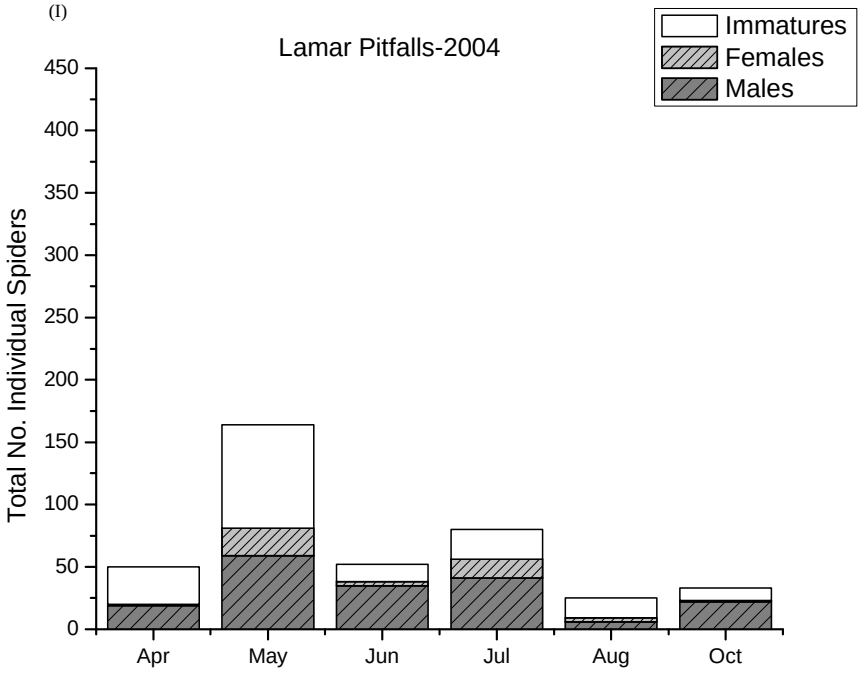
150
200
250
300
350
400
450
Apr
May
Jun
Jul
Aug
Sep
Oct

(I)


Lamar Pitfalls-2004
Immatures
Females
Males
Total No. Individual Spiders
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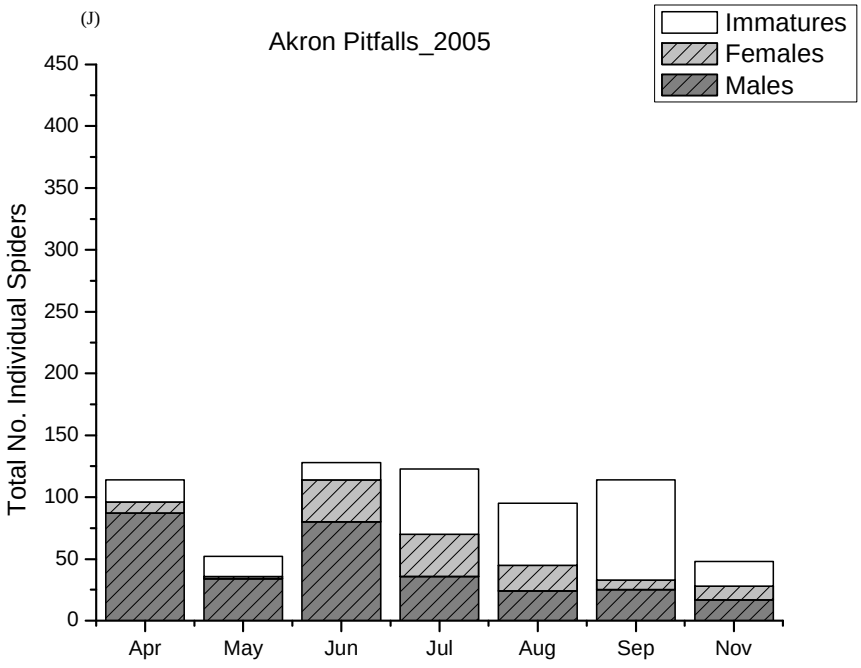
Apr
May
Jun
Jul
Aug
Oct

(J)


Akron Pitfalls_2005
Immatures
Females
Males
Total No. Individual Spiders
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400
450
Apr
May
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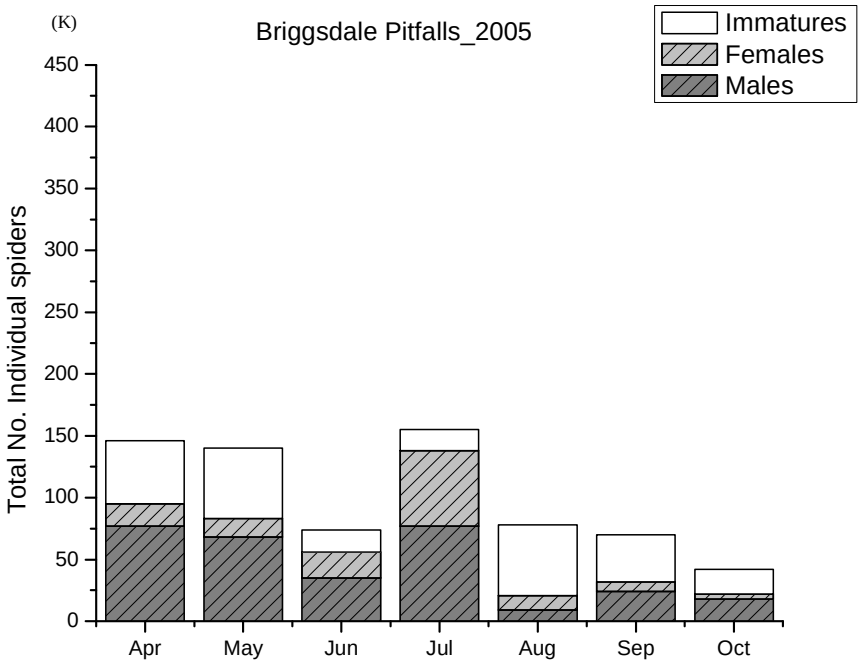
Jul
Aug
Sep
Nov

(K)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Immatures
Females
Males
Total No. Individual spiders
0
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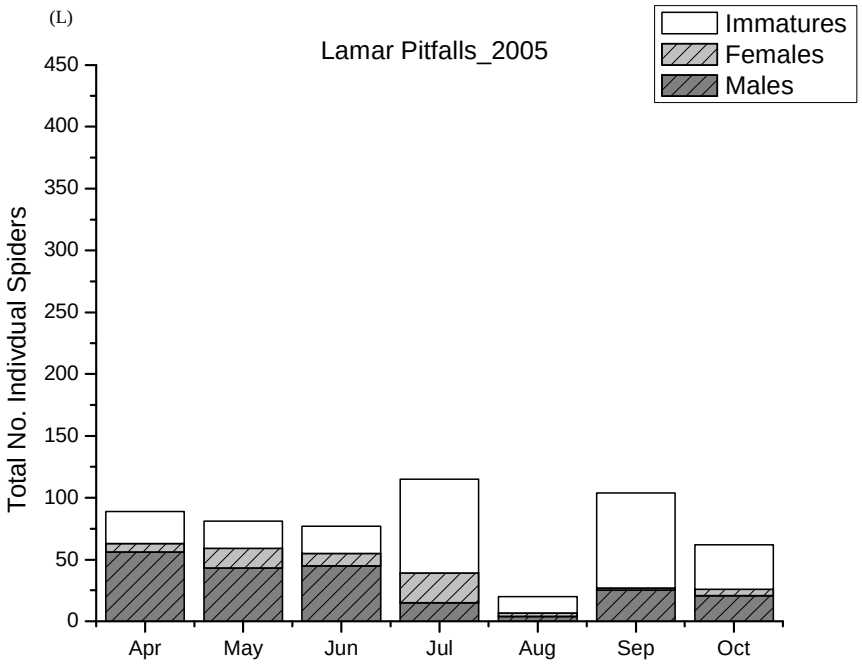
100
150
200
250
300
350
400
450
Apr
May
Jun
Jul
Aug
Sep
Oct

(L)


Lamar Pitfalls_2005
Immatures
Females
Males
Total No. Indivdual Spiders
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100
150
200
250
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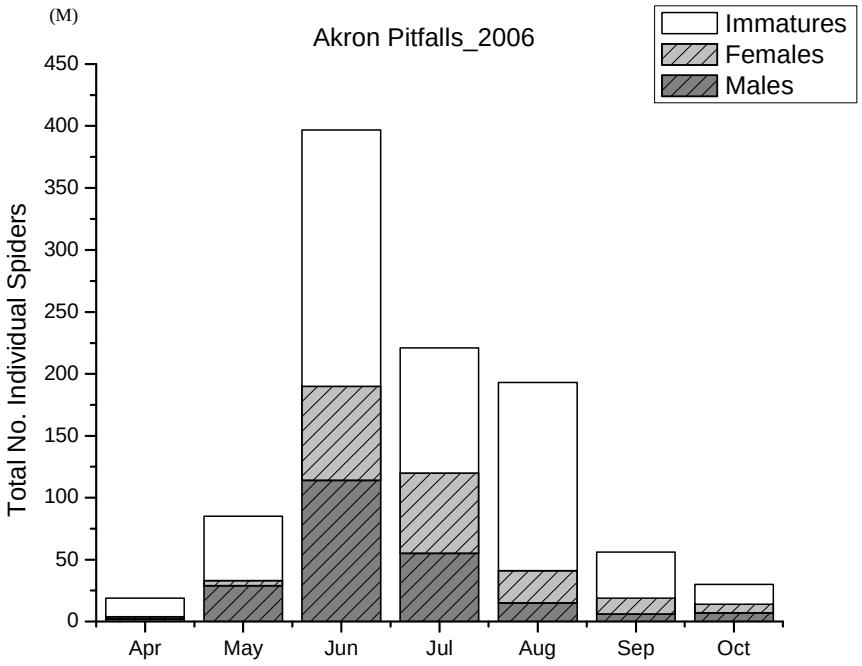
350
400
450
Apr
May
Jun
Jul
Aug
Sep
Oct

(M)


Akron Pitfalls_2006
Immatures
Females
Males
Total No. Individual Spiders
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100
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200
250
300
350
400
450
Apr
May
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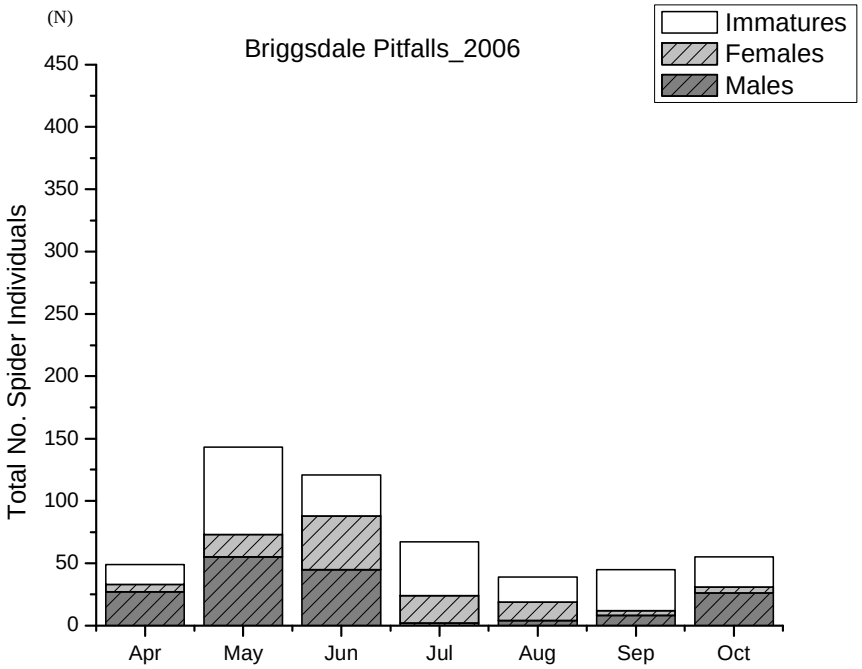
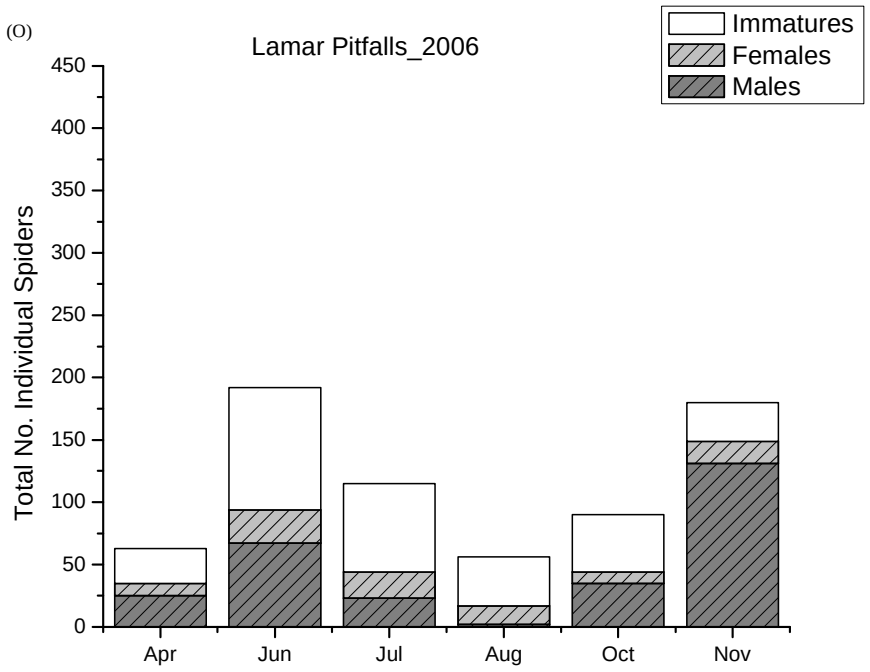
Jul
Aug
Sep
Oct

(N)


Briggsdale Pitfalls_2006
Immatures
Females
Males
Total No. Spider Individuals
0
50
100
150
200
250
300
350
400
450
Apr
May
Jun
Jul
Aug
Sep
Oct



Contrasts showed that spider densities decreased after wheat harvest at all sites and years (Tables 2.6-2.8), except Lamar 2006, where densities were high in both October-November and April-July.

TABLE 2.6. CONTRAST OF DATES COMPARING SPIDER ADULT ACTIVITY DENSITIES PRE AND POST WHEAT HARVEST, AKRON, CO, PITFALLS, 2002-2006. [1]

Year	Effect	df	*t*	*P*-value
2002	Pre (April-July) vs. Post Harvest (August-October)	105	4.46	<0.0001
2003	Pre (April-July) vs. Post Harvest (August-October)	110	5.30	<0.0001
2004	Pre (April-July) vs. Post Harvest (August-October)	90	2.16	0.0337
2005	Pre (April-July) vs. Post Harvest (August-October)	90	5.01	<0.0001
2006	Pre (April-July) vs. Post Harvest (August-October)	90	5.59	<0.0001

[1]Dates averaged over treatments.

TABLE 2.7. CONTRAST OF DATES COMPARING SPIDER ADULT ACTIVITY DENSITIES PRE AND POST WHEAT HARVEST, BRIGGSDALE, CO, PITFALLS, 2002-2006. [1]

Year	Effect	df	*t*	*P*-value
2002	Pre (April-July) vs. Post Harvest (August-October)	95	3.27	0.0015
2003	Pre (April-July) vs. Post Harvest (August-October)	90	6.77	<0.0001
2004	Pre (April-July) vs. Post Harvest (August-October)	90	2.84	0.0055
2005	Pre (April-July) vs. Post Harvest (August-October)	90	8.80	<0.0001
2006	Pre (April-July) vs. Post Harvest (August-October)	90	6.13	<0.0001

[1]Dates averaged over treatments.

TABLE 2.8. CONTRAST OF DATES COMPARING SPIDER ADULT ACTIVITY DENSITIES PRE AND POST WHEAT HARVEST, LAMAR, CO, PITFALLS, 2002-2006. [1,2]

Year	Effect	df	*t*	*P*-value
2002	Pre (April-July) vs. Post Harvest (August-October)	90	4.80	<0.0001
2003	Pre (April-July) vs. Post Harvest (August-October)	89	7.26	<0.0001
2004[2]	Pre (April-July) vs. Post Harvest (August-October)	75	5.47	<0.0001
2005	Pre (April-July) vs. Post Harvest (August-October)	90	5.96	<0.0001
2006[2]	Pre (April-July) vs. Post Harvest (August-October)	75	-0.19	0.8505

[1]Dates averaged over treatments.
[2]No September data, 2004; no May data, 2006.

Family distribution and species list-all sampling techniques

For Akron from 2002-2007, Lycosidae represented 41% of all spiders collected, followed by Gnaphosidae (31%), Thomisidae (9%), Philodromidae (5%), Linyphiidae (5%), and Salticidae (4%) (Table 2.9). Agelenidae, Araneidae, Clubionidae, Corinnidae, Dictynidae, Tetragnathidae, Theridiidae, and Titanoecidae were collected less frequently. At Briggsdale, Gnaphosidae (36%), Lycosidae (30%), Linyphiidae (10%), Thomisidae (9%), Salticidae (6%), Philodromidae (5%) were the most abundant families (Table 2.10). Agelenidae, Araneidae, Clubionidae, Corinnidae, Dictynidae, Pholcidae, Tetragnathidae, Theridiidae, and Titanoecidae were less common. Lamar was dominated by Lycosidae (61%) and Gnaphosidae (25%) (Table 2.11). The families Agelenidae, Araneidae, Clubionidae, Corinnidae, Dictynidae, Linyphiidae, Philodromidae, Pholcidae, Salticidae, Tetragnathidae, Theridiidae, Thomisidae, and Titanoecidae were collected less frequently.

TABLE 2.9. MONTHLY SPIDER COLLECTIONS BY FAMILY AT AKRON, CO, APRIL-OCTOBER, 2002-2007. [1,2]

Month	Family													
	Age	**Ara**	**Clu**	**Cor**	**Dic**	**Gna**	**Lin**	**Lyc**	**Phi**	**Sal**	**Tet**	**The**	**Tho**	**Tit**
April				1	8	107	15	65	20	4		6	49	
May		4			13	195	21	190	24	5	3	6	73	
June	1	5	5	1	29	323	54	321	42	29		9	45	2
July	2	24	5	9	9	124	29	291	44	46		8	50	23
August		3	6	3	18	154	43	365	46	34		9	30	
September	2		1		1	100	11	95	4	17		3	56	
October		1		1	3	28	5	89	1	3			18	
Total	5	37	17	15	81	1031	178	1416	181	138	3	41	321	25
Percentage (%)	0	1	0	0	2	30	5	41	5	4	0	1	9	1

[1]Age=Agelenidae, Ara=Araneidae, Clu=Clubionidae, Cor=Corinnidae, Dic=Dictynidae, Gna=Gnaphosidae, Lin=Linyphiidae, Lyc=Lycosidae, Phi=Philodromidae, Sal=Salticidae, Tet=Tetragnathidae, The=Theridiidae, Tho=Thomisidae, Tit=Titanoecidae.
[2]Adults and immatures collected from all sampling techniques.

TABLE 2.10. MONTHLY SPIDER COLLECTIONS BY FAMILY AT BRIGGSDALE, CO, APRIL-OCTOBER, 2002-2007. [1,2]

Month	Family														
	Age	**Ara**	**Clu**	**Cor**	**Dic**	**Gna**	**Lin**	**Lyc**	**Phi**	**Pho**	**Sal**	**Tet**	**The**	**Tho**	**Tit**
April			1			115	65	41	30		9	1	2	30	
May		1			25	258	61	90	27		37	1	2	55	
June	1	4	1		29	306	60	152	34	1	44	5	4	20	
July	2	7	1	2	17	212	53	317	32	3	61		4	84	2
August	1	1			17	108	68	245	22		19		5	30	
September	1				2	86	10	52	4	1	6		7	42	
October		1	3	2	1	79	4	50	2	2	2	1		26	
Total	5	14	6	4	91	1164	321	947	151	7	178	8	24	287	2
Percentage (%)	0	0	0	0	3	36	10	30	5	0	6	0	1	9	0

[1]Age=Agelenidae, Ara=Araneidae, Clu=Clubionidae, Cor=Corinnidae, Dic=Dictynidae, Gna=Gnaphosidae, Lin=Linyphiidae, Lyc=Lycosidae, Phi=Philodromidae, Pho=Pholcidae, Sal=Salticidae, Tet=Tetragnathidae, The=Theridiidae, Tho=Thomisidae, Tit=Titanoecidae.
[2]Adults and immatures collected from all sampling techniques.

TABLE 2.11. MONTHLY SPIDER COLLECTIONS BY FAMILY AT LAMAR, CO, APRIL-OCTOBER, 2002-2007. [1,2]

Month	Family													
	Age	**Ara**	**Clu**	**Cor**	**Dic**	**Gna**	**Lin**	**Lyc**	**Phi**	**Pho**	**Sal**	**The**	**Tho**	**Tit**
April					16	168	21	78	11		11	9	28	
May		2			6	251	18	105	8		2	9	46	
June	2	6	2		19	257	23	676	17	1	24	11	31	3
July	2				16	168	18	1131	36		25	17	16	
August		2		3	16	85	31	572	35		24	10	10	
September	4		1	1	1	66	7	109	4		6	1	29	
October					5	157	2	132	6		1	1	8	
Total	8	10	3	4	79	1152	120	2803	117	1	93	58	168	3
Percentage (%)	0	0	0	0	2	25	3	61	3	0	2	1	4	0

[1]Age=Agelenidae, Ara=Araneidae, Clu=Clubionidae, Cor=Corinnidae, Dic=Dictynidae, Gna=Gnaphosidae, Lin=Linyphiidae, Lyc=Lycosidae, Phi=Philodromidae, Pho=Pholcidae, Sal=Salticidae, Tet=Tetragnathidae, The=Theridiidae, Tho=Thomisidae, Tit=Titanoecidae.
[2]Adults and immatures collected from all sampling techniques.

Cumulatively from 2002-2007, 77, 78, and 67 species were collected in Akron, Briggsdale, Lamar, respectively (Table 2.12). Thirty-two species were common to all sites, and 16, 12, and 19 species were unique to Akron, Briggsdale, and Lamar, respectively. Twenty-four species were common to Akron and Briggsdale but not Lamar, five were common to Akron and Lamar but not Briggsdale, and ten were common to Briggsdale and Lamar but not Akron. The geographic proximity and agroecological similarity of Briggsdale and Akron may explain why more species were common between these two sites. Overall, Lycosidae and Gnaphosidae dominated the number of individuals collected (Lycosidae 45.0%, Gnaphosidae 29.0%), with the families Thomisidae (6.9%), Linyphiidae (6.1%), Philodromidae (4.3%), and Salticidae (3.7%) representing most of the remaining individuals collected. The number of species contained within a family was dominated by Gnaphosidae (20.2%) and Linyphiidae (14.2%), with Dictynidae (12.6%), Salticidae (10.1%), Lycosidae (9.2%), Thomisidae (8.4%), Philodromidae (8.4%), and Theridiidae (7.6%) representing some of the remaining species. Six families-Lycosidae, Gnaphosidae, Thomisidae, Linyphiidae, Philodromidae, and Salticidae-represented over 75% of the total species for all sites.

TABLE 2.12. SPIDERS COLLECTED AT AKRON (A), BRIGGSDALE (B), AND LAMAR (L), CO, 2002-2007.[1]

Family	Species	A	B	L
Agelenidae	*Agelenopsis aleenae* Chamberlin & Ivie			4
	Agelenopsis oklahoma (Gertsch)	2	2	
Araneidae	*Larinia borealis* Banks	2	4	
Clubionidae	*Clubiona pikei* Gertsch	1		
Corinnidae	*Castianeira alteranda* Gertsch	2		
	Castianeira amoena (Koch)			1
	Castianeira descripta (Hentz)	23	2	
	Phurotimpus certus Gertsch	4		
Dictynidae	*Cicurina* sp. 1			94
	Cicurina sp. 2	5	7	3
	Cicurina sp. 3			1
	Cicurina sp. 4		2	
	Dictyna coloradensis Chamberlin	2	3	

Family	Species	A	B	L
	Dictyna personata Gertsch & Mulaik		9	18
	Dictyna terrestris Emerton	10	33	1
	Dictyna sp.	1	2	
	Emblyna consulta (Gertsch & Ivie)	5	2	
	Emblyna reticulata (Gertsch & Ivie)	4		10
	Emblyna scotta Chamberlin		1	
	Iviella sp. 1	16	1	9
	Iviella sp. 2			8
	Phantyna bicornis (Emerton)	1	13	
	Tricholathys sp.	1		
Gnaphosidae	*Drassodes gosiutus* Chamberlin	1		12
	Drassodes neglectus (Keyserling)	1		
	Drassodes saccatus (Emerton)		1	5
	Drassyllus depressus (Emerton)	14	3	
	Drassyllus lamprus (Chamberlin)	2	3	
	Drassyllus lepidus (Banks)	5		13
	Drassyllus nannellus Chamberlin & Gertsch	137	92	8
	Drassyllus notonus Chamberlin	22	25	65
	Gnaphosa clara (Keyserling)	155	322	
	Gnaphosa parvula Banks	1		
	Gnaphosa saxosa Platnick & Shadab			399
	Gnaphosa sericata (L. Koch)	30	23	
	Haplodrassus chamberlini Platnick & Shadab	61	93	44
	Haplodrassus signifer (C. L. Koch)	8	21	7
	Micaria gertschi Barrows & Ivie	1		
	Micaria gosiuta Gertsch		1	
	Micaria longipes Emerton		2	
	Micaria medica Platnick & Shadab		3	
	Zelotes anglo Gertsch & Riechert	59	36	9
	Zelotes gertschi Platnick & Shadab	33	39	1
	Zelotes hentzi Barrows	4		
	Zelotes lasalanus Chamberlin	23	57	1
	Zelotes nannodes Chamberlin			2
	Zelotes puritanus Chamberlin	18	41	17
Linyphiidae	*Agyneta cf. unimaculata* (Banks)	3		
	Agyneta uta (Chamberlin)		1	
	Agyneta/Meioneta sp. 1	26	15	41
	Agyneta/Meioneta sp. 2		1	1
	Agyneta sp. 3		6	
	Ceratinella brunnea Emerton	28	1	2
	Ceratinops latus (Emerton)		1	
	Coloncus siou Chamberlin	19	2	
	Erigone aletris Crosby & Bishop	1	40	
	Erigone barrows Crosby & Bishop			2
	Grammonota suspiciosa Gertsch & Mulaik			1
	Islandiana flaveola (Banks)	27	10	1
	Islandiana princeps Braendegaard	2		1
	Mythoplastoides exiguus (Banks)	14	1	
	Tennesseellum formicum (Emerton)	38	75	21
	Walckenaeria maesta Millidge			1
	Walckenaeria spiralis (Emerton)	1		
Lycosidae	*Alopecosa kochi* (Keyserling)	50	18	7
	Arctosa rubicunda (Keyserling)	34	2	
	Geolycosa missouriensis (Banks)		1	4

Family	Species	A	B	L
	Geolycosa rafaelana (Chamberlin)			2
	Hogna antelucana (Montgomery)			31
	Hogna coloradensis (Banks)		28	180
	Hogna frondicola (Emerton)	1		
	Schizocosa crassipalpata Roewer	10		
	Schizocosa mccooki (Montgomery)	324	165	186
	Schizocosa minnesotensis (Gertsch)	15	4	
	Varacosa gosiuta (Chamberlin)	2	6	
Philodromidae	*Ebo iviei* Sauer & Platnick		1	
	Ebo parabolis Schick		1	4
	Ebo pepinensis Gertsch		2	10
	Thanatus altimontis Gertsch	1	10	1
	Thanatus coloradensis Keyserling	1	6	
	Thanatus formicinus (Clerck)	21	39	7
	Thanatus rubicellus Mello-Leitão			1
	Tibellus chamberlini Gertsch	2		1
	Tibellus duttoni (Hentz)	33	11	2
	Tibellus oblongus (Walckenaer)	3	1	
Pholcidae	*Psilochorus imitatus* Gertsch & Mulaik	1	6	3
Salticidae	*Habronattus altanus* (Gertsch)	49	31	16
	Habronattus conjunctus (Banks)	15		
	Habronattus cuspidatus Griswold	19	2	1
	Habronattus klauseri (Peckham & Peckham)		53	20
	Pellenes crandalli Lowrie & Gertsch			12
	Pellenes levii Lowrie & Gertsch			2
	Phidippus apacheanus Chamberlin & Gertsch	4	1	
	Phidippus ardens Peckham & Peckham		1	2
	Sassacus papenhoei Peckham & Peckham	2		
	Sitticus dorsatus (Banks)	3	4	
	Synageles occidentalis Cutler		2	
	Talavera minuta (Banks)	9	4	
Tetragnathidae	*Tetragnatha laboriosa* Hentz	1	1	1
Theridiidae	*Enoplognatha joshua* Chamberlin & Ivie	1	6	9
	Euryopis texana Banks	3	45	
	Latrodectus hesperus Chamberlin & Ivie		2	3
	Robertus sp.			2
	Steatoda albomaculata (De Geer)	19	8	24
	Steatoda americana (Emerton)	7	1	
	Steatoda medialis (Banks)			1
	Theridion petraeum Koch			1
	Theridion rabuni Chamberlin & Ivie	6	14	8
Thomisidae	*Misumenops celer* (Hentz)	3		
	Xysticus auctificus Keyserling			2
	Xysticus coloradensis Bryant		7	
	Xysticus cunctator Thorell	5	14	3
	Xysticus ferox (Hentz)	1		
	Xysticus lassanus Chamberlin	9	20	6
	Xysticus nigromaculatus Keyserling		1	
	Xysticus orizaba Banks	114	26	22
	Xysticus pellax O.P.-Cambridge	47	65	6
	Xysticus texanus Banks	17	29	13
Titanoecidae	*Titanoeca nigrella* (Chamberlin)	42	4	7

[1]Only 15 of 17 families are shown because two families, Mimetidae and Miturgidae, were collected in the immature stages and could not be identified further than family.

Mean Spider Densities-Akron, Briggsdale, and Lamar 2002-2007

Akron Pitfall Captures 2002-2006

2002

Mean spider activity densities for pitfall captures were low for all sampling months in Akron 2002 (Table 2.13). Spider activity density was affected by month ($F_{6,84}$=5.62, P<0.0001). There was a treatment by month interaction for mean number of spiders captured in pitfalls ($F_{22,84}$=1.79, P=0.0310). In April and October, spider activity densities were highest in wheat in the crop-intensified rotation. In May, spider activity densities were highest in fallow grown in the crop-intensified rotation. In June-September, spider activity densities were highest in corn grown in the crop-intensified rotations.

TABLE 2.13. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT AKRON, CO, 2002. [1,2,3,4,5]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**[4]	**Oct**[4]
Wheat	Conv.	0.9**ABab**	0.8**Bb**	2.3**ABa**	1.1**Aab**	0.3**Ab**	0.9**ABab**	0.4**Ab**
Fallow	Conv.	1.4**ABab**	3.1**Aa**	1.3**ABb**	1.5**Aab**	0.3**Ab**	0.0**Bc**	
Wheat	Intens.	1.9**Aab**	2.4**Aa**	1.1**Bab**	1.8**Aab**	0.6**Ab**	1.1**ABab**	1.3**Aab**
Corn	Intens.	0.9**ABb**	0.9**Bb**	2.9**Aa**	3.0**Aa**	1.1**Aab**	1.5**ABab**	0.5**Ab**
Fallow	Intens.	0.1**Bb**	1.9**ABa**	1.5**ABa**	1.3**Aab**	0.8**Aab**		

[1]Significant differences have been determined through square-root transformation of the data- raw means are represented in this table.
[2]Means within a column followed by the same capital letters are not significantly different (α = 0.05; PROC MIXED) and represent differences between treatments at each date. Means within rows (months) followed by the same lower case letters are not significantly different (α = 0.05; PROC MIXED). and represent differences between treatments at each month.
[3]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[4]No spiders collected.
[5]Conv. =conventional, intens. =crop intensified.

2003

Month ($F_{6,109}$=9.20, P<0.0001) and treatment ($F_{4,12}$=3.71, P=0.0346) affected spider activity density (Table 2.14). Spider activity densities were highest in April, May, and September and lower in June, August, and October. Activity densities were highest in the wheat in the crop-intensified treatment all months except April and September where wheat in the conventional rotation treatment and fallow in the crop-intensified rotation treatment were highest in April and September, respectively.

TABLE 2.14. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT AKRON, CO, 2003. [1,2,3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep[4]**	**Oct**
Wheat	Conv.	1.6	1.4	0.4	1.5	0.5	0.8	0.4
Fallow	Conv.	1.0	1.3	0.4	0.4	0.3		0.1
Wheat	Intens.	1.1	2.5	0.4	3.1	0.5	1.0	1.3
Corn	Intens.	0.4	1.1	0.0	1.4	0.4	1.0	0.3
Fallow	Intens.	1.5	1.5	0.1	1.8	0.1	1.9	0.3

[1]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[2]No spiders collected in fallow in September.
[3]Conv. =conventional, intens. =crop intensified.

2004

There was no treatment effect ($F_{4,12}$=2.79, P=0.0754) for spider activity density in the 2004 Akron pitfall captures (Table 2.15). Spider activity density was affected by month ($F_{6,114}$=8.37, P<0.0001). Activity densities were highest in April and May and declined thereafter.

TABLE 2.15. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT AKRON, CO, 2004. [1,2,3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	1.3	1.3	1.3	0.4	0.4	0.1	0.8
Fallow	Conv.	1.4	1.4	0.9	0.2	0.6	0.6	1.4
Wheat	Intens.	1.0	2.5	0.5	0.1	0.5	1.1	0.5
Corn	Intens.	1.8	2.3	0.1	1.5	0.6	0.9	0.6
Fallow	Intens.	0.6	1.9	0.4	0.1	0.1	0.3	0.3

[1]Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

2005

Spider activity density was affected by month ($F_{6,114}$=4.51, P=0.0004) and treatment ($F_{4,12}$=7.73, P=0.0025) (Table 2.16). Spider activity densities were relatively high each month. Spider activity density was lowest in May and October for all treatments and higher in the remaining months. Activity densities were highest within the wheat in the conventional rotation treatment, with the exception of May, July, and October where activity densities were highest within the wheat in the crop-intensified treatment.

TABLE 2.16. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT AKRON, CO, 2005. [1,2,3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	3.0	1.0	6.0	2.0	4.5	3.8	1.3
Fallow	Conv.	2.1	1.0	2.1	2.0	0.3	0.5	1.0
Wheat	Intens.	2.5	1.4	3.8	3.4	2.8	3.6	1.8
Millet	Intens.	2.5	1.0	2.1	3.0	1.6	3.3	0.5
Fallow	Intens.	1.3	1.1	1.0	1.5	1.5	1.9	1.1

[1]Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

2006

There was no treatment effect ($F_{4,12}$=0.97, P=0.4578) for spider activity density in the 2006 Akron pitfall captures (Table 2.17). Spider activity density was affected by month for Akron pitfall traps in 2006 ($F_{6,114}$=12.28, P<0.0001). Densities were higher compared to previous years each month. Activity densities were greatest in June for all treatments, except for fallow in the crop-intensified treatment where densities were greatest in July. The densities were lowest for the two wheat and two fallow treatments in April and October, respectively, and for millet in July.

TABLE 2.17. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT AKRON, CO, 2006. [1,2,3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	0.3	0.5	6.0	1.9	0.9	2.0	0.6
Fallow	Conv.	1.0	1.0	4.0	2.8	1.4	1.0	0.8
Wheat	Intens.	0.3	1.8	3.6	1.3	1.1	0.8	0.8
Millet	Intens.	1.5	1.5	3.5	1.0	2.4	1.4	1.3
Fallow	Intens.	0.9	2.1	2.5	2.9	2.3	1.9	0.4

[1]Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

Akron Vacuum Captures 2006-2007

2006

Densities were low each month in 2006 for Akron vacuum samples (Table 2.18). There was no treatment effect ($F_{4,8}$=0.60, P=0.6751). Spider density was affected by month ($F_{3,42}$=4.35, P=0.0093). Densities were highest in May and June for all treatments.

TABLE 2.18. MEAN NO. SPIDERS CAPTURED WITH VACUUM SAMPLES AT AKRON, CO, 2006. [1,2,3]

		Month			
Crop	**Rotation**	**May**	**Jun**	**Jul**	**Aug**
Wheat	Conv.	0.3	2.8	0.2	0.8
Fallow	Conv.	1.5	1.2	0.3	0.5
Wheat	Intens.	0.7	1.8	0.5	0.8
Millet	Intens.	1.7	0.7	0.3	0.3
Fallow	Intens.	0.8	0.3	0.3	0.5

[1]Raw means are represented in this table.
[2]Vacuum means represent an average of two samples per plot, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.
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2007

Spider density was affected by month ($F_{3,42}$=3.65, P=0.0199) and treatment ($F_{4,8}$=4.86, P=0.0277) (Table 2.19). Densities were highest in August for the two wheat treatments and millet in a crop-intensified rotation and highest in May for the other two treatments. Densities were highest in wheat in the conventional rotation treatment in May and July and wheat in the crop-intensified treatment in June and August.

TABLE 2.19. MEAN NO. SPIDERS CAPTURED WITH VACUUM SAMPLES AT AKRON, CO, 2007. [1,2,3]

		Month			
Crop	**Rotation**	**May**	**Jun**	**Jul**	**Aug**
Wheat	Conv.	2.2	1.3	0.7	4.5
Fallow	Conv.	0.3	0.0	0.2	0.2
Wheat	Intens.	0.8	1.5	0.7	5.2
Millet	Intens.	0.8	0.8	0.2	1.5
Fallow	Intens.	1.0	0.2	0.2	0.7

[1]Raw means are represented in this table.
[2]Vacuum means represent an average of two samples per plot, averaged over repetitions
[3]Conv. =conventional, intens. =crop intensified.

Briggsdale Pitfall Captures 2002-2006

2002

Mean activity density was low all months for pitfall captures for Briggsdale in 2002 (Table 2.20). There was no treatment effect on spider activity density ($F_{4,12}$=3.22, P=0.0515). Spider activity density was affected by month ($F_{6,114}$=9.03, P<0.0001). Activity densities were highest from April through July and declined thereafter.

TABLE 2.20. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT BRIGGSDALE, CO, 2002. [1,2,3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	1.5	1.3	1.1	2.6	0.1	1.4	0.5
Fallow	Conv.	2.0	0.4	1.1	1.4	0.0	0.6	0.3
Wheat	Intens.	0.9	1.0	0.4	1.1	0.1	0.8	0.5
Millet	Intens.	2.3	1.8	0.9	2.0	0.0	1.1	0.3
Fallow	Intens.	0.8	1.0	0.4	0.6	0.0	0.4	0.3

[1]Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

2003

Similar to 2002, activity densities were low for all months in 2003 for pitfall captures (Table 2.21). There was no treatment effect on spider activity density ($F_{4,12}$=0.31, P=0.8669). Spider activity density was affected by month ($F_{6,114}$=14.41, P<0.0001). Activity densities were highest for all treatments in June and were low in April, August, and October.

TABLE 2.21. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT BRIGGSDALE, CO, 2003. [1, 2, 3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	0.4	1.1	2.3	1.8	0.6	1.0	0.4
Fallow	Conv.	0.0	0.8	2.1	1.5	0.6	1.3	0.3
Wheat	Intens.	0.8	0.9	1.9	1.5	0.3	1.4	0.6
Millet	Intens.	0.5	0.5	2.3	1.9	0.0	0.9	1.5
Fallow	Intens.	0.5	0.9	3.4	1.8	0.9	1.1	0.4

[1]Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

2004

Activity densities were low each month in 2004 for pitfall captures (Table 2.22). There was no treatment effect ($F_{4,12}$=0.41, P=0.8000). Spider activity density was affected by month ($F_{6,114}$=3.40, P=0.0040).

TABLE 2.22. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT BRIGGSDALE, CO, 2004. [1, 2, 3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	0.3	0.8	0.5	5.1	0.1	0.3	0.3
Fallow	Conv.	1.1	2.0	0.8	0.1	0.4	0.1	0.8
Wheat	Intens.	1.0	1.3	0.4	1.1	0.5	0.3	0.8
Millet	Intens.	0.9	1.5	0.3	0.3	0.1	0.3	0.5
Fallow	Intens.	0.0	1.5	0.3	0.3	0.3	0.4	0.4

[1]Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

2005

Spider activity densities were higher than the previous 2002-2004 years in 2005 for pitfall captures (Table 2.23). Spider activity density was affected by treatment ($F_{4,12}$=6.00, P=0.0069) and month ($F_{6,114}$=11.85, P<0.0001). Activity densities were highest in July for all treatments except for millet and fallow in the crop-intensified rotation treatments and lowest in October. Activity densities were highest in the wheat in the crop-intensified rotation treatment during the months of August through October. Spider activity densities were highest in wheat in the conventional rotation treatment in April, June, and July, which is during the wheat-growing season.

TABLE 2.23. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT BRIGGSDALE, CO, 2005. [1,2,3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	4.4	3.1	3.6	7.3	2.4	2.5	1.1
Fallow	Conv.	3.0	2.9	1.0	3.3	1.3	1.1	1.0
Wheat	Intens.	2.9	4.1	2.3	5.1	2.9	2.8	1.6
Millet	Intens.	4.1	3.6	0.8	2.6	2.3	1.6	0.6
Fallow	Intens.	4.0	3.8	1.6	1.1	1.0	0.8	0.9

1Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

2006

Spider activity density was affected by treatment ($F_{4,12}$=9.52, P=0.0011) for pitfall captures in 2006 (Table 2.24). Spider activity density was affected by month ($F_{6,114}$=11.92, P<0.0001). Activity densities were highest in May and June for all treatments and lowest in August through October. Activity densities were highest in the

fallow in a conventional rotation in April, June, and July and highest in millet in the crop-intensified rotation treatment during May, August, September, and October.

TABLE 2.24. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT BRIGGSDALE, CO, 2006. [1, 2, 3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	0.6	2.8	2.9	1.8	0.8	1.3	1.4
Fallow	Conv.	2.5	4.1	4.6	2.6	0.9	1.3	1.4
Wheat	Intens.	0.9	1.9	2.9	1.6	1.0	1.3	1.5
Millet	Intens.	1.3	6.5	3.6	1.6	2.1	1.5	2.0
Fallow	Intens.	0.9	2.6	1.1	0.8	0.1	0.4	0.6

[1]Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

Briggsdale Vacuum Captures 2006-2007

2006

There was no treatment ($F_{4,8}$=3.68, P=0.0551) or month effect ($F_{3,42}$=1.77, P=0.1675) for spider densities (Table 2.25).

TABLE 2.25. MEAN NO. SPIDERS CAPTURED WITH VACUUM SAMPLES AT BRIGGSDALE, CO, 2006. [1,2,3]

		Month			
Crop	**Rotation**	**May**	**Jun**	**Jul**	**Aug**
Wheat	Conv.	2.0	0.7	3.5	2.5
Fallow	Conv.	0.5	1.2	1.5	2.2
Wheat	Intens.	1.2	1.5	2.0	2.3
Millet	Intens.	1.7	0.7	1.8	1.3
Fallow	Intens.	1.5	0.3	0.0	0.2

[1]Raw means are represented in this table.
[2]Vacuum means represent an average of two samples per plot, averaged over repetitions
[3]Conv. =conventional, intens. =crop intensified.

2007

There was a treatment by month interaction ($F_{12,30}$=0.80, P=0.6479) and a month effect ($F_{3,30}$=3.65, P=0.0236) for 2007 Briggsdale vacuum samples (Table 2.26); however, spider density was not affected by treatment ($F_{4,8}$=3.60, P=0.0515). Densities were highest for the two wheat treatments in July, the two fallow treatments in May, and millet densities were highest in August. Wheat in the conventional and crop-intensified treatments had the highest spider densities in July, and these treatments were significantly different from the others. Similarly, in August, the highest densities were in the wheat in the conventional treatment and in millet.

TABLE 2.26. MEAN NO. SPIDERS CAPTURED WITH VACUUM SAMPLES AT BRIGGSDALE, CO, 2007. [1,2,3,4]

		Month			
Crop	**Rotation**	**May**	**Jun**	**Jul**	**Aug**
Wheat	Conv.	0.3**Ac**	1.0**Ab**	4.7**Aa**	2.8**Bab**
Fallow	Conv.	1.3**Aa**	1.2**Aa**	0.7**Aa**	0.3**Ba**
Wheat	Intens.	0.3**Ab**	0.5**Ab**	5.0**Aa**	0.7**Ab**
Millet	Intens.	1.0**Ab**	0.8**Ab**	0.8**Ab**	4.2**Ba**
Fallow	Intens.	1.2**Aa**	0.8**Aa**	0.0**Aa**	0.3**Ba**

[1]Significant differences have been determined through square-root transformation of the data- raw means are represented in this table.
[2]Means within a column followed by the same capital letters are not significantly different (α = 0.05; PROC MIXED) and represent differences between treatments at each date. Means within rows (months) followed by the same lower case letters are not significantly different (α = 0.05; PROC MIXED). and represent differences between treatments at each month.
[3]Vacuum means represent an average of two samples per plot, averaged over repetitions
[4]Conv. =conventional, intens. =crop intensified.

Lamar Pitfall Captures 2002-2006

2002

With the exception of April, activity densities were low in 2002 (Table 2.27). There was no treatment effect on spider activity densities ($F_{4,12}$=1.51, P=0.2617); however,

spider activity density was affected by month ($F_{6,114}$=19.57, P<0.0001). Activity densities were highest in April for all treatments and were low thereafter.

TABLE 2.27. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT LAMAR, CO, 2002. [1,2,3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	5.0	0.4	1.5	0.9	1.0	0.2	1.1
Fallow	Conv.	2.3	1.3	0.5	0.8	0.3	0.3	0.4
Wheat	Intens.	3.9	0.8	1.9	0.9	0.9	0.6	0.6
Sorghum	Intens.	1.8	0.0	1.1	1.0	0.2	0.0	1.3
Fallow	Intens.	3.5	0.1	1.9	0.9	0.7	0.1	0.8

[1]Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

2003

There was no treatment effect on spider activity densities ($F_{4,12}$=1.78, P=0.1972); however, spider activity density was affected by month ($F_{6,113}$=22.06, P<0.0001) (Table 2.28). Spider activity densities were highest in June and lowest in April and October for all treatments.

TABLE 2.28. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT LAMAR, CO, 2003. [1,2,3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	0.3	2.0	3.6	3.0	0.5	0.1	0.3
Fallow	Conv.	0.3	1.6	3.8	1.5	1.0	0.4	0.1
Wheat	Intens.	0.4	1.3	2.8	2.0	0.9	0.9	0.3
Sorghum	Intens.	0.3	0.6	2.1	1.8	0.8	0.5	0.3
Fallow	Intens.	0.0	0.9	3.3	1.1	0.4	0.1	0.0

[1]Raw means are represented in this table.

[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

2004

Spider activity density was affected by month ($F_{5,75}$=20.12, P<0.0001) and treatment ($F_{4,12}$=4.27, P=0.0223) in pitfall captures in 2004 (Table 2.29). There was a treatment by month interaction with mean number of spiders captured in pitfalls ($F_{20,75}$=2.60, P=0.0015). Activity densities were highest in the fallow in the crop-intensified rotation treatment in April and May, wheat in the conventional rotation in June, July, and October, and wheat in the crop-intensified rotation treatment in August.

TABLE 2.29. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT LAMAR, CO, 2004. [1,2,3,4,5]

		Month					
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Oct**
Wheat	Conv.	1.1**ABbc**	2.5**Bab**	2.8**Aab**	3.9**Aa**	0.6**Ac**	1.0**Abc**
Fallow	Conv.	0.9**ABab**	2.0**Bab**	1.1**ABCab**	1.1**Bab**	0**Ab**	0.9**Aab**
Wheat	Intens.	0.8**Bb**	5.5**Aa**	1.0**BCb**	1.4**Bb**	1.4**Ab**	1.0**Ab**
Sorghum	Intens.	1.1**ABab**	2.5**Ba**	0.3**Cc**	0.8**Bc**	0.9**Ac**	0.4**Ac**
Fallow	Intens.	2.4**Ab**	8.0**Aa**	1.5**ABbc**	2.1**ABc**	0.3**Ac**	0.9**Ac**

[1]Significant differences have been determined through square-root transformation of the data- raw means are represented in this table.
[2]Means within a column followed by the same capital letters are not significantly different (α = 0.05; PROC MIXED) and represent differences between treatments at each date. Means within rows (months) followed by the same lower case letters are not significantly different (α = 0.05; PROC MIXED). and represent differences between treatments at each month.
[3]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[4]Conv. =conventional, intens. =crop intensified.
[5]No September pitfalls collected.

2005

Spider activity density was affected by month in pitfall captures in 2005 ($F_{6,114}$=10.87, P<0.0001) (Table 2.30); however, there was no effect of treatment ($F_{4,12}$=1.45, P=0.2767). Activity densities were highest in April through July for all

treatments and lowest in August. Activity densities were highest in July for most treatments.

TABLE 2.30. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT LAMAR, CO, 2005. [1,2,3]

		Month						
Crop	**Rotation**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**
Wheat	Conv.	2.1	1.4	2.5	3.6	0.6	2.6	1.0
Fallow	Conv.	2.0	3.1	0.3	2.4	0.6	1.8	0.9
Wheat	Intens.	2.8	2.3	3.0	3.0	0.1	2.9	2.3
Sorghum	Intens.	2.8	1.3	1.1	2.0	0.0	2.6	1.9
Fallow	Intens.	1.5	2.3	2.9	3.4	1.1	3.3	1.8

[1]Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

2006

Spider activity density was affected by month ($F_{5,95}$=21.24, P<0.0001) and treatment ($F_{4,12}$=7.36, P=0.0031) (Table 2.31). Activity densities were highest during June and November. Treatment affected spider activity density ($F_{4,12}$=7.50, P=0.0029). Sorghum in the crop-intensified rotation treatment had the highest activity density in April, wheat in the crop-intensified rotation treatment had the highest density in June, July, and October, and wheat in the conventional rotation had the highest activity density in August and November.

TABLE 2.31. MEAN NO. SPIDERS CAPTURED IN PITFALLS AT LAMAR, CO, 2006. [1,2,3,4]

		Month					
Crop	**Rotation**	**Apr**	**Jun**	**Jul**	**Aug**	**Oct**	**Nov**
Wheat	Conv.	1.6	5.3	2.9	2.4	1.9	6.1
Fallow	Conv.	1.4	3.1	2.4	0.6	1.0	3.8

		Month					
Crop	**Rotation**	**Apr**	**Jun**	**Jul**	**Aug**	**Oct**	**Nov**
Wheat	Intens.	1.6	6.0	4.4	2.0	4.3	5.5
Sorghum	Intens.	1.9	4.8	1.4	1.3	1.1	3.0
Fallow	Intens.	1.4	4.8	3.4	0.8	3.0	4.1

[1]Raw means are represented in this table.
[2]Pitfall means represent seven days of total spiders collected, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.
[4]No May pitfalls collected.

Lamar Vacuum Captures 2006-2007

2006

There was no treatment effect ($F_{4,8}$=3.43, P=0.0649) on spider densities for vacuum samples in 2006; however, spider activity density was affected by month ($F_{3,42}$=1.84, P=0.1549) (Table 2.32). Densities were highest in July for the two wheat treatments and highest in June for the other three treatments.

TABLE 2.32. MEAN NO. SPIDERS CAPTURED WITH VACUUM SAMPLES AT LAMAR, CO, 2006. [1,2,3]

		Month			
Crop	**Rotation**	**May**	**Jun**	**Jul**	**Aug**
Wheat	Conv.	2.8	1.2	3.0	1.0
Fallow	Conv.	0.8	1.0	0.8	0.2
Wheat	Intens.	1.2	1.3	3.2	1.8
Sorghum	Intens.	0.7	1.3	0.7	0.7
Fallow	Intens.	0.7	2.5	1.0	0.3

[1]Raw means are represented in this table.
[2]Vacuum means represent an average of two samples per plot, averaged over repetitions.
[3]Conv. =conventional, intens. =crop intensified.

2007

Spider density was affected by month ($F_{1,14}$=14.39, P=0.0020) and treatment ($F_{4,8}$=4.82, P=0.0283) for samples in 2007 (Table 2.33). Densities were not measured in May and June, and densities were high for the month of August for all treatments except fallow in the crop-intensified rotation, where densities were highest in July. The highest densities were present in the wheat in the conventional rotation treatment and sorghum in the crop-intensified rotation.

TABLE 2.33. MEAN NO. SPIDERS CAPTURED WITH VACUUM SAMPLES AT LAMAR, CO, 2007. [1,2,3,4]

		Month	
Crop	**Rotation**	**Jul**	**Aug**
Wheat	Conv.	2.5	14.7
Fallow	Conv.	1.5	3.8
Wheat	Intens.	1.5	3.8
Sorghum	Intens.	1.0	14.5
Fallow	Intens.	4.8	1.7

[1]Raw means are represented in this table.
[2]Vacuum means represent an average of two samples per plot, averaged over repetitions
[3]Conv. =conventional, intens. =crop intensified.
[4]No May or June samples.

Comparison of activity densities and densities between cropping systems

Pitfalls

Akron

With the exception of 2002, spider activity densities in the conventional versus crop-intensified rotations in Akron were similar (Table 2.34). In 2002, because a significant treatment by month interaction occurred (Table 2.13), contrasts of treatments were compared separately for each month-(month 1, t_{84}=-1.01, P=0.3130; month 2, t_{84}=0.19,

P=0.8477; month 3, t_{84}=-0.17, P=0.8667; month 4, t_{84}=1.07, P=0.2869; month 5, t_{84}=-1.57, P=0.1196; month 6, t_{84}=1.06, P=0.2910; month 7, t_{84}=3.38, P<0.0011).

TABLE 2.34. CONTRAST OF TREATMENTS FROM PITFALL SAMPLES COMPARING SPIDER ACTIVITY DENSITIES FROM PITFALLS IN CONVENTIONAL AND CROP-INTENSIFIED TREATMENTS, AKRON, CO, 2002-2006. [1,2]

Year	Effect	df	*t*	*P*-value
2002[2]	Conventional vs. Crop-Intensified Treatments	*see text*	*see text*	*see text*
2003	Conventional vs. Crop-Intensified Treatments	12	-1.55	0.1464
2004	Conventional vs. Crop-Intensified Treatments	12	-0.06	0.9508
2005	Conventional vs. Crop-Intensified Treatments	12	0.06	0.9521
2006	Conventional vs. Crop-Intensified Treatments	12	-0.37	0.7279

[1]Treatments were averaged over months.
[2]Rotations were compared separately for each month.

Briggsdale

Spider activity densities in the conventional versus crop-intensified rotations were similar in Briggsdale (Table 2.35).

TABLE 2.35. CONTRAST OF TREATMENTS COMPARING SPIDER ACTIVITY DENSITIES FROM PITFALLS IN CONVENTIONAL AND CROP-INTENSIFIED TREATMENTS, BRIGGSDALE, CO, 2002-2006.[1]

Year	Effect	df	*t*	*P*-value
2002	Conventional vs. Crop-Intensified Treatments	12	1.66	0.1221
2003	Conventional vs. Crop-Intensified Treatments	12	-0.69	0.5045
2004	Conventional vs. Crop-Intensified Treatments	12	0.62	0.5445
2005	Conventional vs. Crop-Intensified Treatments	12	1.01	0.3346
2006	Conventional vs. Crop-Intensified Treatments	12	1.67	0.1200

[1]Treatments were averaged over months.

Lamar

With the exception of 2004, spider activity densities in the conventional versus crop-intensified rotations in Lamar were similar (Table 2.36). In 2004, because of the significant treatment by month interaction (Table 2.29), contrast of treatments were compared separately for each month-(month 1, t_{75}=2.16, P=0.0339, month 2, t_{75}=1.71, P=0.0910, month 3, t_{75}=4.85, P<0.0001, month 4, t_{75}=2.27, P=0.0261, month 5, t_{75}=-1.97, P=0.0527, month 6, t_{75}=0.48, P=0.6300, month 7, t_{75}=5.75, P<0.0001). A significant

difference between rotations occurred on month 1 (April), month 3 (June), month 4 (July), and month 7 (October).

TABLE 2.36. CONTRAST OF TREATMENTS COMPARING SPIDER ACTIVITY DENSITIES FROM PITFALLS IN CONVENTIONAL AND CROP-INTENSIFIED TREATMENTS, LAMAR, CO, 2002-2006.[1,2]

Year	Effect	df	*T*	*P*-value
2002	Conventional vs. Crop-Intensified Treatments	12	-0.07	0.9419
2003	Conventional vs. Crop-Intensified Treatments	12	1.96	0.0731
2004[2]	Conventional vs. Crop-Intensified Treatments	*see text*	*see text*	*see text*
2005	Conventional vs. Crop-Intensified Treatments	12	-1.16	0.2695
2006	Conventional vs. Crop-Intensified Treatments	12	-1.22	0.2451

[1]Treatments were averaged over months.
[2]Rotations were compared separately for each month.

Vacuum

Akron

Spider densities in the conventional versus crop-intensified rotations in Akron were similar (Table 2.37).

TABLE 2.37. CONTRAST OF TREATMENTS FROM VACUUM SAMPLES COMPARING SPIDER ACTIVITY DENSITIES IN CONVENTIONAL AND CROP-INTENSIFIED TREATMENTS, AKRON, CO, 2002-2006.

Year	Effect	df	*T*	*P*-value
2006	Conventional vs. Crop-Intensified Treatments	8	0.76	0.4676
2007	Conventional vs. Crop-Intensified Treatments	8	-0.17	0.8700

Briggsdale

In 2006, spider densities in the conventional versus crop-intensified rotations in Briggsdale were similar (Table 2.38). In 2007, because of the treatment by month interactions (Table 2.26), contrast of treatments were compared separately for each month-(month 1, t_{75}=2.16, P=0.0339, month 2, t_{75}=1.71, P=0.0910, month 3, t_{75}=0.48, P=0.6330, month 4, t_{75}=5.75, P<0.0001). Thus, in months 1 and 4 (May and August), spider densities differed between the conventional versus the crop-intensified rotations.

TABLE 2.38. CONTRAST OF TREATMENTS FROM VACUUM SAMPLES COMPARING SPIDER DENSITIES IN CONVENTIONAL AND CROP-INTENSIFIED TREATMENTS, BRIGGSDALE, CO, 2006-2007. [1]

Year	Effect	df	*T*	*P*-value
2006	Conventional vs. Crop-Intensified Treatments	8	1.63	0.1409
2007[1]	Conventional vs. Crop-Intensified Treatments	*see text*	*see text*	*see text*

[1] Rotations were compared separately for each month.

Lamar

There were no significant differences between spider densities in the conventional versus crop-intensified rotations in Akron when using contrasts of treatments (Table 2.39).

TABLE 2.39. CONTRAST OF TREATMENTS FROM VACUUM SAMPLES COMPARING SPIDER ACTIVITY IN CONVENTIONAL AND CROP-INTENSIFIED TREATMENTS, LAMAR, CO, 2006-2007.

Year	Effect	df	*T*	*P*-value
2006	Conventional vs. Crop-Intensified Treatments	8	0.20	0.8491
2007	Conventional vs. Crop-Intensified Treatments	8	0.79	0.4507

Species Biodiversity

Inventory completion, sampling intensity, and species accumulation curves

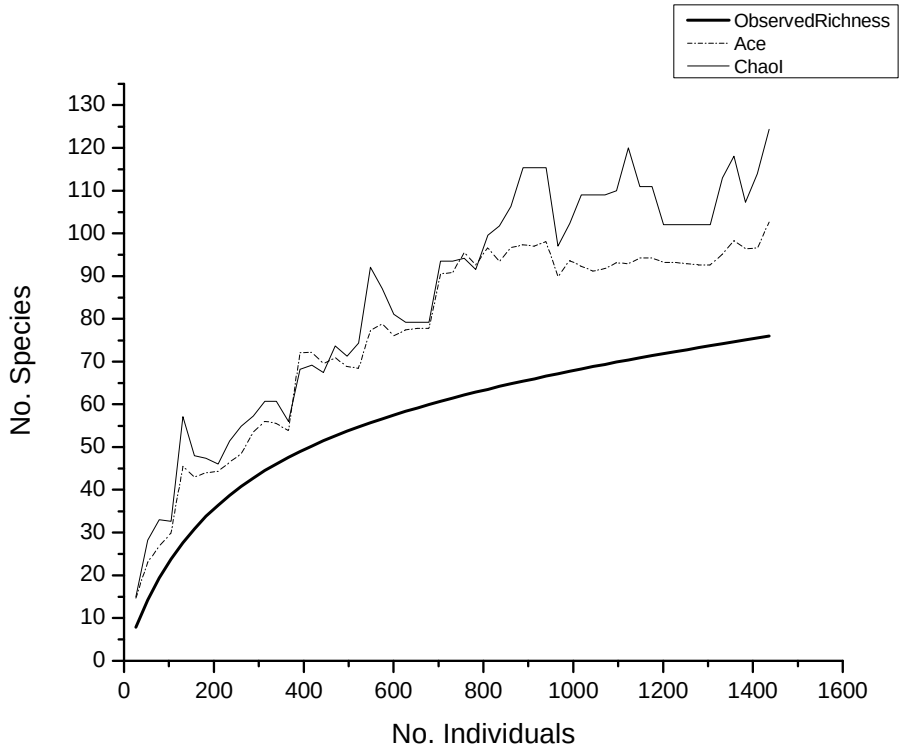
An "inventory completion" calculation was performed by dividing the observed species richness (total number of species collected) by the Chao I estimate (Coddington et al. 2009). This can provide an indication of undersampling, which may affect the interpretation of the biodiversity of the area (Coddington et al. 2009). The inventory completion for all sites was as follows: Akron=61.1%, Briggsdale=82.7%, Lamar=85.2%.

Additionally, the sampling intensity within a system provides an estimate of the number of species retrieved compared to the number of individuals cumulatively sampled (Coddington et al. 2009). The intensities for Akron, Briggsdale, and Lamar were 18.6%, 21.8%, and 20.7%, respectively. The number of singletons (a species represented by only

one individual) in a collection can indicate whether a survey was undersampled. The percentage of singletons for the Akron, Briggsdale, and Lamar sites for 2002 through 2007 was 32.7%, 28.9%, and 34.0% respectively. For comparison, a 10-day extensive survey and collection of over 6000 spiders in Guyana resulted in 29% singletons (Coddington et al. 2009).

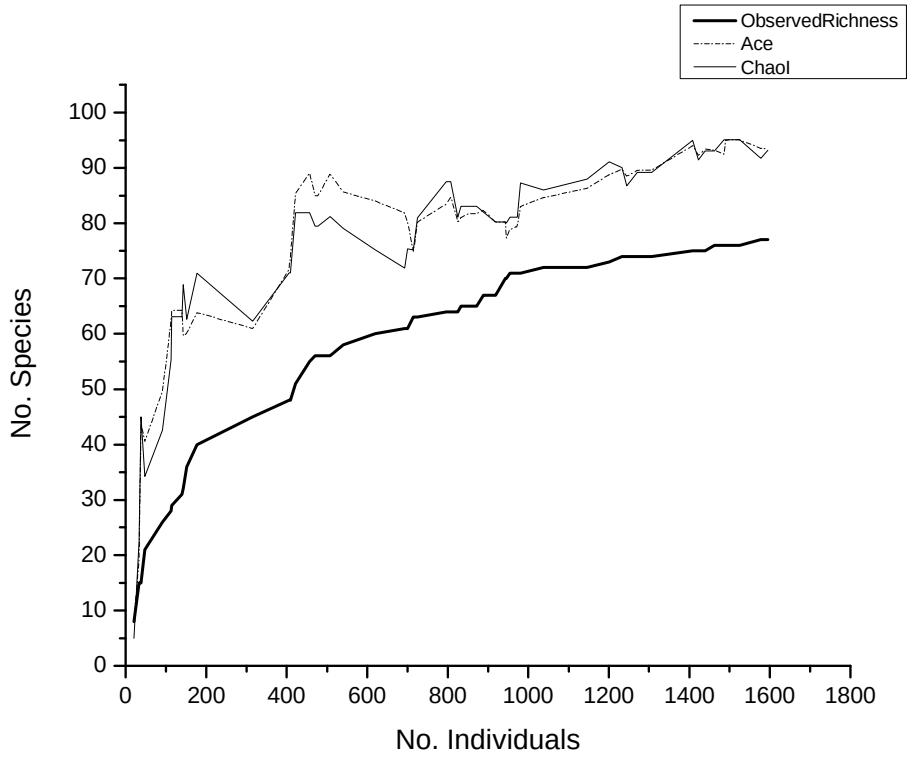
The species accumulation curves for all sites for the years 2002-2007 combined are displayed in Figures 2.9-2.11. The observed richness curves are still rising, which is indicative of incomplete sampling overall (Coddington et al. 2009). The richness estimators (Chao I and Ace) show that the estimated true number of species was higher than the actual observed number of species.

FIGURE 2.9. SPECIES ACCUMULATION CURVE, AKRON, CO, 2002-2007. [1]



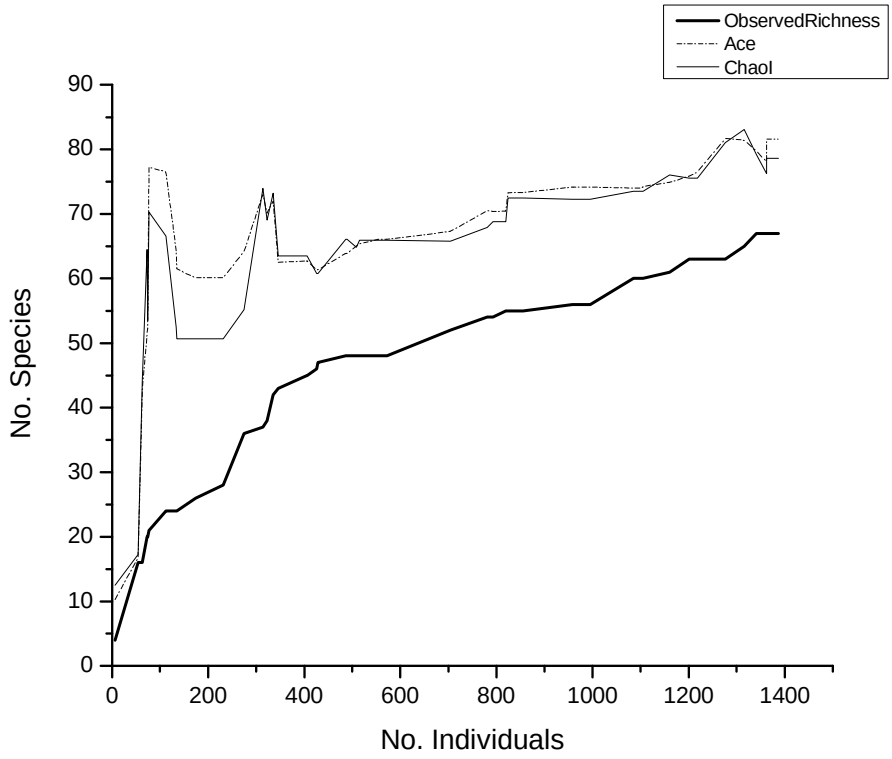
[1]Adult spiders sampled from all treatments from lookdown, pitfall, and vacuum techniques.

FIGURE 2.10. SPECIES ACCUMULATION CURVE, BRIGGSDALE, CO, 2002-2007. [1]



[1]Adult spiders sampled from all treatments from lookdown, pitfall, and vacuum techniques.

FIGURE 2.11. SPECIES ACCUMULATION CURVE, LAMAR, CO, 2002-2007. [1]



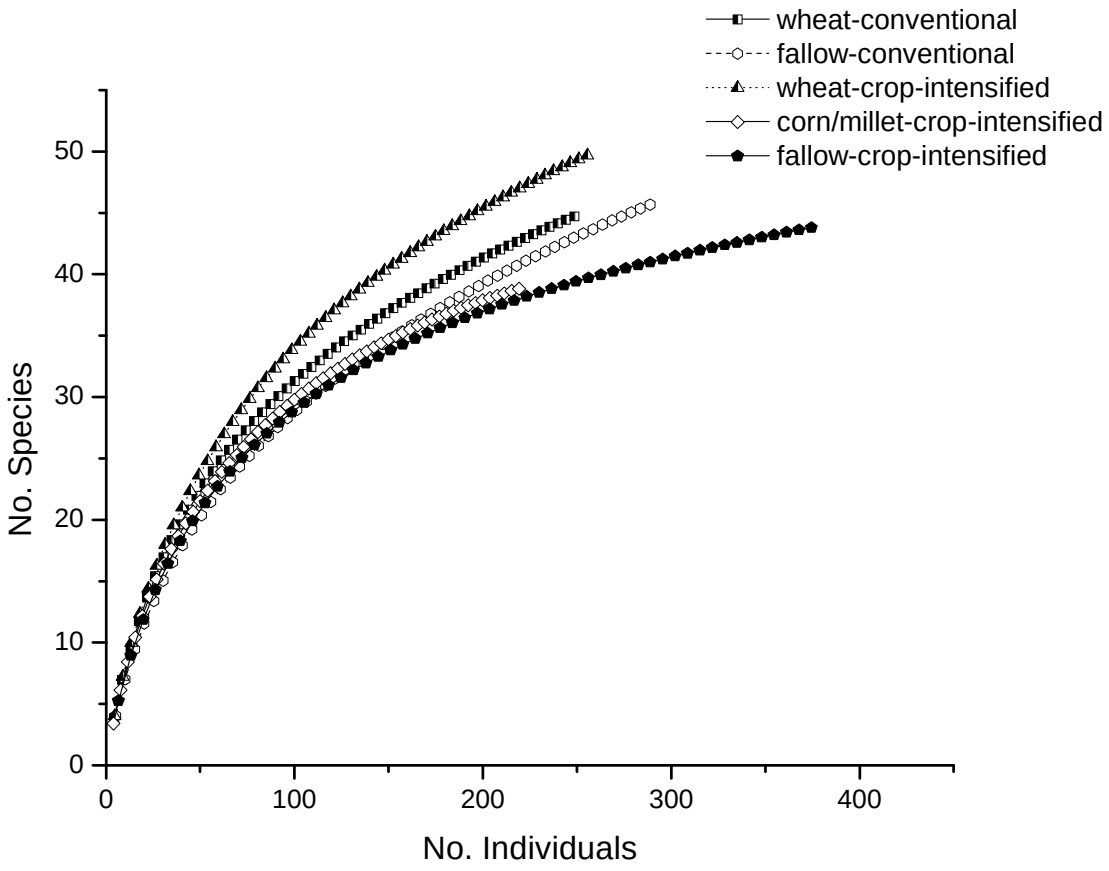
[1]Adult spiders sampled from all treatments from lookdown, pitfall, and vacuum techniques.

Rarefaction curves to assess differences between diversity and treatments

Rarefaction curves for treatments in Akron suggest that more species were collected in wheat in the crop-intensified rotation, followed by fallow in the conventional rotation treatment, and then by wheat in the conventional rotation treatment (Figure 2.12). The summer crops corn and millet hosted the fewest species. For Briggsdale, fallow in the crop-intensified rotation treatment possessed the greatest number of species followed by wheat in the conventional rotation treatment (Figure 2.13). In Lamar, the highest number of species was represented by fallow in the conventional rotation treatment followed by

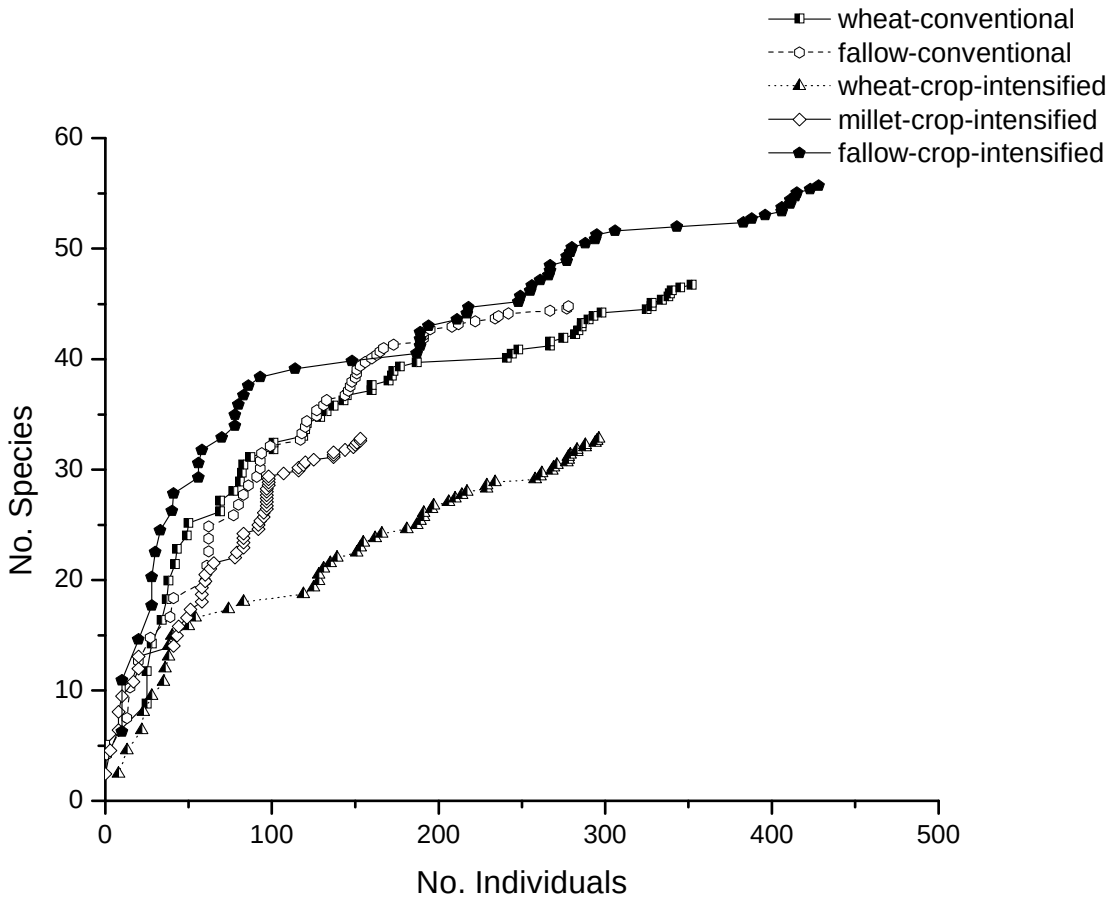
fallow in the crop-intensified rotation treatment. Wheat in the conventional rotation treatment contained the least number of species (Figure 2.14).

FIGURE 2.12. SPECIES RAREFACTION CURVES AKRON, CO, 2002-2007.[1]



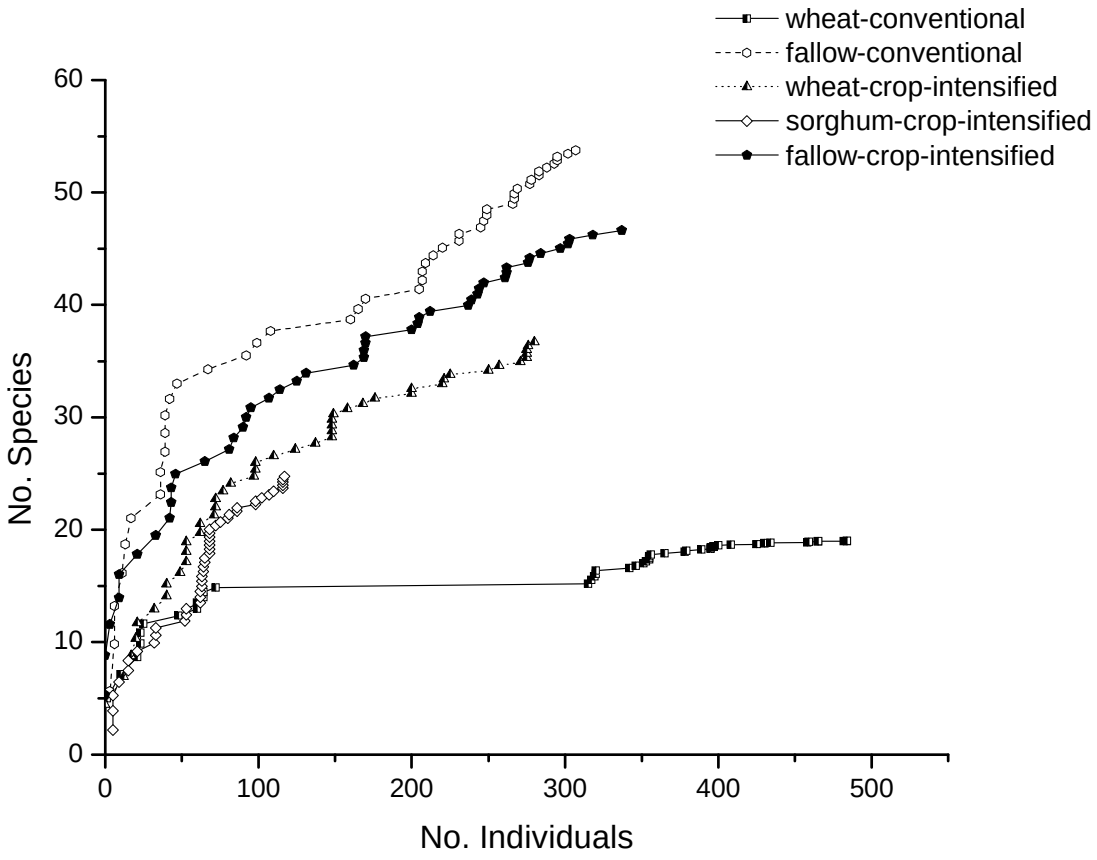
[1]Adult spiders sampled from all treatments from lookdown, pitfall, and vacuum techniques.

FIGURE 2.13. SPECIES RAREFACTION CURVES BRIGGSDALE, CO, 2002-2007. [1]



[1]Adult spiders sampled from all treatments from lookdown, pitfall, and vacuum techniques.

FIGURE 2.14. SPECIES RAREFACTION CURVES LAMAR, CO, 2002-2007. [1]



[1]Adult spiders sampled from all treatments from lookdown, pitfall, and vacuum techniques.

Shannon diversity indices

Akron

Spider biodiversity was highest in corn in all years, with the exception of 2005 (Table 2.40). In 2005, biodiversity was highest in wheat in the crop-intensified treatments. With the exception of corn in 2006, spider biodiversity was highest for all treatments in 2005. Biodiversity was lowest in 2007, which is likely because only one sampling method, vacuuming, was conducted during this year. Biodiversity was similar among

treatments for all years except 2004-(2002-$F_{4,12}$=0.03, P=0.9756), (2003-$F_{4,12}$=0.85, P=0.5194), (2004-$F_{4,12}$=12.69, P=0.0003), (2005-$F_{4,1}$=0.24, P=0.9109), (2006-$F_{4,12}$=1.18, P=0.3669), (2007-$F_{4,5}$=0.34, P=0.8379).

TABLE 2.40. SHANNON DIVERSITY INDEX (H) FOR AKRON, CO, 2002-2007.[1,2,3]

	Conventional		Crop-Intensified		
Year	**Wheat**	**Fallow**	**Wheat**	**Corn[2]**	**Fallow**
2002	5.66**a**	4.30**a**	5.20**a**	6.17**a**	3.45**a**
2003	6.09**a**	5.15**a**	4.95**a**	6.17**a**	3.85**a**
2004	4.84**b**	5.81**b**	5.97**b**	8.64**a**	5.71**b**
2005	10.15**a**	8.16**a**	10.39**a**	9.41**a**	9.41**a**
2006	6.92**a**	5.52**a**	8.98**a**	9.71**a**	5.52**a**
2007	1.67**a**	1.31**a**	1.67**a**	2.00**a**	1.00**a**

[1]From pitfall, lookdown, and vacuum sampling.
[2]Means within rows (months) followed by the same lowercase letters are not significantly different (α = 0.05; PROC MIXED).
[3]Corn changed to millet in 2005-2006 crop year.

Briggsdale

Spider biodiversity varied between treatments each year (Table 2.41). Biodiversity was highest in 2005 and 2006, with the exception of high spider biodiversity in fallow in the crop-intensified treatment in 2003. Similar to Akron, biodiversity was lowest in 2007. Treatments differed in 2002 ($F_{4,12}$= 3.61, P=0.0375) and 2003 ($F_{4,12}$= 4.48, P=0.0191), respectively, but not during 2004-2007 (2004-$F_{4,11}$=0.87, P=0.5115), (2005-$F_{4,12}$=0.60, P=0.6703), (2006-$F_{4,12}$=1.84, P=0.1853), (2007-$F_{4,4}$=2.04, P=0.2535).

TABLE 2.41. SHANNON DIVERSITY INDEX (H) FOR BRIGGSDALE, CO, 2002-2007.[1,2]

	Conventional		Crop-Intensified		
Year	**Wheat**	**Fallow**	**Wheat**	**Millet**	**Fallow**
2002	5.36**a**	4.64**ab**	3.43**b**	5.82**a**	3.75**b**
2003	5.48**b**	3.59**b**	5.02**b**	4.13**b**	8.43**a**
2004	3.20**a**	2.82**a**	3.84**a**	4.04**a**	4.52**a**
2005	12.39**a**	10.90**a**	9.61**a**	12.10**a**	12.34**a**
2006	9.41**a**	9.87**a**	11.64**a**	14.05**a**	6.11**a**
2007	2.83**a**	3.46**a**	2.00**a**	2.76**a**	1.25**a**

[1]From pitfall, lookdown, and vacuum sampling.
[2]Means within rows (months) followed by the same lower case letters are not significantly different ($\alpha = 0.05$; PROC MIXED).

Lamar

Similar to Briggsdale, spider biodiversity varied between treatments each year in Lamar (Table 2.42). Biodiversity was highest in 2006, with the exception that spider biodiversity was highest in wheat in the conventional treatment in 2005. Biodiversity was again lowest in 2007 and similar among all treatments each year (2002-$F_{4,12}$=0.10, P=0.9790), (2003-$F_{4,12}$=1.43, P=0.1787), (2004-$F_{4,12}$=1.78, P=0.1980), (2005-$F_{4,12}$=0.87, P=0.5078), (2006-$F_{4,12}$=2.33, P=0.1150), (2007-$F_{4,12}$=3.19, P=0.1054).

TABLE 2.42. SHANNON DIVERSITY INDEX (H) FOR LAMAR, CO, 2002-2007.[1,2,3]

	Conventional		Crop-Intensified		
Year	**Wheat**	**Fallow**	**Wheat**	**Sorghum**	**Fallow**
2002	4.08	3.50	3.45	3.90	3.92
2003	4.47	3.19	3.83	2.07	3.05

	Conventional		Crop-Intensified		
Year	**Wheat**	**Fallow**	**Wheat**	**Sorghum**	**Fallow**
2004	2.44	4.58	3.56	2.73	4.31
2005	7.40	6.54	6.84	5.59	6.41
2006	6.87	6.84	8.87	6.75	10.29
2007	0.33	2.27	[2]	1.82	0.67

[1]From pitfall, lookdown, and vacuum sampling.
[2]No spiders in wheat in the crop-intensified treatment in 2007.
[3]Means within rows (months) followed by the same lower case letters are not significantly different (α = 0.05; PROC MIXED).

Comparison of diversity between cropping systems

Akron

Spider biodiversity in the conventional versus crop-intensified rotations was similar among treatments, with the exception of 2004 (Table 2.43). This may be because the biodiversity of the corn treatment within the crop-intensified rotation was significantly higher than the biodiversity for all other treatments (Table 2.40), and this high biodiversity index value might have contributed to a higher mean index value for this crop-intensified rotation.

TABLE 2.43. CONTRAST OF TREATMENTS FROM PITFALL SAMPLES COMPARING SPIDER DIVERSITY IN CONVENTIONAL AND CROP-INTENSIFIED TREATMENTS, AKRON, CO, 2002-2007. [1]

Year	Effect	df	*t*	*P*-value
2002	Conventional vs. Crop-Intensified Treatments	12	0.03	0.9756
2003	Conventional vs. Crop-Intensified Treatments	12	0.67	0.5162
2004	Conventional vs. Crop-Intensified Treatments	12	-3.93	0.0020
2005	Conventional vs. Crop-Intensified Treatments	12	-0.36	0.7254
2006	Conventional vs. Crop-Intensified Treatments	12	-1.13	0.2795
2007	Conventional vs. Crop-Intensified Treatments	12	-0.10	0.9269

[1]From pitfall, lookdown, and vacuum samples.

Briggsdale

Spider biodiversity was similar among all treatments in the conventional versus crop-intensified rotations each year (Table 2.44).

TABLE 2.44. CONTRAST OF TREATMENTS COMPARING SPIDER DIVERSTIY IN CONVENTIONAL AND CROP-INTENSIFIED TREATMENTS, BRIGGSDALE, CO, 2002-2007.[1]

Year	Effect	df	*t*	*P*-value
2002	Conventional vs. Crop-Intensified Treatments	12	1.36	0.1981
2003	Conventional vs. Crop-Intensified Treatments	12	-1.63	0.1295
2004	Conventional vs. Crop-Intensified Treatments	12	-1.76	0.1067
2005	Conventional vs. Crop-Intensified Treatments	12	0.21	0.8387
2006	Conventional vs. Crop-Intensified Treatments	12	-0.49	0.6352
2007	Conventional vs. Crop-Intensified Treatments	12	1.82	0.1423

[1]From pitfall, lookdown, and vacuum samples.

Lamar

Spider biodiversity was similar in the conventional versus crop-intensified rotations in Briggsdale for all years (Table 2.45).

TABLE 2.45. CONTRAST OF TREATMENTS COMPARING SPIDER DIVERSITY IN CONVENTIONAL AND CROP-INTENSIFIED TREATMENTS, LAMAR, CO, 2002-2007. [1,2]

Year	Effect	df	*t*	*P*-value
2002	Conventional vs. Crop-Intensified Treatments	12	0.04	0.9679
2003	Conventional vs. Crop-Intensified Treatments	12	1.43	0.1787
2004	Conventional vs. Crop-Intensified Treatments	12	-0.04	0.9702
2005	Conventional vs. Crop-Intensified Treatments	12	1.07	0.3604
2006	Conventional vs. Crop-Intensified Treatments	12	-1.87	0.0858
2007[2]		*see text*	*see text*	*see text*

[1]From pitfall, lookdown, and vacuum samples.
[2]No adult spiders collected in Lamar in 2007.

Aphids

At Akron, in 2002, no *D. noxia* were observed during Zadoks (based on Zadoks scale, a widely used cereal development scale in agriculture (Zadoks et al. 1974)) 20, 30, or 50 in either the conventional or crop-intensified plots (Table 2.46). Similarly, in 2003, no aphids were present at Zadoks 20, 30, or 50, with the exception of 0.01 *D. noxia* sampled per tiller at Zadoks 50 in the conventional rotation. Densities measured 0-0.02 aphids per

tiller during 2002-2003 and 2007. In 2004, the number of aphids per tiller was highest at Zadoks 80-87 in both rotations. In 2005, the number of aphids per tiller was highest during Zadoks 50-59 and Zadoks 80-87. In 2006, the number of aphids per tiller was highest at Zadoks 70-79. No *D. noxia* were present in 2007 in Akron.

At Briggsdale, in 2002-2003 and 2005-2006, no *D. noxia* were present during sampling (Table 2.47). In 2004, *D. noxia* densities were highest at Zadoks 50-59 in both rotations. In 2007, *D. noxia* were present at Zadoks 30 in low densities.

At Lamar, no *D. noxia* were present during sampling in 2002 and 2007 (Table 2.48). In 2003, *D. noxia* was present at Zadoks 40. Aphid densities were not recorded in 2004. In 2005, *D. noxia* densities were highest at Zadoks 30, and *D. noxia* were present at Zadoks 40 in 2006. In 2007, the number of aphids per tiller measured 0-0.02 at all sites, and no measurements were made past Zadoks 40.

TABLE 2.46. NO. OF *D. NOXIA* PER TILLER IN WHEAT IN THE CONVENTIONAL AND CROP-INTENSIFIED ROTATIONS IN AKRON, CO, 2002-2007.

Year	Wheat Stage (Zadoks)	Rotation	
		Conventional	Crop-Intensified
2002	20	0	0
	30	0	0
	50	0	0
2003	20	0	0
	30	0	0
	50	0.01	0
2004	20	0	0
	30	0	0.03

Year	Wheat Stage (Zadoks)	Rotation	
		Conventional	Crop-Intensified
	50	0.08	0.10
2005	30	0.01	0
	50	0.02	0.08
	80	0.15	0.13
2006	30	0	0
	50	0.03	0
	70	0.03	0.07
2007	30	0	0
	50	0	0

TABLE 2.47. NO. OF *D. NOXIA* PER TILLER IN WHEAT IN THE CONVENTIONAL AND CROP-INTENSIFIED ROTATIONS IN BRIGGSDALE, CO, 2002-2007.

Year	Wheat Stage (Zadoks)	Rotation	
		Conventional	Crop-Intensified
2002	20	0	0
2003	20	0	0
	30	0	0
2004	20	0	0
	30	0	0.03
	50	0.07	0.15
2005	20	0	0
2006	20	0	0
2007	30	0.02	0.01

TABLE 2.48. NO. OF *D. NOXIA* PER TILLER IN WHEAT IN THE CONVENTIONAL AND CROP-INTENSIFIED ROTATIONS IN LAMAR, CO, 2002-2007.

Year	Wheat Stage (Zadoks)	Rotation	
		Conventional	Crop-Intensified
2002	20	0	0
	40	0	0
2003	40	0.05	0.05
2004[1]			
2005	20	0	0
	30	0.02	0.04
2006	40	0.03	0.01
2007	40	0	0

[1]No aphid data collected for 2004.

Discussion

This study assessed spider density and biodiversity in a multi-year, multi-site crop-intensified farming system with potential economic and environmental benefits. Because much available land is dedicated to agriculture, it is logical to adopt sustainable practices for optimal agronomic, environmental, and biological properties. It also was important to determine whether the spider fauna, a potential source of biological control, benefitted from a crop-intensified system. Overall, crop intensification had little effect on spider density or biodiversity. If spider densities were affected, then the treatments within the crop-intensified rotations should maintain higher densities or Shannon index values than those treatments within the conventional rotation, which was not the case. The contrast of treatments also verified that no differences were apparent between the two rotations

for spider density. If spiders are preferentially residing within the crop-intensified rotation, densities should also gradually increase in these treatments over time, and this was not evident. Aside from 2005 and 2006, mean spider densities and activity densities were low. Furthermore, treatment effects may not be apparent with such low spider densities.

Sustainable crop management practices often have no effect on spider density and diversity. For example, there were no differences in spider density and richness when comparing conventional, integrated, and organic farming systems in the Netherlands (Booij and Noorland 1992) and no differences in spider density and biodiversity between conventional and organic cropping systems in Germany (Clough et al. 2005, Schmidt et al. 2005, Diekotter et al. 2010).

Because undisturbed habitats adjacent to cropping systems in Colorado are not typically available or economically justifiable, an adjacent summer crop could provide a suitable alternative to maintain and enhance spider densities in wheat production. Additional crops within the rotation provide a level of connectivity between habitats, which is crucial for maintaining natural enemy populations (Duelli 1988). If the summer crops (corn, millet, and sorghum) provide a source of connectivity or dispersal during agricultural disturbances, activity densities should increase within these treatments following wheat harvest in June and July at all sites. With the exception of Akron pitfalls in 2002 (Table 2.13) and 2006 (Table 2.17), there was no indication that total activity densities were higher in wheat fields prior to harvest and summer crops adjacent to wheat after harvest. Treatment effects were affected by date for pitfalls in Akron, 2002, Lamar,

2004, and vacuum samples for Briggsdale, 2007; however, these effects were unpredictably associated with crop rotation and date, providing no suggestion that spiders were dispersing to summer crops.

The lack of treatment effects between the crop-intensified and conventional rotations might be explained by several factors. The experimental plots might have been an unrealistic size for predator studies. In eastern Colorado farming systems, crops are typically planted into 51 hectare fields or larger. Plots in this study represented only a small portion of these normal sizes (Table 2.1). Thus, it may be that experimental plots were too small, and predators could easily move between the different treatments, regardless of what crop was present. If the study was conducted on a typical size farm, it is possible that differences might have been seen between the different crops within the two rotations. Also, tillage operations differed at each site. Only herbicides were used to control weeds at Briggsdale. At Akron, only the conventional rotation was tilled. In Lamar, tillage was performed on all treatments. For future studies, it would be important to include residue measurements within treatments and also to include vegetation height and structure measurements to understand how these factors might be correlated with spider density and biodiversity.

Mean spider activity density was higher in 2005 and 2006 at all sites. Precipitation might have been a factor with increased density. In 2005 at Akron, several heavy rains occurred in late May. Briggsdale and Lamar precipitation was also above average prior to wheat harvest in 2005. Similarly, for 2006, precipitation was above average, particularly in Lamar. Increased precipitation can improve microhabitats or increase

humidity below crop canopies, having a positive effect on spider densities (Sunderland and Samu 2000). The increased precipitation for May 2004 in Lamar and all sites for 2005 and 2006 also allowed for enhanced weed growth (Kerzicnik, pers. obs). Weeds dominated the cropping systems in Lamar in 2004. Weeds and increased weediness can be a source of insect diversity (van Emden and Wearing 1965), providing more prey for generalist predators. Similarly, increased weed density has been correlated with increased spider densities (Altieri et al. 1985, Carter and Rypstra 1995, Jmhasly and Nentwig 1995, Balfour and Rypstra 1998, Wardle et al. 1999) and also can provide more web-building spider attachment sites (McNett and Rypstra 2000). Aphid densities were highest at Zadoks 80 in Akron in 2005, which also might have attracted additional Linyphiidae. Linyphiid spiders non-randomly located their webs where prey densities were highest in winter wheat fields in the United Kingdom (Harwood et al. 2001, Harwood et al. 2003).

Spider biodiversity was also highest at all sites from 2005-2006, particularly in 2005 (Tables 2.40-2.42). Increased spider richness and biodiversity in the northwestern Negev desert in Israel was associated with increased precipitation (Opatovsky et al. 2010). Linyphiidae activity densities were higher in Briggsdale and Akron in 2005 (18% and 21% of pitfall collections, respectively), which may be correlated with the precipitation. Linyphiids prefer increased humidity for establishment (Nyffeler and Sunderland 2003). In Akron, 2005, Linyphiidae densities and species richness were higher than other years. In particular, the species *Agyneta/Meioneta* sp. 1, *Ceratinella brunnea* Emerton, *Coloncus siou* Chamberlin, *Islandiana flaveola* (Banks), *Mythoplastoides exiguus*

(Banks), *Tennesseellum formicum* (Emerton), *Agyneta cf. unimaculata* (Banks), *Agyneta uta* (Chamberlin), *Erigone aletris* Crosby & Bishop, *Islandiana princeps* Braendegaard, and *Walckenaeria spiralis* (Emerton) were present in 2005 in higher densities than other years with the latter five species present in 2005 only. At Briggsdale, 2005, the number of adult Linyphiidae was over triple the number present in other years, with *M. exiguus* and *E. aletris* dominating. In Lamar, 2005, biodiversity may have been higher due to several species present within the families Theridiidae, Thomisidae, Philodromidae, Lycosidae, Salticidae, Linyphiidae, and Gnaphosidae. In Lamar, 2006, *Schizocosa mccooki* (Araneae: Lycosidae), *H. coloradensis* (Araneae: Lycosidae), *Gnaphosa saxosa* (Araneae: Gnaphosidae), and *Cicurina* sp. 1 (Araneae: Dictynidae) densities were relatively high. Furthermore, the only other time *Cicurina* sp. 1 was sampled was in April pitfalls in 2002 with just four specimens collected. Although the natural history of *Cicurina* sp. 1 is not known, *Cicurina bryantae* Exline (Araneae: Dictynidae) has been exclusively associated with retreats within rotting wood (Bennett 1985). The excessive rain in November may have created optimal conditions for *Cicurina* sp. 1, such as those described for *C. bryantae*.

Predicted disturbances within the agroecosystem, particularly crop harvest, are important for identifying spider density patterns. It is important to know the life histories of the dominant spider fauna to comprehend survival strategies of spiders within agroecosystems (Thorbek et al. 2004). Month of sampling affected spider densities in pitfalls and vacuum samples. Furthermore, with the dominant fauna in this study, adult densities were highest in April-July prior to harvest, and activity densities decreased

thereafter at all sites. This phenology pattern associated with frequent disturbances is typical of agrobiont spiders; the first generation of the most common agricultural Hungarian spiders coincided with the vegetation period of the crops (Samu and Szinetar 2002). One exception to the high density of spiders pre-wheat harvest versus post-wheat harvest occurred in Lamar in 2006, which can be explained by the unusually high density of *Cicurina* sp. 1 in November. In a 10-year survey of spiders in arable habitats in Hungary, the phenology curves of the dominant agrobionts revealed that the spiders' first generation coincided with the main vegetative period of the crop (Samu and Szinetar 2002). *Pardosa agrestris* (Westring) (Araneae: Lycosidae) is a dominant European species that exemplifies adaptability to periodic disturbances, reproducing in June to avoid the temporary and predictable flooded marshes in its habitat (Richter 1970). Likewise, the dominant spider fauna in New Mexico adapt to periodic flood irrigation and several cuttings of alfalfa (Richman et al. 1990). In Sweden, Lycosidae can tolerate agricultural disturbances, as both adults and juveniles were not affected by the disturbance of wheat planting and immediately recolonized fields post harvest (Oberg and Ekbom 2006).

Over 72% of the spiders from all collection methods from 2002-2007 were represented by the families Gnaphosidae and Lycosidae. The dominant species at the three sites were the following: Akron-*Schizocosa mccooki* (Araneae: Lycosidae), *Gnaphosa clara* (Araneae: Gnaphosidae), and *Drassyllus nannellus* (Araneae: Gnaphosidae); Briggsdale-*G. clara*, *S. mccooki*, and *Haplodrassus chamberlini* (Araneae: Gnaphosidae); Lamar-*Gnaphosa saxosa* (Araneae: Gnaphosidae), *S. mccooki*, and

Hogna coloradensis (Araneae: Lycosidae). Two spider families, Gnaphosidae and Lycosidae, represented over 85% of the spider fauna collected from a shortgrass steppe ecosystem in Weld County, CO (Weeks and Holtzer 2000). Five families contained 61% of the species identified in a field crop survey of spiders in North America (Young and Edwards 1990). In Hungary, 10% of the spider species made up 60-90% of the entire community in a 10-year spider survey (Samu and Szinetar 2002). In a New Mexico biodiversity study of spiders in alfalfa, four species, *Pardosa sternalis* (Thorell) (Araneae: Lycosidae), *Misumenops* spp. (Araneae: Thomisidae), *Grammonota cf. pictilis* (O.P.-Cambridge) (Araneae: Linyphiidae), and *Tetragnatha laboriosa* Hentz (Araneae: Tetragnathidae) comprised 95% of the collection (Richman et al. 1990). As a result of the domination of a few individuals representing the majority of the collection, the remaining individuals can cause drastic differences between species diversity values (Samu and Szinetár 2002).

The species accumulation curves and the high percentage of singletons present indicate that the biodiversity of spiders was underestimated. This may be due to the sampling methods utilized. Each sampling method has limitations, thus, it is difficult to capture the entire species composition within an area. For example, vacuum sampling can vary based on the site, climate, and degree of weed cover (Sunderland and Topping 1995). Vacuum sampling for all sites also was dominated by immature spiders (83%), which could not be identified to species. Additionally, immature spiders are typically more active within the vegetation during the daytime, and adult activity is limited (Sunderland and Topping 1995). Although three sampling techniques were employed,

lookdown and vacuum techniques were only utilized for four months of the two years. Some dispersing spiders from neighboring fields might have been missed in early spring. Many of the singletons sampled could have drifted in from adjacent fields and only been sampled occasionally.

In Lamar, the increased biodiversity in fallow indicated in the rarefaction curves may be due to cracks and holes throughout the soil, which spiders could reside. Holes (10 cm deep) were created in a study in Belgian maize fields, which resulted in a significant increase in the linyphiid spiders *Bathyphantes gracilis* (Blackwall) and *Lepthyphantes tenuis* (Blackwall). Similarly, *L. tenuis* densities were enhanced when additional holes were created in soils within a wheat crop (Samu et al. 1996). In Briggsdale, no tillage was conducted, which allowed for more residue in the fallow. Crop residue, mulch, and thatch can increase habitat availability for spiders, providing protection from extreme climatic conditions and predation (Riechert and Bishop 1990, Schmidt et al. 2004, Langellotto and Denno 2004, Finke and Denno 2006). It may be that the soil cracks and crop residues within the fallow provided suitable habitat for colonization, and crop type was not important for establishment. Spider density and biodiversity may be more dependent upon areas for colonization and not particularly associated with particular crops.

The composition of spiders in eastern Colorado agroecosystems is drastically different from that reported in Western Europe. Spider densities in European cereal agroecosystems can potentially reach up to 600 per m^2; however, in the United States, densities average a maximum of 2 per m^2 in cropping systems (Nyffeler and Sunderland

2003). In a study of spider biodiversity and density in Lamar, CO, during one wheat-growing season using a D-vac suction sampler and hand search, spider densities were estimated at 0.7 spiders per m^2 with 14 species collected within 11 families (Greenstone 2001).

Similarly, the faunal composition of spiders differs between the United States and Europe. Spiders in western European agroecosystems are generally dominated by Linyphiidae, which can comprise over 90% of the total spider fauna (Nyffeler and Sunderland 2003). In contrast, in the United States cropping systems, the spider fauna is generally more diverse with hunters (spiders that catch prey without a web) comprising over 50% of the total spiders (Nyffeler and Sunderland 2003). This study compliments previous literature from cropping systems in the United States (Young and Edwards 1990, Nyffeler 1999); the number of spider individuals within the families Gnaphosidae and Lycosidae families combined dominated the majority of the spider collection at all sites for this study (71% at Akron, 66% in Briggsdale, and 85% in Lamar).

The difference in faunal composition suggests important implications for biological control of pests in Colorado agroecosystems. In European cropping systems, the Linyphiidae are not only aggregating to patches with high aphid densities (Harwood et al. 2001) and consuming aphids (Nyffeler and Benz 1988), they also are killing numerous aphids within their webs, regardless of whether or not they are consuming them (Sunderland et al. 1986). The fraction of web-building spiders compared to hunting spiders may be suggestive of the functional biological control within the agroecosystem (Nyffeler and Sunderland 2003). As this ratio was low for this study, spiders may be of

limited value for the biological control of crop pests in eastern Colorado dryland production systems. Because spider density and biodiversity was not enhanced within these systems and the percentage of hunting spiders was high in comparison to web-building spiders with a greater biological control function, it could be that the potential of the indigenous spider community for cereal aphid management in eastern Colorado is limited.

Conclusions

1. A total of 11,207 spiders were collected from Akron, Briggsdale, and Lamar, CO. Of these spiders, 119 species from 17 families were represented; 32 species were common to all sites, 16, 12, and 19 unique to Akron, Briggsdale, and Lamar, respectively.
2. Activity density of spiders was consistently affected by date but rarely by the conventional or crop-intensified treatments.
3. The spider fauna in eastern Colorado agroecosystems were predominately hunting species, suggesting that the biological control function of these fauna may not be as important as the dominant web-building fauna in western European agroecosystems.

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APPENDIX A. PLOT MAP FOR AKRON, CO, 2002-2007.

	Rep 4											
	401	**402**	**403**	**404**	**405**	**406**	**407**	**408**	**409**	**410**	**411**	**412**
2002		**Wheat**			**Corn**		**Sunfl**	**Fallow**		**Wheat**		**Fallow**
2003		**Fallow**			**Fallow**			**Wheat**		**Corn**	**Sunfl**	**Wheat**
2004		**Wheat**		**Sunfl**	**Wheat**			**Fallow**		**Fallow**		**Corn**
2005	**Sunfl**	**Fallow**			**Corn**			**Wheat**		**Wheat**		**Fallow**
2006		**Wheat**			**Fallow**		**Sunfl**	**Fallow**		**Millet**		**Wheat**
2007		**Fallow**			**Wheat**			**Wheat**		**Fallow**		**Millet**
	Rep 3											
	301	**302**	**303**	**304**	**305**	**306**	**307**	**308**	**309**	**310**	**311**	**312**
2002			**Fallow**	**Corn**			**Wheat**		**Fallow**	**Sunfl**	**Wheat**	
2003			**Wheat**	**Fallow**	**Sunfl**		**Corn**		**Wheat**		**Fallow**	
2004			**Fallow**	**Wheat**			**Fallow**		**Corn**		**Wheat**	**Sunfl**
2005		**Sunfl**	**Wheat**	**Corn**			**Wheat**		**Fallow**		**Fallow**	
2006			**Fallow**	**Fallow**			**Millet**		**Wheat**		**Wheat**	
2007			**Wheat**	**Wheat**			**Fallow**		**Millet**		**Fallow**	
	Rep 2											
	201	**202**	**203**	**204**	**205**	**206**	**207**	**208**	**209**	**210**	**211**	**212**
2002	**Fallow**			**Wheat**	**Fallow**	**Wheat**	**Sunfl**		**Corn**			
2003	**Wheat**	**Sunfl**		**Corn**	**Wheat**	**Fallow**			**Fallow**			
2004	**Fallow**			**Fallow`**	**Corn**	**Wheat**			**Wheat**	**Sunfl**		
2005	**Wheat**			**Wheat**	**Fallow**	**Fallow**			**Corn**		**Sunfl**	
2006	**Fallow**			**Millet**	**Wheat**	**Wheat**	**Sunfl**		**Fallow**			
2007	**Wheat**	**Sunfl**		**Fallow**	**Millet**	**Fallow**	**Sunfl**		**Wheat**	**Sunfl**	**Sunfl**	
	Rep 1											
	101	**102**	**103**	**104**	**105**	**106**	**107**	**108**	**109**	**110**	**111**	**112**
2002			**Wheat**	**Fallow**		**Corn**			**Fallow**	**Wheat**		**Sunfl**
2003			**Corn**	**Wheat**	**Sunfl**	**Fallow**			**Wheat**	**Fallow**		
2004	**Sunfl**		**Fallow**	**Corn**		**Wheat**			**Fallow**	**Wheat**		
2005			**Wheat**	**Fallow**		**Corn**	**Sunfl**		**Wheat**	**Fallow**		
2006			**Millet**	**Wheat**		**Fallow**			**Fallow**	**Wheat**		**Sunfl**
2007	**Sunfl**		**Fallow**	**Millet**	**Sunf**	**Wheat**	**Sunfl**		**Wheat**	**Fallow**		**Sunfl**

APPENDIX B. PLOT MAP FOR BRIGGSDALE, CO, 2002-2010.

Insects & Dryland Cropping Systems Experiment

Briggsdale, CO

	Rep 3												Rep 4											
Plot	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
Rot.	3	4	4	2	4	2	4	3	4	1	4	3	3	3	2	4	4	4	2	4	3	4	4	1
Trtmt.	3	6	7	2	11	1	9	5	10	12	8	4	4	5	1	7	9	8	2	6	3	10	11	12
Var.	R S	R S	S R	S R	S R	R S	R S	R S	R S	S R	S R	S R	R S	R S	R S	S R	S R	S R	S R	S R	R S	R S	S R	RS
2002	W	C	Sf	W	W1	F	C	F	W2	GS	F	M*	M*	F	F	Sf	C	F	W	C	W	W2	W1	GS
2003	M*	Sf	F	F	W2	W	C	W	C		W1	F	F	W	W	F	C	W1	F	Sf	M*	C	W2	
2004	F	F	W1	W	C	F	Sf	M*	C		W2	W	W	F	F	W1	Sf	W2	W	F	F	C	C	
2005	W	W1	W2	F	C	W	M*	F	Sf		C	M*	M*	F	W	W2	F	C	F	W1	W	Sf	C	
2006	M*	W2	C	W	Sf	F	W1	W	F		C	F	F	W	F	C	W1	C	W	W2	M*	F	Sf	
2007	F	C	C	F	F	W	W2	M*	W1		Sf	W	W	M*	W	C	W2	Sf	F	C	F	W1	F	
2008	W	C	Sf	W	W1	F	C	F	W2		F	M*	M*	F	F	Sf	C	F	W	C	W	W2	W1	
2009	M*	Sf	F	F	W2	W	C	W	C		W1	F	F	W	W	F	C	W1	F	Sf	M*	C	W2	
2010	F	F	W1	W	C	F	Sf	M*	C		W2	W	W	F	F	W1	Sf	W2	W	F	F	C	C	

County Road 84 on north edge of plots

➦ Plot 1 starts at first REA pole west of the trees.

	Rep 1												Rep 2											
Plot	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Rot.	4	2	4	3	2	4	4	1	3	4	4	3	3	4	3	2	1	4	3	2	4	4	4	4
Trtmt.	9	2	10	3	1	7	6	12	4	8	11	5	5	6	4	1	12	10	3	2	7	9	11	8
Var.	S R	S R	S R	S R	S R	R S	R S	R S	S R	R S	S R	S R	S R	R S	R S	S R	S R	R S	S R	S R	R S	S R	S R	S R
2002	C	W	W2	W	F	Sf	C	GS	M*	F	W1	F	F	C	M*	F	GS	W2	W	W	Sf	C	W1	F
2003	C	F	C	M*	W	F	Sf		F	W1	W2	W	W	Sf	F	W		C	M*	F	F	C	W2	W1
2004	Sf	W	C	F	F	W1	F		W	W2	C	M*	M*	F	W	F		C	F	W	W1	Sf	C	W2
2005	F	F	Sf	W	W	W2	W1		M*	C	C	F	F	W1	M*	W		Sf	W	F	W2	F	C	C
2006	W1	W	F	M*	F	C	W2		F	C	Sf	W	W	W2	F	F		F	M*	W	C	W1	Sf	C
2007	W2	F	W1	F	W	C	C		W	Sf	F	M*	M*	C	W	W		W1	F	F	Sb	W2	F	Sf
2008	C	W	W2	W	F	Sf	C		M*	F	W1	F	F	C	M*	F		W2	W	W	Sf	C	W1	F
2009	C	F	C	M*	W	F	Sf		F	W1	W2	W	W	Sf	F	W		C	M*	F	F	C	W2	W1
2010	Sf	W	C	F	F	W1	F		W	W2	C	M*	M*	F	W	F		C	F	W	W1	Sf	C	W2

County Road 84 on north edge of plots

Rotations
1 = Opportunity
2 = Wheat/Fallow
3 = Wheat/Millet*/Fallow
4 = Wheat/Wheat/Corn/Corn/Sunflower/Fallow (Changed from Wheat/Wheat/Corn/RRSoybean/Sunflower/Austrian Winter Pea in 2002)

Variety Treatments
S = Susceptible Variety
R = Resistant Variety

GS = Grain Sorghum

APPENDIX C. PLOT MAP FOR LAMAR, CO, 2002-2008.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	W-F		W-S-F			W-S-F			W-F		W-S-F			W-F		W-S-F				W-F
2002	W	F	S	F	W	W	F	S	F	W	S	W	F	F	W	S	W	F	W	F
2003	F	W	F	W	S	S	W	F	W	F	F	S	W	W	F	F	S	W	F	W
2004	W	F	W	S	F	F	S	W	F	W	W	F	S	F	W	W	F	S	W	F
2005	F	W	S	F	W	W	F	S	W	F	S	W	F	W	F	S	W	F	F	W
2006	W	F	F	W	S	S	W	F	F	W	F	S	W	F	W	F	S	W	W	F
2007	F	W	W	S	F	F	S	W	W	F	W	F	S	W	F	W	F	S	F	W
2008	W	F	S	F	W	W	F	S	F	W	S	W	F	F	W	S	W	F	W	F
	REP 1					REP 2					REP 3					REP 4				

W-F=Wheat-fallow rotation
W-S-F=Wheat-sorghum-fallow rotation

CHAPTER 3-IMPLICATIONS OF RUSSIAN WHEAT APHID, *DIURAPHIS NOXIA*, FALLING RATES FOR BIOLOGICAL CONTROL IN RESISTANT AND SUSCEPTIBLE WHEAT

Abstract

The restriction of aphid reestablishment onto plants by epigeal predators represents a critical component of integrated pest management. To further realize the potential that these predators might have in control programs, it is necessary to quantify such behavior as aphid falling rate to reveal the number of aphids that are available as potential prey. This study calculated the falling rate of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae), and tested whether this aphid more likely fell from wheat plants that differed between flat leaf architecture versus those with furled leaves. Specifically, the hypothesis was tested that a resistant wheat line (flat leaves) will have a higher aphid falling rate than a susceptible closely-related line (furled leaves). The experiment was performed at Fort Collins and Akron, Colorado, USA, from May through July, 2008. Aphids were sampled from infested wheat rows to estimate aphid density, and sticky traps were used to capture falling aphids and to measure falling rate. Falling rates ranged from 0.7% to 69.5% in Fort Collins and from 1.4% to 59.5% in Akron. The falling rate of *D. noxia* was more influenced by plant growth stage than aphid densities, with the highest falling rate occurring after wheat senescence.

Wheat plants with flat leaf architecture did not significantly increase aphid falling rate. *Diuraphis noxia* falls at a higher rate at lower aphid densities, which is when epigeal predators could have their greatest biological control impact.

Introduction

The high diversity of natural enemies frequenting agroecosystems are often purported to translate into improved regulation of pest species (Straub and Snyder 2006, Straub et al. 2008) through mechanisms of niche partitioning in space (Finke and Snyder 2008) and time (Lundgren et al. 2009). Such diverse foraging within a complex community of natural enemies can therefore allow for co-existence of species most adept at pest suppression. Those species that inhabit different strata within the crop thereby have the capacity to impact pests at multiple levels, and it is the community of natural enemies that act in synchrony with one another, rather than individual species acting alone, that provide greatest value in biological control (Sunderland et al. 1997). The epigean fauna that consist of, amongst others, ground beetles (Coleoptera: Carabidae), rove beetles (Coleoptera: Staphylinidae), and spiders (Araneae) should therefore be considered when developing a robust integrated pest management strategy for aphids, given that up to 90% of falling aphids will successfully recolonize the plant if not preyed upon (Sopp et al. 1987, Winder et al. 1994). This is further highlighted by the abundance of such predators in agricultural systems (Luff 1983, Riechert and Lockley 1984, Booij and Noorlander 1992, Fan et al. 1993, Lövei and Sunderland 1996, Kromp 1999). For example, infestation of wheat tillers by English grain aphids, *Sitobion avenae* (F.) (Hemiptera: Aphididae), has been negatively related to the activity density of linyphiid spiders,

carabids, and staphylinids (Winder 1990, Duffield et al. 1996). Ground beetles can also aggregate to areas with high aphid densities (Bryan and Wratten 1984), and post-mortem analysis of predator feeding behavior has revealed that many ground-active species consume large numbers of aphids (e.g., Sunderland et al. 1987, Harwood et al. 2004, Winder et al. 2005). In Europe, experimentally manipulated ground predator densities resulted in aphid reduction in maize (Lang et al. 1999), barley (Chiverton 1986, Ekbom et al. 1992, Ostman et al. 2003), and wheat (Collins et al. 2002, Lang 2003, Schmidt et al. 2004). In addition, early season activity by generalist predators can also help prevent a rapid increase in pest numbers, a time of year when generalists feed and have greatest impact on pest population dynamics (Chiverton 1987, Landis and van der Werf 1997; Harwood et al. 2004, 2007).

In order to examine the potential role of ground-active predators on aphid population dynamics, it is first necessary to quantify the availability (i.e., number) of aphids that fall to the ground and, thus, become potential prey. Many aphids exhibit a dropping defense mechanism triggered by disturbance from natural enemies, by the release of an alarm pheromone, or by weather (Hughes 1963, Cannon 1986, Sunderland et al. 1986, Winder 1990, Ferran and Deconchat 1992, Gowling and van Emden 1994, Winder et al. 1994, Mann et al. 1995, Clark and Messina 1998, Shah et al. 1999). Falling rates have, for example, been studied in species such as *S. avenae*, where daily dropping rates in the United Kingdom ranged from 95% (growth stage: Zadoks 30; based on Zadoks scale, a widely used cereal development scale in agriculture (Zadoks et al. 1974)) to 20% and below (growth stage: Zadoks 71-94) and the following year from 15% (growth stage: Zadoks 69) to 35% (growth stage: Zadoks 90) (Sunderland et al. 1986). In another study,

falling rates of this same aphid (and in the same country) ranged from 18-30% throughout the wheat growing season (Winder 1990), and the density of falling *S. avenae* peaked at 348 m^{-2} per day (Winder et al. 1994). Similarly, other aphids also fall from the crop with high frequency; the rose grain aphid, *Metopolophium dirhodum* (Walker) (Hemiptera: Aphididae), can fall at a rate from approximately 40% (growth stage: Zadoks 69) to 100% (growth stage: Zadoks 90) (Sunderland et al. 1986); while the pea aphid, *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae), has been documented as falling at a rate of 7% (over 24 h) from alfalfa in the presence of the hemipteran predators *Nabis americoferus* Carayon (Heteroptera: Nabidae), *Geocoris punctipes* (Say) (Heteroptera: Geocoridae), and *Orius insidiosus* (Say) (Heteroptera: Anthocoridae) and 60% in the presence of the coccinellid, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) (Losey and Denno 1998). Given such high dropping rates in aphid populations, ground-active predators are likely exposed to significant numbers of aphids and may subsequently feed on these prey, thereby preventing them from reestablishing on crop plants. Such suppression could delay or, at least, limit the rapid increase in aphid populations that afflict many agroecosystems, possibly reducing the reliance on pesticide applications for aphid control.

Accurate quantification of the falling rate of aphids is therefore essential to provide an insight into the role of epigeal predators in the biological control of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae). *Diuraphis noxia* is a common pest of wheat, *Triticum aestivum* L. (Poales: Poaceae), and other susceptible small grains in all major wheat growing countries except Australia (Elliott et al. 1998). To date, *D. noxia* damage has been managed by aphid-resistant wheat cultivars and

pesticides. Further complicating management issues in the United States, however, has been the discovery of a new biotype of *D. noxia* in 2003, (RWA2) (Haley et al. 2004). Biotype RWA2 emerged based on its virulence to cultivars containing the genes *Dn4* and *Dny*, which confer resistance to biotype RWA1 (Haley et al. 2004, Collins et al. 2005, Jyoti and Michaud 2005, Qureshi et al. 2006, Peng et al. 2007). In addition, several biotypes have since been identified (Burd et al. 2006, Weiland et al. 2008), and further studies quantifying the efficacy of biological control agents are therefore required.

Architectural traits may enhance the falling rate of aphids. Plant damage that is induced by *D. noxia* feeding prevents the wheat leaf from unfurling (Webster et al. 1987a, Burd and Burton 1992, Archer et al. 1998), which presents challenges for natural enemies and insecticides to reach the aphids. *Diuraphis noxia*-resistant cultivars are characterized by having unfurled leaves and no leaf streaking (Hawley et al. 2003, Lapitan et al. 2007), and *D. noxia* tends to feed within the rolls of furled immature wheat leaves or within the sheath of mature leaves (Burd and Burton 1992). Therefore, leaf unfurling may allow for increased exposure of *D. noxia* to chemical and biological controls (Hawley et al. 2003). Additionally, aphids might have difficulty remaining on the leaf or increase their exposure to external disturbances, possibly triggering a higher falling rate than in a susceptible cultivar.

The resistant wheat line used for this study (STARS 02RWA2414-11/5*CO00554) contains the gene *Dn7*, which has demonstrated resistance to RWA2 (Haley et al. 2004, Collins et al. 2005, Jyoti and Michaud 2005, Qureshi et al. 2006, Peng et al. 2007). Wheat plants from this line expressed approximately 50% resistance to RWA2 (J. Rudolph, Colorado State University, unpublished data). Wheat lines with a similar 50%

resistance expression in a study with biotype RWA1 yielded more and supported fewer aphids per tiller than populations that were 100% susceptible (Randolph et al. 2007). Therefore, 50% resistance should be sufficient to reduce aphid densities and symptoms of *D. noxia* infestations, i.e., chlorosis and leaf rolling, and the lower level of leaf rolling should lead to greater falling rates than the susceptible line.

Architectural traits may also influence aphid-predator interactions since the combination of natural enemies and plant resistance may have an additive or synergistic effect on aphid suppression. Selection of wheat plants that allow unfurling is preferable so that natural enemies and parasitoids can easily access *D. noxia* (Webster et al. 1987b, Burd et al. 1993, Kauffman and Laroche 1994) given that several species of coccinellids have difficulty accessing *D. noxia* within furled leaves (Kauffman and Laroche 1994). For example, contact and capture efficiency of *D. noxia* by the fourteen-spotted ladybeetle, *Propylea quatuordecimpunctata* (L.) (Coleoptera: Coccinellidae), (Messina et al. 1997, Clark and Messina 1998) and the larvae of the green lacewing, *Chrysoperla plorabunda* (Fitch) (Neuroptera: Chrysopidae), (Messina et al. 1997), increased on Indian ricegrass, *Oryzopsis hymenoides* (Roemer and Schultes) Ricker (Poales: Poaceae), with narrow, tightly-rolled leaves with fewer areas for shelter in comparison to crested wheatgrass, *Agropyron cristatum* L. Gaertn. (Cyperales: Poaecae), with wide, flat leaves. A synergistic effect was also observed between a *D. noxia*-resistant wheat line and predation by *C. plorabunda*, resulting in the reduction of *D. noxia* densities on wheat (Messina and Sorenson 2001). Furthermore, a positive synergism in reducing aphid densities was demonstrated with parasitoids and resistant wheat lines for the rose-grain aphid, *M. dirhodum* (Gowling and van Emden 1994) and the greenbug, *Schizaphis*

graminum (Rondani) (Hemiptera: Aphididae) (Starks et al. 1972). Parasitoids and a *D. noxia*-resistant line of slender wheatgrass, *Elymus trachycaulus ssp. trachycaulus* (Link) Gould ex Shinners, had the effect of reducing *D. noxia* densities (Reed et al. 1992). It is therefore apparent that resistant cultivars may increase the efficiency of biological control and the overall reduction of aphid densities.

Consequently, there is a clear need to understand mechanisms associated with aphid dropping rates in order to fully realize the potential of epigeal natural enemies in biological control. The prevention of plant re-establishment by aphids forms a crucial component of integrated pest management programs given the likelihood for successful recolonization of the plant if the fallen aphids are not preyed upon. This study was designed to determine the likelihood for *D. noxia* to fall from resistant and susceptible wheat plants with flat leaves and rolled leaves, respectively. We hypothesized that a resistant wheat line will have a higher aphid falling rate than a susceptible closely-related line because resistant wheat lines have flat leaf architecture, making it difficult for *D. noxia* to remain on the tiller.

Methods

Study site and planting regime

Research was conducted in winter wheat fields at Fort Collins (40.65099°N, -104.99671°W; 1534 m) and Akron (40.16033°N, -103.14161°W; 1421 m), Colorado, USA, throughout the wheat growing season (May-July) in 2008. The wheat lines used for this study consisted of two closely-related lines from the Colorado State University wheat breeding program. One of these, CO00554, (TAM 302/Akron//Halt pedigree) is

susceptible to biotype RWA2, but carries the *Dn4* gene from Halt for biotype RWA1 resistance. The other line (STARS 02RWA2414-11/5*CO00554) carries the *Dn7* gene effective against both biotype RWA1 and biotype RWA2 from STARS 02RWA2414-11 and was derived through backcrossing with CO00554 as the recurrent parent.

The Fort Collins site was irrigated once prior to planting on 3 September 2007 to insure uniform plant emergence, and wheat was grown according to standard agronomic practices for the region. Plots were 3.24 m^2 with six wheat rows in Fort Collins and 4.56 m^2 with seven wheat rows each in Akron due to differences in local production practices. The wheat was planted on 11 September 2007 in Fort Collins and on 23 September 2007 in Akron. "Hatcher" wheat (Haley et al. 2005) was planted as a buffer between and outside of the plots at Fort Collins, and "Prairie Red" wheat (Quick et al. 2001) was planted in Akron. On 6 May 2008, plots at Akron were treated with 430 g (AI)/ha 2,4-D (2,4-D Lo-V 6E, Universal Crop Protection Alliance, Eagan, MN), 12.7 g (AI)/ha triasulfuron + 79.2 g (AI)/ha dicamba (Rave, Syngenta Crop Protection, Greensboro, NC), and 0.25% v/v non-ionic surfactant (Activator 90, Loveland Industries, Greeley, CO) for weed control. No herbicides were applied at the Fort Collins site.

Insect sampling protocols

Within each plot, winter wheat plants were infested with greenhouse-reared (16:8 L:D cycle, 24°C, 65% humidity) biotype RWA2 using a Davis inoculator (Davis and Oswalt 1979). Three, one-meter rows in the center of each plot were infested with a 1x and 10x aphid infestation level at both Akron and Fort Collins, which corresponded to

approximately 246 and 2,460 aphids at the Fort Collins site on 7 March 2008 and 210 and 2,100 aphids on 20 March 2008 at Akron. Past experience with establishment of *D. noxia* infestations has shown that these levels are adequate for initiating a range of aphid densities sufficient for regression analysis (Randolph et al. 2005a, 2005b; Randolph et al. 2006, Randolph et al. 2007). Infestation numbers to be applied in the field were estimated by using the Davis inoculator to deliver aphids to 10 Petri dishes. The number of *D. noxia* delivered per inoculator delivery unit was averaged, providing an estimate of the number of aphids delivered to wheat in the field. The three infested rows were used to estimate absolute aphid densities and to quantify the frequency of aphid dropping behavior using ground-based sticky traps that also simulate web-site interception frequencies of epigeal linyphiid spiders (Harwood et al. 2001, 2003;Harwood and Obrycki 2007), major predators of falling aphids in the field (Sunderland et al. 1987, Harwood et al. 2004). At Fort Collins, experimental plots were sampled at Zadoks growth stages 33, 42, 59, 77 and 87, and the Akron research site was sampled at Zadoks growth stages 37, 66, and 87.

The mean density of aphids on wheat tillers was estimated by removing 14 cm^2 of wheat tillers randomly from one of the three infested rows every two weeks from each plot. Tillers were cut and removed at ground level, placed into a 3.8 L plastic bag, and held on ice until they were transferred into Berlese funnels for 24 h. Aphids were extracted into 75% ethanol for long-term storage and subsequent counting.

Fallen aphids were sampled using acrylic sheeting (surface area of 141 and 182 cm^2 at Fort Collins and Akron, respectively) coated with a thin film of Tangletrap Insect Trap Coating® (Tanglefoot Co., Grand Rapids, Michigan, USA) using a medium-consistency

brush-on formulation. Surface area of the squares differed by location because width was determined by row spacing. The sticky traps were placed on the ground in between rows, taking care not to disturb aphids on adjacent tillers. Sticky traps are highly efficient at catching falling, rather than crawling, aphids (Fraser 1982) and, thus, are appropriate to measure aphid dropping rate and possible spider web-site interception frequencies (Harwood et al. 2001, 2003). The sticky traps were left *in situ* for 24 h on each sample date at each site, collected, and transported to the laboratory in a cooler for counting. All invertebrates (including *D. noxia*) were counted and identified under a dissecting microscope. Aphids that were on the sides of the traps were ignored to avoid counting crawling aphids. In total, 32 sticky traps were set at each sampling period at each location (two wheat lines, two infestation levels, and eight repetitions).

Aphid falling rate, as a percentage of total aphid population, was calculated as the number of aphids per ha intercepted by sticky traps divided by the total activity density of aphids per ha estimated from both the sticky trap and wheat tiller samples. Aphid counts from the wheat tiller samples and sticky traps were converted to aphid density per ha. The falling rate percentage was calculated as ST/(WT+ST) where ST is the calculated sticky trap density per ha and WT is the wheat tiller density per ha.

Given that setting traps might dislodge some aphids, thus leading to an overestimation of aphid falling rates (Sunderland et al. 1986), additional traps were set out at each date in one repetition of each treatment, removed immediately after placement, and the number of aphids counted. The percentage of dislodged aphids, averaged over date, at Fort Collins and Akron was extremely low, signifying the

negligible likelihood for overestimating falling rates of aphids using ground-based interception traps.

Data Analysis

The data were analyzed as a split-plot design with repeated measures, with the whole-plot factor as infestation level and the subplot factor as level of resistance. The effects of date, infestation level, and level of resistance were analyzed with three response variables: (1) density of *D. noxia* on wheat tillers; (2) density of *D. noxia* on sticky traps; and (3) falling rate. Sites were analyzed separately because sampling dates varied between sites. For the density of *D. noxia* on both wheat tillers and sticky traps, mixed models with autoregressive errors and unequal variances across dates were considered. A model was selected based on the lowest Akaike's Information Criterion (AIC) value, which is used to measure the best fit model, and restricted maximum likelihood (REML) was used as a method for estimating the parameters of the model (SAS Institute 2002-2003). A mixed model with an autoregressive order 1 covariance structure with heterogeneous variances across dates (ARH(1)) was chosen as the appropriate model for the density of aphids on wheat tillers at the Fort Collins site. A mixed model with unstructured covariance with heterogeneous variances across dates (un (1)) was chosen for the density of aphids on wheat tillers at Akron and the density of aphids on sticky traps at both Fort Collins and Akron. For the falling rate response variable, a generalized linear mixed model (GLMM) with an autoregressive order 1 covariance structure with heterogeneous variances across dates was chosen, which fits models to data with correlations (SAS Institute 2002-2003). Degrees of freedom for comparisons were estimated using the Kenward-Roger method (Kenward and Roger 1997) for all response

variables. Aphid densities were $\log_{10}$ transformed for the number of aphids on the wheat tillers and log (x + 1) for the number of aphids on the sticky traps to homogenize the variances. Aphid densities were not transformed for the falling rate response variable. If any fixed effects within the model were significant ($P \leq 0.05$), means were separated and compared with t-tests using the "lsmeans" procedure, which controlled for comparisonwise error ($\alpha = 0.05$) (SAS Institute 2002-2003). Untransformed means ± one standard error are presented in tables and figures.

Results

Diuraphis noxia was the most abundant aphid present in this study. Other aphids that were present included the bird cherry-oat aphid, *Rhopalosiphum padi* L. (Hemiptera: Aphididae) (mean 176 ± 34 cm^{-2} in Fort Collins and 27 ± 7 cm^{-2} in Akron, averaged over date, resistance, and infestation level), and the English grain aphid, *S. avenae* (mean 1.3 ± 0.03 cm^{-2} in Fort Collins and 0.07 ± 0.07 cm^{-2} in Akron, averaged over date, resistance, and infestation level). These aphids were not included in any of the analyses.

Fort Collins Research Site

Aphid densities on wheat tillers differed between the two original infestation levels ($F_{1,8.6}$=42.03, P=0.0001), and there was a interaction between date and infestation level for aphid density ($F_{4,54.1}$=9.83, P< 0.0001). The 10x infestation level was higher than the 1x level, averaged over the level of resistance on May 4 ($t_{25.6}$=-6.43, P< 0.0001), May 21 ($t_{19.1}$=-6.29, P<0.0001), and June 4 ($t_{23.7}$=-4.80, P< 0.0001). The density of aphids on wheat tillers at Fort Collins varied with sample date ($F_{4,54.1}$=386.08, P< 0.0001), peaking

on June 4, and then declining in both the resistant and susceptible lines, and at both infestation levels (Table 3.1). Aphid densities varied with the level of resistance ($F_{1,29.1}$=31.92, P< 0.0001), with densities in susceptible treatments at least double those in the resistant treatments, although there was also a interaction between date and resistance for aphid density ($F_{4,54.1}$=3.55, P=0.0121). Additionally, there was a difference between the resistant and susceptible treatments, averaged over infestation levels, on May 21 ($t_{24.3}$=-4.59, P=0.0001), June 4 ($t_{25.2}$=-5.18, P<0.0001), and June 18 ($t_{21.2}$=-6.59, P<0.0001).

TABLE 3.1 MEAN DENSITY (M-2 D-1) OF BIOTYPE RWA2 *D. NOXIA* ON WHEAT TILLERS AT TWO INFESTATION LEVELS FOR RESISTANT (R) AND SUSCEPTIBLE (S) WHEAT LINES IN FORT COLLINS, CO, 2008[1,2].

	1xR	1xS	10xR	10xS
Date				
4 May	19	38	86	116
21 May	189	394	504	1227
4 June	835	1686	1764	5974
18 June	749	1405	635	1987
2 July	19	23	20	22
$F_{4,54.1}$ date		386.08		
$P > F$ date		< 0.0001		
$F_{1,29.1}$ resistance		31.92		
$P > F$ resistance		< 0.0001		

[1]Three, one-meter rows in the center of each plot were infested with a 1x and 10x aphid infestation level on 7 March 2008, which corresponded to approximately 246 and 2,460 aphids.
[2]Means within a column followed by the same capital letters are not significantly different (α = 0.05; PROC MIXED). Means within rows within each infestation level followed by the same lower case letters are not significantly different (α = 0.05; PROC MIXED).

Similarly, aphid activity density on sticky traps differed between infestation levels ($F_{1, 26.2}$=53.04, P<0.0001), and there was an interaction between date and infestation level for aphid activity density ($F_{4,48.1}$=35.17, P<0.0001). The 10x infestation level maintained a higher activity density of aphids on May 4 ($t_{25.2}$=-3.49, P<0.0018), May 21 ($t_{27.3}$=-8.98, P<0.0001) and June 4 ($t_{19.8}$=-9.62, P<0.0001), averaged over resistance. The number of aphids changed over time ($F_{4,48.1}$=269.56, P<0.0001) (Table 3.2), and a further

interaction occurred between date and resistance ($F_{4,48.1}$=2.55, P=0.0508). Interestingly, the interactions occurred on June 4 ($t_{21.1}$=-3.05, P=0.0060) and June 18 ($t_{29.6}$=-4.63, P<0.0001), where aphid activity-densities were at their highest on the traps.

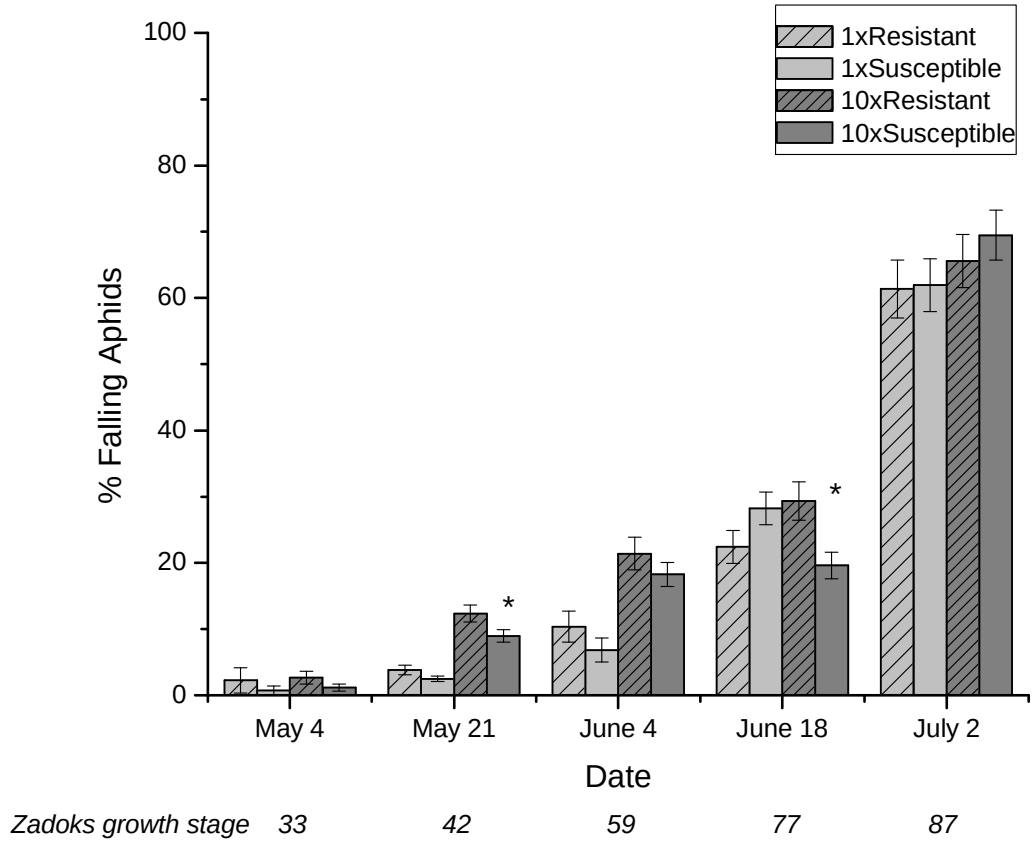
TABLE 3.2. MEAN DENSITY (M-2 D-1) OF BIOTYPE RWA2 *D. NOXIA* ON STICKY TRAPS AT TWO INFESTATION LEVELS FOR RESISTANT (R) AND SUSCEPTIBLE (S) WHEAT LINES IN FORT COLLINS, CO, 2008[1,2].

	1xR	1xS	10xR	10xS
Date				
4 May	0.4	0.4	2.7	2.0
21 May	9.3	12.4	84.8	147.6
4 June	120.9	149.5	474.9	1207.7
18 June	275.0	730.9	320.8	612.2
2 July	40.9	45.8	45.0	61.4
$F_{4,48.1}$ date			269.56	
$P > F$ date			< 0.0001	
$F_{1,32.5}$ resistance			2.69	
$P > F$ resistance			0.1105	

[1]Three, one-meter rows in the center of each plot were infested with a 1x and 10x aphid infestation level on 7 March 2008, which corresponded to approximately 246 and 2,460 aphids.
[2]Means within a column followed by the same capital letters are not significantly different (α = 0.05; PROC MIXED). Means within rows within each infestation level followed by the same lower case letters are not significantly different (α = 0.05; PROC MIXED).

Falling rates at Fort Collins ranged from 0.7% on May 4 to 69.5% on July 2 (Figure 3.1). For both the resistant and susceptible line and at each infestation level, falling rates increased over time ($F_{4,135}$=384.76, P<0.0001). Falling rates were higher at the 10x infestation level (16.9% ± 1.38) versus the 1x infestation level (10.4% ± 1.4), averaged over resistance ($F_{1,115.8}$=9.51, P=0.0026), and a further interaction occurred between resistance and date ($F_{4,53}$=2.84, P=0.0267). Additionally, there was an interaction that occurred between the factors date, resistance, and infestation level for falling rate ($F_{4,135}$=4.88, P=0.0010), as falling rates differed between the susceptible and resistant treatments on May 21 ($t_{19.42}$=2.20, P=0.0403) and on June 18 ($t_{34.11}$=2.81, P=0.0081).

FIGURE 3.1. MEAN FALLING RATE (± SE) OF BIOTYPE RWA2 *D. NOXIA* IN RESISTANT AND SUSCEPTIBLE WHEATS AT FORT COLLINS, CO, 2008.



*Significant difference between resistant and susceptible treatments α=0.05.

Akron Research Site

As with the Fort Collins site, aphid densities on wheat tillers differed between infestation levels ($F_{1,73.7}$=22.32, P <0.0001), and a further interaction occurred between infestation level and date ($F_{2,52}$=10.45, P=0.0002), which was apparent on May 13 (t_{28}=6.09, P<0.0001) and June 11 (t_{28}=-2.21, P=0.0353). The density of aphids on wheat tillers at Akron varied over time ($F_{2,52}$=96.48, P<0.0001) (Table 3.3), peaking on June 11

for all treatments and declining thereafter. Aphid densities varied with resistance at both the 1x and 10x infestation levels ($F_{1,73.7}$=54.28, P<0.0001).

TABLE 3.3. MEAN DENSITY (M-2 D-1) OF BIOTYPE RWA2 *D. NOXIA* ON WHEAT TILLERS AT TWO INFESTATION LEVELS FOR RESISTANT (R) AND SUSCEPTIBLE (S) WHEAT LINES IN AKRON, CO, 2008[1,2].

	1xR	1xS	10xR	10xS
Date				
13 May	35	89	137	441
10 June	351	984	424	1790
25 June	99	193	121	228
$F_{2,52}$ date		96.48		
$P > F$ date		< 0.0001		
$F_{1,73.7}$ resistance		54.28		
$P > F$ resistance		< 0.0001		

[1]Three, one-meter rows in the center of each plot were infested with a 1x and 10x aphid infestation level on 20 March 2008, which corresponded to approximately 210 and 2,100 aphids.
[2]Means within a column followed by the same capital letters are not significantly different (α = 0.05; PROC MIXED). Means within rows within each infestation level followed by the same lower case letters are not significantly different (α = 0.05; PROC MIXED).

As with the aphid densities on wheat tillers, more aphids were caught on the sticky traps at the 10x infestation level than the 1x infestation level ($F_{1,35.4}$=23.76, P<0.0001), and there was an interaction between date and infestation level ($F_{2,35.1}$=15.73, P<0.0001), specifically on May 13 ($t_{27.4}$=-4.19, P=0.0003) and June 11 ($t_{30.7}$=-3.84, P=0.0006), averaged over resistance. Similar to the Fort Collins site, an interaction occurred between date and level of resistance ($F_{2,35.1}$=6.06, P=0.0055) when the sticky traps contained the highest aphid-activity densities (Table 4) on June 11 ($t_{23.1}$=-5.32, P<0.0001) and June 25 ($t_{16.9}$=-4.59, P=0.0003). Additionally, aphid densities changed significantly over time ($F_{2,35.1}$=99.99, P<0.0001) (Table 3.4).

TABLE 3.4. MEAN DENSITY (M-2 D-1) OF BIOTYPE RWA2 *D. NOXIA* ON STICKY TRAPS AT TWO INFESTATION LEVELS FOR RESISTANT (R) AND SUSCEPTIBLE (S) WHEAT LINES IN AKRON, CO, 2008[1,2].

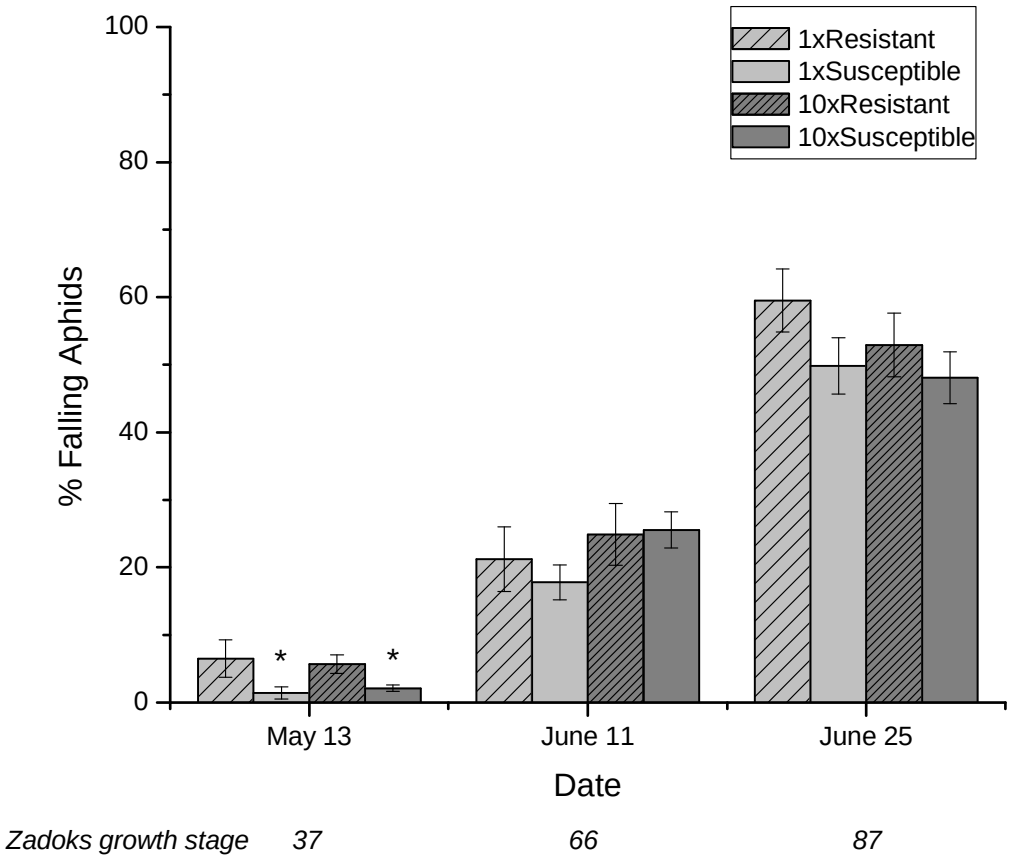
	1xR	1xS	10xR	10xS
Date				
13 May	2.3	1.4	8.0	9.9
10 June	86.3	165.9	131.9	605.1
25 June	140.4	190.4	131.9	202.6
$F_{2,35.1}$ date		99.99		

	1xR	1xS	10xR	10xS
Date				
$P > F$ date		< 0.0001		
$F_{1,30.2}$ resistance		1.94		
$P > F$ resistance		0.1743		

[1]Three, one-meter rows in the center of each plot were infested with a 1x and 10x aphid infestation level on 20 March 2008, which corresponded to approximately 210 and 2,100 aphids.
[2]Means within a column followed by the same capital letters are not significantly different (α = 0.05; PROC MIXED). Means within rows within each infestation level followed by the same lower case letters are not significantly different (α = 0.05; PROC MIXED).

The falling rates at Akron ranged from 1.4% on May 13 to 59.5% on June 25 (Figure 3.2) with falling rates changing over time for all treatments ($F_{2,38.1}$=99.13, P<0.0001). As anticipated, the falling rates were higher in the resistant treatments (22.6% ± 2.0) versus the susceptible treatments (14.3% ± 1.6) ($F_{1,60.4}$=10.49, P=0.0019). Similar to the Fort Collins site, there was an interaction between resistance and date ($F_{2,83}$=3.07, P=0.0518), which was apparent at the 1x infestation level resistant treatment compared with its paired susceptible treatment on May 13 (t_{83}=2.00, P=0.0487) and the 10x susceptible and resistant treatments on May 13 (t_{83}=2.95, P=0.0041).

FIGURE 3.2. MEAN FALLING RATE (± SE) OF BIOTYPE RWA2 *D. NOXIA* IN RESISTANT AND SUSCEPTIBLE WHEATS AT AKRON, CO, 2008.



*Significant difference between resistant and susceptible treatments α=0.05.

Discussion

The ability of epigeal natural enemies to contribute to valuable regulation of pests that dwell, for the most part, in the higher strata of the plant relies on the rate at which those prey fall from the crop and thus become "potential" prey for ground-based fauna. In recent years, considerable attention has focused on the role of management practices

and landscape diversity that promote ecosystem services provided by natural enemy communities (e.g., Fiedler et al. 2008, Gardiner et al. 2009), but understanding the role of these communities in biological control programs relies on our fundamental knowledge of the ecology and behavior of the prey, i.e. the pests. It is acknowledged that the community of natural enemies in agroecosystems contributes to pest suppression (Sunderland et al. 1997) by impacting prey though the partitioning of their resources. Additionally, many ground-based predators are generalists (e.g., carabids, staphylinids and spiders) and thereby impact pest communities early during the colonization phase of population growth by subsisting on alternative, non-pest prey when pests are scarce.

Understanding the falling rates of aphids, therefore, not only quantifies the variability that exists between resistant and susceptible wheat lines, but potentially provides valuable information for pest management programs. Falling rates of *D. noxia* ranged from less than 5% early in the season to over 60% for all treatments at the Fort Collins site and from 5% to over 50% in Akron. The falling rates of other aphid species also demonstrate a wide range of variability; *Sitobion avenae* falling rates ranged from 20-95% in one study (Sunderland et al. 1986) and 18-30% in another study (Winder 1990); *Metopolophium dirhodum* varied from 40-100% throughout the wheat-growing season; and *Acyrthosiphon pisum* falling rates varied from 7-60%, depending on which predator species was present (Losey and Denno 1998). Such variation has a profound implication for the ability of ground-based predators in biological control; generalists are unlikely to regulate pest populations during exponential phases of population growth, but early in the season when densities are low. However, at this time of year, the proportion falling from the crop was low, and, relative to the total population of aphids, few were likely to

become “potential” prey. If generalists preferentially forage on these scarce falling prey items as a means of diversifying their diet and optimizing the intake of essential nutrients and amino acids (Greenstone 1979, Mayntz et al. 2005), a greater level of biological control may result, as fewer would be likely to recolonize the plant. Indeed, ground-based predators often forage at disproportionately high levels on scarce falling aphids (Harwood et al. 2004), suggesting such mechanisms operate under open-field conditions.

Interestingly, the falling rate of *D. noxia* was influenced more by plant growth stage than by abundance as the highest falling rate coincided with plant senescence at both sites. Following senescence, *D. noxia* utilizes alternate hosts in between wheat harvest and fall planting (Kriel et al. 1986, Kindler and Springer 1989), thus aphids are most likely fleeing wheat plants in search of new hosts. This behavior was evident in dryland wheat in Texas, where the highest dispersal of *D. noxia* followed wheat senescence (Archer and Bynum 1993). Furthermore, the utilization of alternate hosts may be related to a decline in host quality. For example, *A. pisum* was more likely to drop from the broad bean plant *Vicia faba* L. (Fabales: Leguminosae) when food quality decreased (Dill et al. 1990). Consequently, host quality may be a cue for initiation of *D. noxia* dispersal to new hosts since falling rate appears to increase with decreasing host quality.

Diuraphis noxia falling rates were highest when aphid densities were lower in all treatments at both sites. Mean aphid densities on wheat tillers were greatest at Fort Collins on June 4 (growth stage: Zadoks 59), but the greatest falling rate occurred at the last growth stage with the lowest aphid densities sampled on July 2 (growth stage: Zadoks 87). In Akron, the greatest density of aphids was recorded on June 10 (growth stage: Zadoks 56) with the greatest falling rate occurring on June 25 (growth stage:

Zadoks 87) when aphid densities were lower. Both *S. avenae* (Sopp et al. 1987, Winder 1990) and *M. dirhodum* (Walker) (Sopp et al. 1987) fell at a higher rate at lower aphid densities. Similarly, Losey and Denno (1998) reported that *A. pisum* falling rate did not increase with increasing aphid densities. The fact that higher falling rates occur at lower aphid densities profoundly impacts the role of generalist predators in biological control because pressure is only likely to be exerted when pest densities are low.

The number of fallen *D. noxia* available as potential prey for ground predators was substantial for all treatments at all sampling dates. This ranged from less than 1 m^{-2} d^{-1} early in the season to >1200 aphids m^{-2} d^{-1} at wheat senescence at Fort Collins and from 2 m^{-2} d^{-1} to >600 aphids m^{-2} d^{-1} at senescence in Akron for all treatments. In British agricultural systems, the number of fallen *S. avenae* during natural infestations ranged from approximately 10 aphids m^{-2} d^{-1} at stem elongation of wheat (growth stage: Zadoks 30) and peaked at approximately 150 m^{-2} d^{-1} during flowering (growth stage: Zadoks 62), and the following year the number of fallen aphids ranged from 184 m^{-2} d^{-1} at stem elongation and increased to 348 m^{-2} d^{-1} during late heading/flowering (growth stage: Zadoks 50-62) (Sunderland et al. 1986). When measuring the recolonization rate of *S. avenae*, 90% of the aphids returned to the wheat canopy after release on the soil surface (Sopp et al. 1987, Winder et al. 1994). Although recolonization onto wheat tillers is likely for *D. noxia* after falling, many fall to the ground and represent a likely food source for epigeal predators.

Diuraphis noxia-resistant wheat lines did not influence falling rate. A wheat line conferring close to 100% biotype RWA2 resistance might enhance the unfurling of the

wheat leaf, further complicating shelter areas and colonization for *D. noxia*. This may increase the likelihood of aphids falling from the leaf.

Ultimately, the establishment of *D. noxia* falling rates is essential to understanding its management by generalist predators. The availability of *D. noxia* as potential prey for the epigeal fauna suggests that these abundant natural enemies may be an important component of biological control. Although the falling rate of *D. noxia* is highest at the senescence of the wheat, the greatest impact of generalist predation is more likely to be during colonization when aphid densities are low, preventing recolonization of *D. noxia* on wheat tillers and subsequent increases to economic injury levels. This research has clearly demonstrated the potential role of the epigeal community in the biological control of the Russian wheat aphid, and further research is now required to identify, in a quantitative manner, the impact of predator communities on pest population dynamics in the field.

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CHAPTER 4-MOLECULAR ELUCIDATION OF FOOD WEB PROCESSES EXHIBITED BY SPIDERS IN EASTERN COLORADO WINTER WHEAT

Abstract

The Russian wheat aphid, *Diuraphis noxia* (Hemiptera: Aphididae) is a major pest of wheat and has caused over $893 million in losses in the United States from 1987 to 1993. Determining effective predators of pests can allow for the conservation of key species. The goal of this study was to track the predation of two dominant spider species in northern Colorado wheat agroecosystems on *D. noxia* using PCR and species-specific primers. A partial 1146 bp sequence from the mitochondrial cytochrome oxidase I (COI) gene was used and aligned with other non-target sequences to create two primer pairs that amplified a 227 bp fragment of *D. noxia* DNA. Three *D. noxia* infestation levels, 0x, 1x and 10x, were established within winter wheat, and *T. laboriosa* and *P. sternalis* were collected from May-July within these plots. Of the *T. laboriosa* and *P. sternalis* collected, 32% and 48% screened positive for the presence of *D. noxia* DNA, respectively. Over 92% of *T. laboriosa* were collected at the 1x or 10x *D. noxia* infestation levels combined, demonstrating that *T. laboriosa* was preferentially residing in plots with high *D. noxia* densities. *Pardosa sternalis* was more evenly distributed between aphid infestation levels. This study confirms the role of *T. laboriosa* and *P. sternalis* as predators of *D. noxia* in Colorado agroecosystems.

Introduction

Understanding the interactions between predators and prey in the field is complex. Observations of predator-prey interactions are often disruptive to the study system. A predator's dietary composition is difficult to quantify or describe accurately in the laboratory, as prey preferences often do not relate to the prey composition in the field (Nyffeler and Benz 1987). Using molecular tools to analyze predation can alleviate these concerns and contribute to the understanding of food webs and the biological control potential of specific predators. Furthermore, the identification of effective predators can allow for the conservation of key species for pest management.

Molecular techniques, particularly the polymerase chain reaction (PCR), have been used to study invertebrate predator-prey systems (Hoogendoorn and Heimpel 2001, Agustí et al. 2003a,b, Harwood et al. 2007, 2009; Juen and Traugott 2007, Kuusk et al. 2008, Monzó et al. 2010). Field sampling followed by gut-content analysis is an efficient way of measuring naturally-occurring predation (Sunderland 1988, Sheppard and Harwood 2005). Few studies using PCR have been performed with predation in the field (Agustí et al. 2003a, Harwood et al. 2007, 2009; Juen and Traugott 2007, Kuusk et al. 2008, Monzó et al. 2010).

Successful detection of prey contents through PCR has been performed using primers created from genes comprising several copies per cell, such as nuclear (Zaidi et al. 1999, Hoogendoorn and Heimpel 2001) or mitochondrial (Chen et al. 2000, Agustí et al. 2003a, 2003b, Harwood et al. 2007) genes. These provide numerous target areas for primer attachment (Hoy 1994). This is of particular importance when working with partially

degraded DNA, allowing for more successful detection of target prey (Agustí et al. 2003b). DNA breaks down into smaller fragments during digestion, which is a particular concern with predators such as spiders that predigest their prey (King et al. 2008). Thus, target DNA detection has been successful with primers that amplify fragments of 300 bp or less (Zaidi et al. 1999, Hoogendoorn and Heimpel 2001, Juen and Traugott 2005, Monzó et al. 2010).

Little is known about the role of generalist predators in reducing densities of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Hemiptera: Aphididae). This pest has caused over $893 million in losses in the United States from 1987 to 1993 (Morrison and Peairs 1998) and continues to be a common pest in Colorado. Spiders are a major part of the generalist predator community in agroecosystems (Riechert and Lockley 1984) and feed on crop pests, including aphids (Chiverton 1987, Sunderland et al. 1987, Winder et al. 1994; Harwood et al. 2004, 2005).

Ideal candidates for biological control should show preference for the pest as prey and the ability to aggregate to high pest density areas (Monsrud and Toft 1999). For example, linyphiid spiders (Araneae: Linyphiidae) (Harwood et al. 2001, Harwood et al. 2003) and carabid and staphylinid beetles (Coleoptera: Carabidae and Staphylinidae) (Bryan and Wratten 1984, Monsrud and Toft 1999) can aggregate to areas of high prey density. The web-building spider *Achaearanea tepidariorum* (Koch) (Araneae: Theridiidae) relocates web sites frequently until finding areas with high prey densities (Turnbull 1964), and *Argiope trifasciata* Simon (Araneae: Araneidae) leaves its web less frequently in old-field habitats when prey availability was sufficient (McNett and Rypstra

1997). Additionally, spiders may need to prey on a variety of prey, including aphids, to optimize their intake of essential amino acids (Greenstone 1979).

Tetragnatha laboriosa Hentz (Araneae: Tetragnathidae) is a dominant predator within several agroecosystems (Young and Edwards 1990, Nyffeler and Sterling 1994), can tolerate agricultural disturbances such as alfalfa cutting, and can readily reestablish within the agroecosystem (Howell and Pienkowski 1971). For successful agrobionts, dominant species within agroecosystems (Luczak 1979), it is important to have efficient dispersal capabilities. While many spider families are only capable of dispersing by ballooning as spiderlings or immatures, Tetragnathidae can disperse throughout its lifetime (Bell et al. 2005). *Tetragnatha laboriosa* builds small webs, capturing mainly aphids and small flies (Provencher and Coderre 1987) and has a narrow host range (Culin and Yeargan 1982). For *T. laboriosa*, aphids represent 78% of the prey captured within their webs in cotton during predatory visual observations (Nyffeler and Sterling 1994), 12% of its prey during observations in soybean (Culin and Yeargan 1982), and 12% of its prey in winter wheat (Jmhasly and Nentwig 1995). The spider *Pachygnatha degeeri* Sundevall (Araneae: Tetragnathidae) preferentially consumed aphids despite relatively low associated aphid densities reported from the field (Harwood et al. 2005). It is also likely that spiders will migrate and remain in areas where a pest represents a consistent food source (Nyffeler and Sunderland 2003). If *T. laboriosa* is a dominant predator in these systems and is associated with aphids and other small prey, it is likely that *T. laboriosa* is feeding on *D. noxia* and contributing to the integrated pest management of aphids in wheat fields.

Spiders in the genus *Pardosa* also are commonly found in agroecosystems (Marshall and Rypstra 1999, Samu and Szinetár 2002, Öberg and Ekbom 2006), and *Pardosa sternalis* (Thorell) (Araneae: Lycosidae) is a common spider in northern Colorado agroecosystems. *Pardosa* spp. spiders are not negatively affected by mechanical disturbances, such as sowing, which is an important attribute for an agrobiont species (Öberg and Ekbom 2006). *Pardosa* spp. are active hunters that do not build a web to capture prey and have a broad feeding niche (Bailey and Chada 1968).

Since *T. laboriosa* is a known aphid predator and both spider species are dominant in wheat agroecosystems, it is hypothesized that these two spider species will feed on *D. noxia*. Using PCR and species-specific primers, the goal of this study was to confirm their predation on *D. noxia*.

Materials and Methods

Study Site and Planting Regime

Research was conducted in winter wheat at Colorado State University's Agricultural, Research, Development and Education Center (ARDEC) four miles north of Fort Collins, Colorado, USA, (40.65099°N, -104.99671°W; 1534 m), May-July, 2008. The wheat lines used for this study consisted of two closely-related lines from the Colorado State University wheat breeding program. One of these, CO00554, (TAM 302/Akron/Halt pedigree) is susceptible to biotype RWA2, but carries the *Dn4* gene from Halt for biotype RWA1 resistance. The other line (STARS 02RWA2414-11/5*CO00554) carries the *Dn7* gene effective against both biotype RWA1 and biotype RWA2 from STARS

02RWA2414-11 and was derived through backcrossing with CO00554 as the recurrent parent.

The site was irrigated once prior to planting on 3 September 2007 to insure uniform plant emergence, and wheat was grown according to standard agronomic practices for the region. The wheat was planted on 11 September 2007. Plots were 3.24 m^2 with six wheat rows. "Hatcher" wheat (Haley et al. 2005) was planted as a buffer between and outside of the plots. No herbicides were applied.

Preliminary Research

Prior to the experiment, live pitfall traps were placed within four repetitions of both the susceptible and resistant wheat lines. The traps were two-liter plastic bottles with the top cut, inverted, and placed flush with the soil surface to form a funnel. A 500 mL cup was placed inside the bottom half of the plastic bottle. An inch of soil was placed in the bottom of the traps to help maintain live spiders. *Pardosa sternalis* (the cursorial hunter) was the dominant spider present within the live pitfall traps, and *T. laboriosa* (the web-building spider) did not appear until early June.

Aphid Field Infestation

Within each plot, winter wheat plants were infested with greenhouse-reared (L16:D8 cycle, 24°C, 65% humidity) biotype RWA2 using a Davis inoculator (Davis and Oswalt 1979). It is important to understand predation rates at several infestation levels as rates are frequently underestimated at low prey levels (Nyffeler et al. 1994). In this study, three infestation levels, 0x, 1x and 10x were established. Three, one-meter rows in the center of the 1x and 10x plots were infested with approximately 246 and 2,460 biotype

RWA2 aphids, respectively, on 7 March 2008. Infestation numbers to be applied in the field were estimated by using the Davis inoculator to deliver aphids to 10 Petri dishes. The number of *D. noxia* per inoculator delivery per Petri dish was averaged, providing an estimate of the number of aphids delivered to wheat in the field.

Spider Feeding Experiment

Feeding experiments are necessary to determine how long DNA survives within the predator gut following digestion. A laboratory feeding study was performed to verify that *D. noxia* could be detected within the guts of the spiders after feeding. Spiders were collected daily from live pitfall traps set in wheat adjacent to the plots. Over 50 spiders were collected for each species to conduct positive controls.

The spiders for positive controls were maintained in 100 x 15 mm Petri dishes with Plaster of Paris as a substrate on the bottom of the dish for humidity within a plant growth chamber (Lab-Line Biotronette Plant Growth Chamber, Lab-Line Instrument, Inc.) on a L16:D8 cycle with day and night temperatures at 24ºC and 20ºC, respectively, mimicking natural field conditions. Moisture was provided by spraying the inside of the dish twice daily with water. The spiders were fed two to three *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) every other day for approximately two weeks to reduce stress and maintain the health of the spider prior to the start of the experiment. They were starved for seven to ten days, and then fed one biotype RWA2 aphid. The spiders were individually observed under a microscope at 10x to assure that the aphid was captured within the spiders' chelicerae. If the spider dropped the aphid or failed to feed within 20 minutes, it was returned to the plant growth chamber. Spiders that fed on an aphid were then frozen at the following post-feeding times to represent positive controls

(in h): 0, 4, 8, 12, 16, and 24, with eight individuals represented for each time period. Spiders were maintained in the plant growth chamber during their digestion period before freezing. Spiders were identified in chilled 100% ethanol under the microscope, and any visible aphid remains found within the spiders' chelicerae or surrounding areas were removed. Eight spiders from both species were also starved for ten days to serve as negative controls.

Spider Field Collection

Spiders were collected from May-July 2008 from within the designated 0x, 1x, and 10x aphid density treatments (Appendix A) during five main wheat stages (Zadoks 40, 50, 60, 70, and 80, respectively) (Zadoks et al. 1974) . Both *T. laboriosa* and *P. sternalis* were collected within wheat rows, within plants, webs within plants, and around the plots. *Tetragnatha laboriosa* was sampled between the hours of 07:30-09:00, where dew allowed for easy web detection. It was also observed to feed more frequently in the morning (07:50 to 11:00 h) compared with evening observations (20:00 to 23:50 h) (Culin and Yeargan 1982). *Pardosa sternalis* spiders were also sampled at this time and additionally as time allowed between the hours of 09:00 and 15:00. Spiders were sampled by hand or with an aspirator to reduce the risk of false positives with the target prey (King et al. 2008). The spiders were then transferred live individually into microcentrifuge tubes filled with chilled 100% ethanol, and transferred to the laboratory in a cooler, maintained at 4ºC or below. The spiders were identified in ethanol on ice, placed in sterilized Eppendorf tubes with 100% ethanol, and stored at -80ºC until further processing. *Tetragnatha laboriosa* spiders were not present past 19 June 2008, so

collection of both spider species was discontinued after this date. A total of 64 *T. laboriosa* and 71 *P. sternalis* were collected from the field.

Aphid Sampling

The mean density of *D. noxia* on wheat tillers was estimated by removing wheat tillers from a 14 cm^2 area every two weeks from each 0x, 1x and 10x plot. Tillers were cut and removed at ground level, placed into a 3.8 L plastic bag, and held on ice until they were transferred into Berlese funnels for 24 h. Aphids were extracted into 75% ethanol for long-term storage and subsequent counting.

DNA Extraction

Spider and aphid individuals were smashed along the edge of a tube with sterile pipette tips, and extraction of DNA from field-collected and control spiders was performed with Qiagen DNeasy Animal Tissue kits (Qiagen) following the manufacturer's protocol. DNA extraction using DNeasy Tissue kits was successful with the detection of Collembola and aphid DNA from inside the guts of field-collected spiders (Agustí et al. 2003a, Kuusk et al. 2008).

The DNA concentration from the extractions was quantified with a NanoDrop ND-1000 Spectrophotometer using 1 µL of template. The ratio of sample absorbance at 260 and 280 nm was used to assess the purity of the DNA. DNA concentrations from the spiders ranged from 50 ng/µL-450 ng/µL. DNA concentrations from single aphids ranged from 1-6 ng/µL. After measurement, total spider DNA extractions were diluted to 50 ng/µL for standardization and subsequently stored at -20ºC.

Primer Design

A partial 1146 bp sequence from the mitochondrial cytochrome oxidase I (COI) gene was retrieved from the GenBank database (Accession #FJ232620, *D. noxia*) (to see previous molecular work/troubleshooting, see Appendix B). This sequence, sequences from *P. sternalis* and *T. laboriosa* (sequenced from general COI primers), and those of the following aphid species derived from GenBank: *Diuraphis frequens* (Walker) (Hemiptera: Aphididae), *D. tritici* (Gillette) (Hemiptera: Aphididae), *Rhopalosiphum padi* L. (Hemiptera: Aphididae), *R. maidis* (Fitch) (Hemiptera: Aphididae), and *Sipha elegans* del Guercio (Hemiptera: Aphididae) were aligned using ClustalW (Larkin et al. 2007) within the BioEdit sequence alignment editor (Version 7.0.5, Tom Hall, Ibis Therapeutics). The goal of the alignment of these sequences was to select species-specific *D. noxia* primers and to prevent the primers from amplifying the spider species. Several pairs of primers were created and tested. A pair of primers was selected and optimized by performing a gradient PCR and by adjusting reagent concentrations, number of cycles, and the denaturation, annealing, and extension times (Table 4.1).

TABLE 4.1. PRIMER SEQUENCE, AMPLICON SIZE (BP) OF AMPLIFIED FRAGMENT, AND ANNEALING TEMPERATURE (ºC) OF PRIMERS (T_A) FOR *D. NOXIA*.

Primer	Forward Sequence	Reverse Sequence	Size	T_a
RWACOI	CACTTATTATGTAGTAGCACATTTTCAT	TTAGGATAATCTGTATATCGTCGTGGT	227	60

PCR Amplification and Purification

PCR reactions were conducted in a total volume of 25 µL, which included the following reagents: 2.5µL of Takara 10x Buffer (100 mM Tris-HCl (pH 8.3), 500 mM

KCl, 15 mM $MgCl_2$) 1.0 µL of each primer (0.4 µM), 2 µL of Takara dNTP mixture (dATP, dGTP, dTTP) (2.5 mM each dNTP), 5 units/µL of Takara *Taq* HS DNA polymerase, and 5 µL of template DNA (250 ng/µL). The PCR protocol included the following: an initial denaturation step of 3 min at 94°C; followed by 35 cycles of denaturing for 30s at 94°C, annealing for 30s at 45°C, and extension for 60s at 72°C; and a final extension step of 72°C for 5 min. PCR products were separated by electrophoresis in 2% agarose gels for 35 min at 100 volts, post-stained with ethidium bromide for 30 min to 1hr, and photographed under UV light. Each PCR was run with a positive control (*D. noxia*) and a negative control (all reagents except the template DNA) to ensure the PCR was contamination-free.

The PCR product from one positive control *D. noxia* was purified with a Mo Bio Ultraclean Purification Kit following the manufacturer's protocol and sequenced following the dideoxychain-termination method at University of Washington's High-Throughput Sequencing Solutions. The nucleotide identity for both primer pairs matched 100% with *D. noxia*, indicating that the correct region was amplified for PCR.

Cross-Reactivity Testing

Primer specificity testing is necessary to reduce the occurrence of false positives due to the cross-reactivity of primers (Harper et al. 2005, Admassu et al. 2006). False positives are of particular concern when it comes to generalist predators, such as spiders, that feed on a variety of prey (Sheppard and Harwood 2005, Gariepy et al. 2007). Other prey were collected by sweepnet or by hand, placed in 100% EtOH, and transferred to the laboratory on ice (Table 4.2). DNA from these individuals were extracted with Qiagen

kits, and PCRs were conducted with the *D. noxia*-specific primers to ensure that these other prey were not amplified with these particular primers.

TABLE 4.2. ARTHROPODS TESTED AGAINST PRIMER PAIRS.

Order	Family	Species
Acari	Tetranychidae	*Oligonychus pratensis* (Banks), *Petrobia latens* (Müller)
Araneae	Gnaphosidae	*Drassyllus nannellus* Chamberlin & Gertsch
	Lycosidae	*Schizocosa mccooki* (Montgomery)
	Thomisidae	*Xysticus pellax* O.P.-Cambridge
Coleoptera	Carabidae	*Bembidion quadramaculatum* sp., *Poecilus* sp.
	Coccinellidae	*Coccinella septempunctata* L., *Hippodamia convergens* Guérin-Méneville, *Hippodamia parenthesis* Say, *Coccinella transversoguttata* Faldermann, *Scymnus* sp.
Collembola	Isotomidae	
Diptera	Culcidae	*Culex pipiens* L., *Culex tarsalis* Coquillett
	Tachinidae	*Phasia* sp.
Hemiptera	Anthocoridae	*Orius* sp.
	Lygaeidae	*Nysius* cf. *raphanus* Howard
	Miridae	*Lygus* sp.
	Nabidae	
	Pentatomidae	
	Rhopalidae	*Arhyssus lateralis* (Say)
Homoptera	Aphididae	*Acyrthosiphon pisum* Harris, *Diuraphis frequens* Walker, *Diuraphis tritici* (Gillette), *Rhopalosiphum padi* (L.), *Schizaphis graminum* (Rondani), *Sitobion avenae* (F.), *Sipha elegans* del Guercio, *Rhopalosiphum maidis* Fitch
Thysanoptera	Thripidae	*Anaphothrips obscurus* (Muller)

Analyses

Statistical Analysis

Data were analyzed for both spider species for the effects of wheat stage, infestation level, and levels of resistance using the "Mixed" procedure in SAS (SAS Institute 2002-2008) with the REML estimation method and the Kenward-Roger approximation for degrees of freedom (Kenward 1997). Repeated measures models with autoregressive errors and unequal variances across dates were evaluated and used when justified by AIC

values (Burnham and Anderson 2002). Statistical computations were performed using the "Mixed" procedure in SAS (SAS Institute 2002-2008) with the REML estimation method and the Kenward-Roger approximation for degrees of freedom (Kenward 1997). Because spider densities were low, spiders were pooled into the following five wheat stages: Zadoks 40, 50, 60, 70, and 80. Spider densities were square-root transformed ($x + 0.5$) to homogenize the variances. When significant effects were observed ($P \leq 0.05$), least squares means were separated using t-tests. Untransformed means are presented in tables and figures.

Molecular half-lives, the amount of time post-feeding where half of the predators are positively identified with prey DNA (Greenstone and Hunt 1993), were calculated using the "probit" procedure in SAS (SAS Institute 2002-2008) for each species and can be used to compare results from positive control feeding studies. Fisher exact tests were performed using the "PROC FREQ" procedure in SAS (SAS Institute 2002-2008) to determine whether the percent of positive field spiders was correlated with increasing aphid densities.

For the density of *D. noxia* on both wheat tillers, mixed models with autoregressive errors and unequal variances across dates were considered. A model was selected based on the lowest Akaike's Information Criterion (AIC) value, which is used to measure the best fit model, and restricted maximum likelihood (REML) was used as a method for estimating the parameters of the model (SAS Institute 2002-2008). A mixed model with an autoregressive order 1 covariance structure with heterogeneous variances across dates (ARH(1)) was chosen as the appropriate model.

Results

Feeding trials

Results of the spider feeding experiment show that 100% of *T. laboriosa* tested positive for *D. noxia* zero hours after feeding. At 4 hours, 62.5% of the spiders tested positive for *D. noxia* after feeding. At 12, 16, and 24 hours post-feeding, 0% of the spiders tested positive for *D. noxia*. The molecular half-life for *T. laboriosa* was 4.2 ± 1.1 hrs. Starved *T. laboriosa* did not amplify *D. noxia*-specific primers (negative controls).

For the spider feeding experiment, 100% of *P. sternalis* tested positive for *D. noxia* zero hours after feeding. At 4, 12, 16, and 24 hours post-feeding, 0% of the spiders tested positive for *D. noxia*. The molecular half-life for *P. sternalis* was 2.0 ± 0.4 hrs. Starved *P. sternalis* did not amplify *D. noxia*-specific primers (negative controls).

Field collected spiders

Tetragnatha laboriosa

Sixty-four total *T. laboriosa* were collected in 2008. Of these, 3% were male, 53% were immature (22% penultimate males), and 44% were either immature or female. Since *T. laboriosa* is a haplogyne spider, the identification of females requires epigynal dissection for accuracy (Ubick et al. 2005). This dissection could contaminate the abdomen and result in DNA degradation. Because of this, these spiders were grouped into an "immature or female" category. The immatures collected were assumed to be *T. laboriosa* as no other *Tetragnatha* spp. are present at this site.

Wheat stage and level of resistance did not affect *T. laboriosa* densities ($F_{4,211}$=0.47, P=0.7572). Spider densities also were not affected by the combination of resistance and infestation level ($F_{2,211}$=0.42, P=0.6555). Level of aphid resistance within the wheat did not affect *T. laboriosa* densities ($F_{1,211}$=1.67, P=0.1974). *Tetragnatha laboriosa* densities were affected by wheat stage and infestation level combined ($F_{8,218}$=7.07, P<0.0001). The highest mean spider density occurred during Zadoks 60 at the 10x aphid infestation level and subsequently declined after this stage, and mean densities of *T. laboriosa* were greatest at Zadoks 60 at all aphid infestation levels (Table 4.3). *Tetragnatha laboriosa* was not present within any of the aphid infestation levels at Zadoks 40 or at the 0x level during inflorescence. Spider densities were lower at the 1x and 10x infestation levels at Zadoks 50, peaked at Zadoks 60, and declined at Zadoks 70 and 80. *Tetragnatha laboriosa* densities were highest at the 10x infestation level for all wheat stages. Of the total *T. laboriosa* collected, 8%, 39%, and 53% were present at the 0x, 1x, and 10x aphid infestation levels, respectively.

TABLE 4.3. MEAN NO. OF *T. LABORIOSA* PER WHEAT STAGE AND INFESTATION LEVEL, FORT COLLINS, CO, 2008.[1,2]

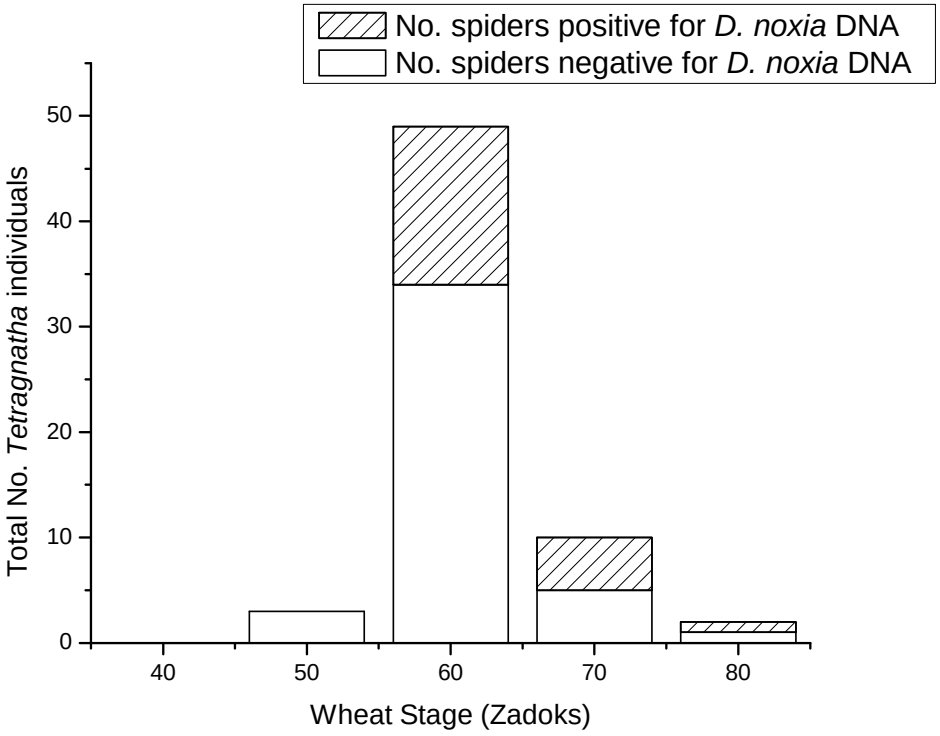
Wheat Stage (Zadoks)	Infestation level		
	0x	1x	10x
40	0.00 **Aa**	0.00 **Ba**	0.00 **Ba**
50	0.00 **Aa**	0.06 **Ba**	0.06 **Ba**
60	0.13 **Ab**	1.19 **Ab**	1.69 **Aa**
70	0.06 **Aa**	0.31 **Ba**	0.13 **Ba**
80	0.00 **Aa**	0.00 **Ba**	0.00 **Aa**

[1]Means within a column followed by the same capital letters are not significantly different and represent differences between wheat stages within each infestation level (α = 0.05; PROC MIXED). Means within rows within each infestation level followed by the same lower case letters are not significantly different and represent differences between infestation levels at each wheat stage. (α = 0.05; PROC MIXED).
[2]Means averaged over resistance; 0x,1x, and 10x refer to the respective aphid infestation levels.

Of the 64 total *T. laboriosa* collected from all wheat stages and infestation levels, 32.8% were positive for the presence of *D. noxia*. The number of spiders testing positive

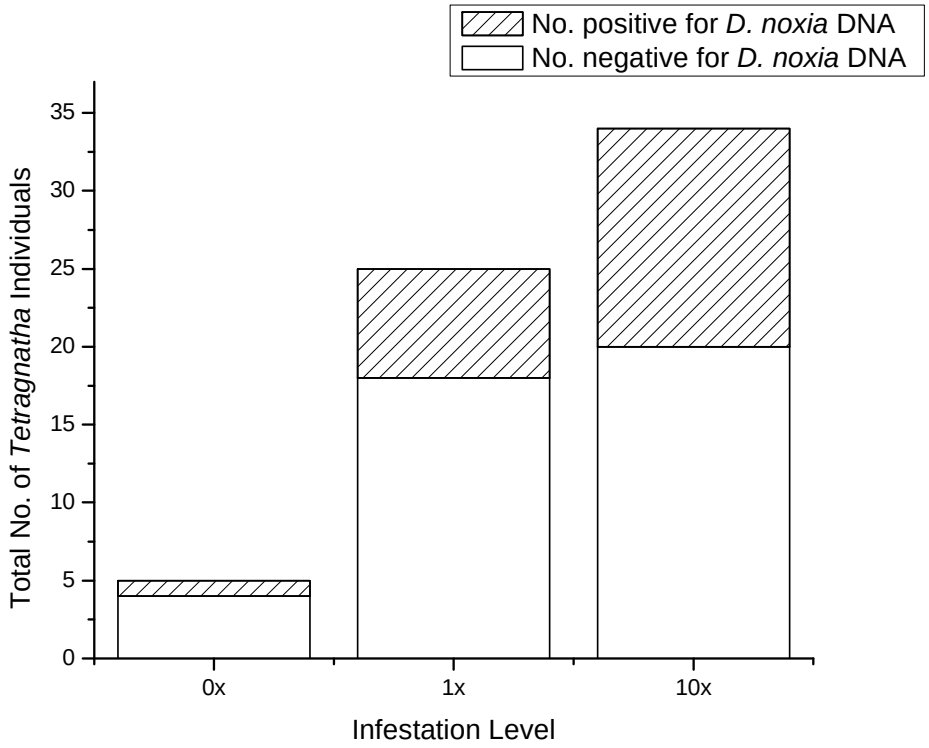
for the presence of *D. noxia* DNA was highest at Zadoks 60 (Figure 4.1). However, this was not significantly different from the other wheat stages (Fisher's Exact Test, P=0.2998).

FIGURE 4.1. TOTAL *T. LABORIOSA* COLLECTED PER WHEAT STAGE AND NO. POSITIVE FOR *D. NOXIA* DNA, FORT COLLINS, CO, 2008.



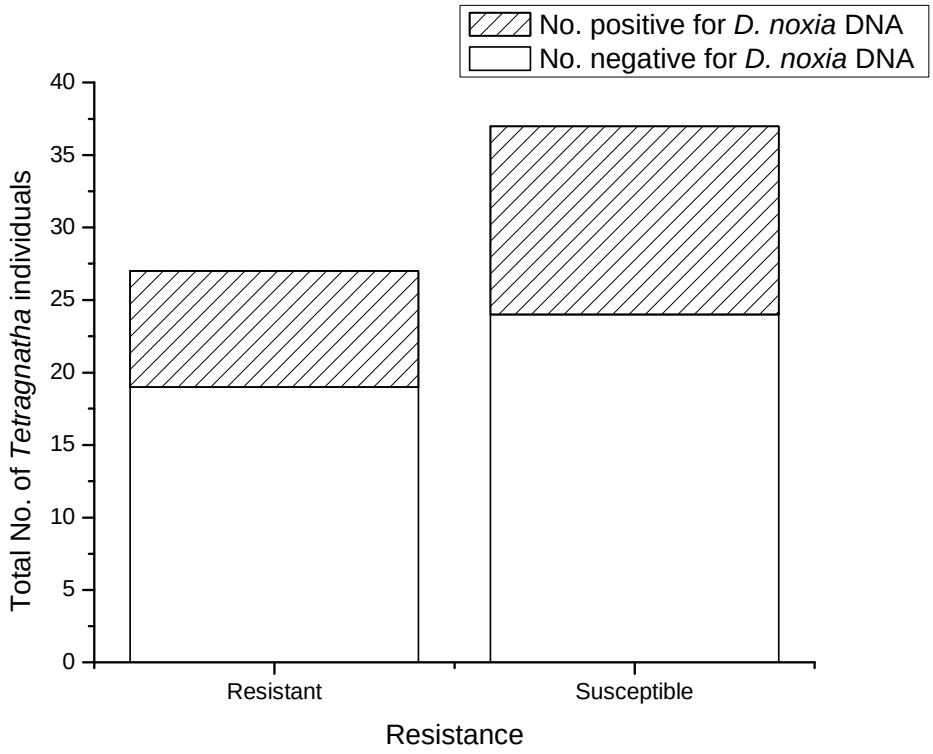
At Zadoks 40, 50, 60, 70, and 80 crop stages, 0%, 0%, 44%, 50%, and 50% were positive for the presence of *D. noxia* DNA, respectively. The number of *T. laboriosa* testing positive for *D. noxia* DNA was not significantly related to infestation level (Fisher's Exact Test, P=0.5542) (Figure 4.2).

FIGURE 4.2. TOTAL *T. LABORIOSA* COLLECTED PER APHID INFESTATION LEVEL AND NO. POSITIVE FOR *D. NOXIA* DNA, FORT COLLINS, CO, 2008.



Although the number of spiders testing positive increased with increasing aphid infestation level, this relationship was not significant (Mantel-Haenszel Chi Square, DF=1, Q=2.31, P=0.1287). At the 0x, 1x, and 10x aphid infestation levels, 20%, 28%, and 41% were positive for the presence of *D. noxia* DNA, respectively (Figure 4.2). The percentage of *T. laboriosa* testing positive for *D. noxia* DNA was not significantly different between the susceptible and resistant wheat varieties (35% and 30%, respectively) (Fisher's Exact Test, P=0.0788) (Figure 4.3).

FIGURE 4.3. TOTAL *T. LABORIOSA* COLLECTED PER CROP RESISTANCE LEVEL AND NO. POSITIVE FOR *D. NOXIA* DNA, FORT COLLINS, CO, 2008.



Pardosa sternalis

Seventy-one total *P. sternalis* were collected in 2008. Of these, 28% were male, 51% were immature, and 21% were female. The immatures collected were assumed to be *P. sternalis*, as no other *Pardosa* spp. were present in the field.

Wheat stage, infestation level, and level of aphid resistance did not interact to affect *P. sternalis* densities ($F_{8,203}$=1.45, P=0.1770). *Pardosa sternalis* densities were not affected by wheat stage and infestation level combined ($F_{8,203}$=0.77, P=0.6335), wheat stage and level of resistance ($F_{4,203}$=1.41, P=0.2329), or resistance and infestation level combined ($F_{2,203}$=0.81, P=0.4473). Infestation level also did not affect *P. sternalis* densities ($F_{2,203}$=1.96, P=0.1433). Wheat stage affected the density of *P. sternalis*

collected ($F_{4,277}$=7.43, P<0.0001). Mean densities were highest at the Zadoks 40 (Table 4.4). Level of aphid resistance affected *P. sternalis* densities ($F_{1,227}$=5.83, P=0.0165) (Table 4.5). *Pardosa sternalis* densities were higher in the resistant treatments, averaged over infestation level and wheat stage.

TABLE 4.4. MEAN *P. STERNALIS* PER WHEAT STAGE, FORT COLLINS, CO, 2008.[1,2]

Wheat Stage (Zadoks)	# *P. sternalis*
40	0.583**a**
50	0.271**b**
60	0.167**bc**
70	0.396**b**
80	0.042**c**

[1]Means within a column followed by the same lowercase letters are not significantly different (α = 0.05; PROC MIXED).
[2]Significant differences have been determined through square-root transformation of the data- raw means are represented in this table.

TABLE 4.5. MEAN *P. STERNALIS* PER RESISTANCE LEVEL, FORT COLLINS, CO, 2008.[1,2]

Resistance Level	# *P. sternalis*
Resistant	0.38**a**
Susceptible	0.21**b**

Unlike *T. laboriosa*, *P. sternalis* was more uniformly distributed until Zadoks 80 (Figure 4.4). For all *P. sternalis* collected, 39%, 18%, 11%, 30%, and 28% were collected at Zadoks 40, 50, 60, 70, and 80 wheat growth stages, respectively.

Of the 71 total *P. sternalis* collected, 47.9% were positive for the presence of *D. noxia*. The number of *P. sternalis* found within each infestation level are shown in Figure 4.5. At the 0x aphid infestation level, 29 individuals were collected (40% of the total collection) of which 21% were positive for *D. noxia* DNA. At the 1x infestation level, 26 individuals were collected (23.6% of the total collection) of which 27% were positive for the presence of *D. noxia* DNA. At the 10x infestation level, 17 individuals were collected (36% of the total collection), and 65% were positive for the presence of *D. noxia* DNA. The number of spiders testing positive for the presence of *D. noxia* DNA

was highest at Zadoks 40 (Figure 4.4); however, this was not significantly different from other wheat stages (Fisher's Exact Test, P=0.1809). At Zadoks 40, 50, 60, 70, and 80, 36%, 39%, 0%, 75%, and 0% were positive for the presence of *D. noxia* DNA, respectively (Figure 4.4).

FIGURE 4.4. TOTAL *P. STERNALIS* COLLECTED PER WHEAT STAGE AND NO. POSITIVE FOR *D. NOXIA* DNA, FORT COLLINS, CO, 2008.

As aphid density increased, the percentage of spiders testing positive for *D. noxia* increased (Mantel-Haenszel Chi Square, DF=1, *Q*=8.169, P=0.0043). At the 0x, 1x, and 10x aphid infestation levels, 21%, 27%, and 65% were positive for the presence of *D. noxia* DNA, respectively (Figure 4.5). The percentage of spiders testing positive for *D. noxia* DNA was similar between resistance levels (Fisher's Exact Test, P=0.8100) (Figure 4.6).

FIGURE 4.5. TOTAL *P. STERNALIS* PER INFESTATION LEVEL AND NO. POSITIVE FOR *D. NOXIA* DNA, FORT COLLINS, CO, 2008.

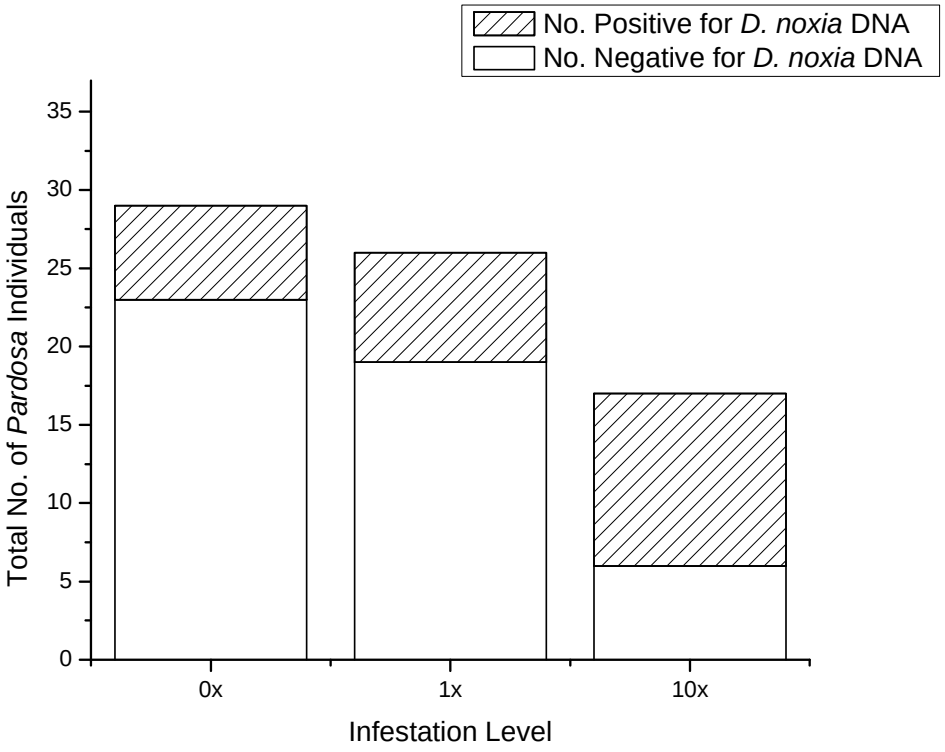


FIGURE 4.6. TOTAL *P. STERNALIS* PER CROP RESISTANCE LEVEL AND NO. POSITIVE FOR *D. NOXIA* DNA, FORT COLLINS, CO, 2008.

Aphids

A date by resistance by infestation level interaction occurred for aphid density ($F_{8,85.9}$=4.04, P=0.0004) (Table 4.5). The highest aphid densities occurred at the 10x susceptible treatment at Zadoks 50, followed by the 10x resistant, 1x susceptible, and 1x resistant treatments at Zadoks 50. Aphid densities within the 0x resistant and susceptible treatments remained close to or at zero for all dates. Aphid densities were on average twice as high at each date in the susceptible treatments versus the resistant treatments within the same infestation level for the 1x and 10x infestation level treatments. Similarly, the aphid densities were approximately two times higher on average between infestation levels within the same resistance for the 1x and 10x infestation level

treatments. Aphid densities gradually increased from stem elongation until inflorescence, peaked at inflorescence, and subsequently declined at milk and dough.

TABLE 4.6. MEAN DENSITY *D. NOXIA* ON WHEAT TILLERS PER CM^{-2} D^{-1}AT THE 1X AND 10X INFESTATION LEVELS FOR RESISTANT (R) AND SUSCEPTIBLE (S) WHEAT LINES IN FORT COLLINS, CO, 2008 [1,2].

Date	Wheat Stage (Zadoks)	0xR	0xS	1xR	1xS	10xR	10xS
4 May	30	0.00**Bc**	0.00**Cc**	0.19**Cb**	0.38**Cb**	0.86**Ca**	1.16**Ca**
21 May	40	0.00**Bd**	0.01**Cd**	1.89**Bc**	3.94**Bb**	5.04**Bb**	12.27**Ba**
4 June	50	0.10**Ad**	0.22**Bd**	8.35**Ac**	16.86**Ab**	17.64**Ab**	59.74**Aa**
18 June	70	0.01**Bc**	0.49**Ac**	7.49**Ab**	14.05**Aa**	6.35**Bb**	19.87**Ba**
2 July	80	0.02**Bb**	0.20**Ba**	0.19**Ca**	0.23**Ca**	0.20**Ca**	0.22**Da**

[1]Significant differences have been determined through square-root transformation of the data- raw means are represented in this table.
[2]Means within a column followed by the same capital letters are not significantly different ($\alpha = 0.05$; PROC MIXED) and represent differences between treatments at each date. Means within rows followed by the same lower case letters are not significantly different ($\alpha = 0.05$; PROC MIXED) and represent differences between treatments at each date.

Discussion

The determination of key natural enemies is an important component of the integrated pest management of *D. noxia*. In particular, it is important to establish whether *D. noxia* is consumed by common predators, such as spiders, in the field. *Diuraphis noxia* densities reached economically damaging levels in this study, and various levels of aphid infestation were tested in the field. Of the *T. laboriosa* and *P. sternalis* collected, 33% and 48% screened positive for the presence of *D. noxia* DNA, confirming these species as *D. noxia* predators.

Tetragnatha laboriosa appeared to respond to aphid densities. These spiders peaked during Zadoks 60 and within the 10x and 1x aphid infestation level treatments, which corresponded with the highest density of *D. noxia*. Over 92% *T. laboriosa* were collected at the 1x or 10x infestation levels combined, and over half of the total *T. laboriosa*

collected were collected within the 10x infestation level, indicating a clear preference for treatments containing *D. noxia* densities. *Diuraphis noxia* densities decreased over 97% from Zadoks 70-80 wheat stages within each infestation level. Interestingly, *T. laboriosa* collections were close to zero by this time. Thus, *T. laboriosa* arrived in wheat fields at peak aphid densities and dispersed to an adjacent corn crop as *D. noxia* densities declined (Kerzicnik, pers. obs.). *Pardosa sternalis* did not respond to aphid densities; over 40% of *P. sternalis* were collected within the 0x infestation levels, suggesting that the spiders were not preferentially residing within the aphid-infested plots.

For *T. laboriosa*, the highest percentage of spiders collected at peak aphid densities also corresponded with the highest number of spiders screening positive for *D. noxia* DNA. Over 41% of the spiders collected at the 10x infestation level tested positive for *D. noxia*. Using ELISA tests, 95-100% of *Pterostichus cupreus* L. (Coleoptera: Carabidae) individuals were positively identified with *R. padi* remains during peak aphid densities (Chiverton 1987). With gut dissection, 11% of staphylinid and carabid beetles were positively identified with aphid remains with the percentage of positives increasing with increasing aphid densities (Sunderland and Vickerman 1980).

Biological control is most efficient when generalist predators arrive within the crop early before pests reach peak densities (Edwards et al. 1979, Ekbom and Wiktelius 1985, Chiverton et al. 1986, Birkhofer et al. 2008). *Pardosa sternalis* was most abundant at Zadoks 40, prior to peak aphid densities, and demonstrated a very high aphid consumption rate. Furthermore, *P. sternalis* may have been present even earlier in the season, as sampling did not commence until early May. On the contrary, *T. laboriosa* arrived after aphid densities were at economically damaging levels. However, spiders

can occasionally increase predation rates when pest densities are high, demonstrating a type III functional response (Riechert and Lockley 1984). The spiders *Phidippus audax* (Hentz) (Araneae: Salticidae), *Oxyopes salticus* Hentz (Araneae: Oxyopidae), and *Misumenops celer* Hentz (Araneae: Thomisidae) increased predation rates when densities of the leafhopper *Pseudatomoscelis seriatus* (Reuter) increased incrementally (Breene et al. 1990).

The retention times for the detection of target DNA post-feeding, as determined by the feeding trials, were low for both species-4.0 and 2.0 h for *T. laboriosa* and *P. sternalis*, respectively. The predator to prey size relationship might have affected the molecular half-life. Using monoclonal antibodies and ELISA, pink bollworm eggs, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), were detected for a longer period of time from inside the guts of a minute pirate bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), compared with a ladybeetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae) (Hagler and Naranjo 1997). Both *P. sternalis* and *T. laboriosa* were substantially larger than a single *D. noxia* prey. Other studies that indicate longer retention times with prey DNA in feeding trials were more representative of a smaller predator-prey ratio (Agustí et al. 2003a, Monzó et al. 2010). As a result, the implications of spider consumption rates in the field must be appropriately assessed. For this study, molecular half-lives of around four hours indicate that the spiders that screened positive for *D. noxia* DNA fed on an aphid within just a few hours of collection.

Because predation rates were high for both spiders, it is possible that rates were overestimated due to secondary predation. Through PCR, DNA as a result of secondary

predation could be detected for up to eight hours with the carabid beetle *Pterostichus melanarius* Illiger (Coloeptera: Carabidae) feeding on a spider *Tenuiphantes tenuis* (Blackwall) (Araneae: Linyphiidae) that had just fed on an English grain aphid, *S. avenae* (Sheppard et al. 2005). Because *D. noxia* densities were so high, several *D. noxia*-specific and other generalist predators might have been attracted to the treatments to consume aphids. Subsequently, both *T. laboriosa* and *P. sternalis* might have fed on these predators, resulting in an inaccurate determination of a trophic link with *D. noxia*. Scavenging also can contribute to false positives (Juen and Traugott 2005). However, because retention times of DNA were low with the primers used, it is unlikely that either secondary predation or scavenging is a major source of overestimation.

Tetragnatha laboriosa and *P. sternalis* had high predation rates on *D. noxia*, indicating a contribution to its biological control. The presence of *P. sternalis* earlier in the season suggests that this species may be a more effective predator than *T. laboriosa*. The latter species responded to densities when *D. noxia* had already reached economically damaging levels. Although its consumption of *D. noxia* was noteworthy, *T. laboriosa* also might have been attracted to very high-density aphid treatments for the alternative prey present, i.e., other predators that were attracted to high aphid densities. At lower aphid densities, it is possible that *T. laboriosa* would not be attracted to the aphid-infested treatments.

This study confirms *T. laboriosa* and *P. sternalis* predation on *D. noxia* in Colorado agroecosystems. Understanding how spiders function as consumers can allow for more sustainable integrated management and can provide further insight as to how biological control contributes to the ecology of the food web.

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APPENDIX A. PLOT MAP AT ARDEC, FORT COLLINS, CO, 2008.

Infest. Level	Wheat Variety	Resis.	REP 1	REP 2	REP 3	REP 4	REP 5	REP 6	REP 7	REP 8
0x	554	S	106	201	307	409	504	607	711	807
	554	R	105	202	308	410	503	608	712	808
1x	554	S	101	208	310	402	505	604	705	803
	554	R	102	207	309	401	506	603	706	804
10x	554	S	110	211	303	408	509	611	704	812
	554	R	109	212	304	407	510	612	703	811

	0x					**N^**					
	1x	B	B	B	B		B	B	B	B	
	10x	B	B	B	B		B	B	B	B	
		B	B	B	B		B	B	B	B	
		B	B	B	B		B	B	B	B	
				711	**712**				**811**	**812**	
		705	**706**						**807**	**808**	
				703	**704**				**803**	**804**	
		509	**510**						**611**	**612**	
BUFFER						BUFFER					BUFFER
		505	**506**						**607**	**608**	
				503	**504**				**603**	**604**	
		309	**310**				**409**	**410**			
				307	**308**				**407**	**408**	
				303	**304**		**401**	**402**			
		109	**110**						**211**	**212**	
		105	**106**						**207**	**208**	
		101	**102**				**201**	**202**			
		B	B	B	B		B	B	B	B	
		B	B	B	B		B	B	B	B	
		B	B	B	B		B	B	B	B	
		B	B	B	B		B	B	B	B	

APPENDIX B. PREVIOUS MOLECULAR WORK AND TROUBLESHOOTING

In January, 2009, I traveled to the University of Kentucky to work in James Harwood's lab and learn molecular techniques from him and his postdoctoral research associate, Eric Chapman. I shipped all of my samples to Kentucky prior to extraction, and I performed the extractions of my field and control spiders with Qiagen kits. I was there for two weeks and learned the extraction technique thoroughly using both aphids and spiders.

Following the extractions, with Eric's assistance, I performed a PCR with general primers with my eight *D. noxia* extractions to ensure that the extractions worked. Since the extractions were successful, the RWACOIIF2 and RWACOIIR1 primers were used for PCRs (see below).

Primer Pair I: RWACOIIF2 and RWACOIIR1, gene=mitochondrial, Cytochrome Oxidase II (COII)

RWACOIIF2 and RWACOIIR1 were originally targeted to begin the molecular gut-content analysis work (Chen et al. 2000). Since the extractions were successful, the RWACOIIF2 and RWACOIIR1 primers were further optimized at the University of Kentucky by running gradient PCRs, testing different concentrations of dNTPs, primers, and *Taq*. I then used spiders fed an aphid and frozen immediately (0 hr positive controls) in PCRs to see if aphids were successfully amplified within spider guts. These PCRs were successful. Both spider species *T. laboriosa* and *P. sternalis* were tested; however,

P. sternalis bands were very weak and showed early signs of DNA degradation. This may be because of the hairs on *P. sternalis*. Jan Stephens found that the hairs of mosquitoes degraded their DNA during extractions (personal observation). I ran about 10 PCRs, trying to optimize the PCRs with the 0hr positive controls. I ran into a contamination problem where I had streaking in the negative control. I tried a new kit/new reagents, and I continued to have the same problem. I decided to ship my extractions back to CSU and continue there.

I continued with these primers at CSU in the Leach lab. I ordered a new kit (same reagents as I used in Kentucky) and proceeded with PCRs using the same procedure. I had some operator error issues. For example, it took some practice learning how to set up reactions properly, using a new thermal cycler, and the running and staining of gels. Regardless, even after some trial-and-error and using the same procedures in Kentucky and new equipment, I was unable to get these primers to work for even total aphid extractions after about 20 PCRs. I corresponded with Eric describing the problems, and he suggested that I try a new pair of primers. I didn't with new primers or any other molecular work until winter, 2009, as I had to finish the identification of my spiders.

Primer Pair 2: cytbF_1 and cytbR_1 (342bp), gene=mitochondrial, Cytochrome B (cytb)

Per Eric's advice, I tried a new pair of primers. I met with Myron Bruce to search for available *D. noxia* sequences on GenBank. We found a large sequence from the cytb gene (Thao et al. 2004) and created candidate primers with ITDNA software and this sequence. The primers created amplified a fragment of 342bp. This primer was blasted against spiders and other aphid species.

(Forward=CCATCACCCATTGGTTGTAAAGCACC, Reverse = TGAGTTCAAACCGGTGTAAGCCAG). When blasting these individually and just alone, the selected primers came up with a 100% hit for *D. noxia*, and no other species of interest were close to a match.

I went down to the Denver Museum of Nature and Science to try these new primers with aphid extractions, working with Kayce Bell, a Research Associate knowledgeable about PCR. I used her kit/reagents and my kit/reagents to test out the new primer pair (Primer Pair 2) and the previous primer pair (Primer Pair I). Kayce's kit and the new primers amplified *D. noxia* DNA. The 1st primer pair also amplified *D. noxia* DNA successfully; however, these bands were weak. I decided to continue optimizing these primers down at the museum. I optimized these primers by adjusting the annealing temperature and concentrations of reagents. However, after about 10 PCRs, I was successful amplifying pure aphid DNA and was unsuccessful amplifying aphid DNA from spider guts. After talking with James Harwood and further researching primers used for other gut-content studies, I found that target DNA detection has been successful with primers that amplify fragments of 300 bp or less (Zaidi et al. 1999, Hoogendoorn and Heimpel 2001, Juen and Traugott 2005, Monzó et al. 2010). These primers were designed to amplify a much larger fragment, 342bp. Thus, this might have been the problem.

Additionally, I spoke with Jan Stephens, and she indicated that I try several things.

1. It was a necessity to quantify my DNA to make sure I was putting the appropriate amount of DNA template within my PCR reactions. Thus, using the NanoDrop in the Leach lab, I measured all of my aphid and spider extractions.

2. I also ran gels on my genomic DNA to see if my DNA was degraded (Figure 4.7). For quality DNA, the gel will ideally show one bright band at the top and should be clear from that band down. Otherwise, smearing is indicative of DNA degradation. Because the aphid DNA measured less than 10ng/ul, the total DNA was not apparent on the gel. The *P. sternalis* DNA shows bright bands at the bottom but also shows considerable smearing throughout the rest of the lane. With *T. laboriosa*, bright bands were apparent at the top of the gel and looked clear throughout the rest of the lanes. Although I saw degradation from this gel with *P. sternalis*, I still had bright enough bands and decent-quality DNA.

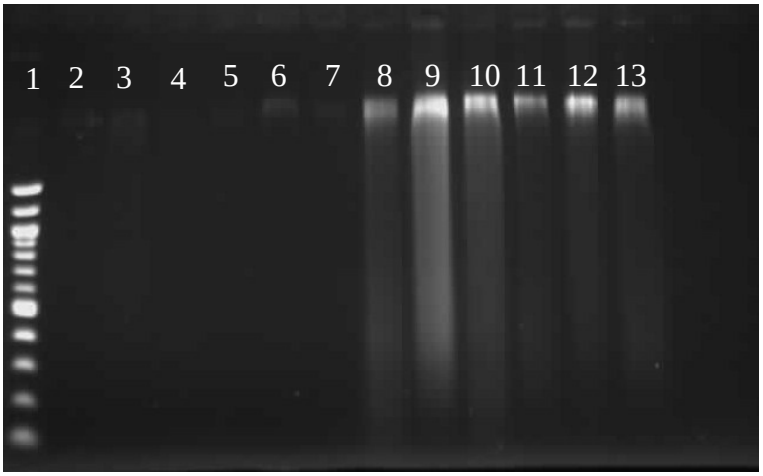

FIGURE 4.7. AGAROSE GEL OF TOTAL GENOMIC DNA.

Lane 1=100bp ladder, Lanes 2-7=*D. noxia* total DNA, Lanes 8-10=*P. sternalis* 0hr positive controls, Lanes 11-13=*T. laboriosa* 0hr positive controls.

3. Also, my gels were showing lots of streaking at the Denver Museum, which was indicative of using too much DNA in my PCRs. I diluted all of my spider extractions to 50 µg/ul to standardize the amount of template I used in each reaction and tried amounts from 50-400µg/ul within the reactions. In particular, I used starved spider

DNA mixed with aphid DNA to determine optimal concentrations of spider DNA to use in PCRs (Figure 4.8).

a.

b.

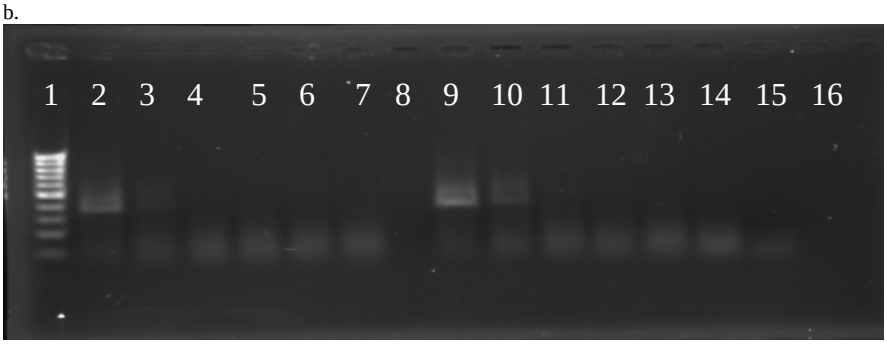

FIGURE 4.8. TESTING DIFFERENT CONCENTRATIONS OF *D. NOXIA* DNA MIXED WITH STARVED SPIDER *P. STERNALIS* AND *T. LABORIOSA* DNA.

4. We also tried shearing the DNA to make sure that degraded DNA could be detected with these primers (Figure 4.9). We sheared DNA with a 25 gauge needle, extracting and expelling the DNA several times.

FIGURE 4.9. TEST OF DNA SHEARING

Lane 1=100bp ladder, Lanes 2=non-sheared *D. noxia* DNA, Lane 3=*D.noxia* DNA sheared 40 times, Lane 4=*D. noxia* DNA sheared 100 times.

5. I tried to work with these primers again with Jan, but I was still unable to get any spiders fed aphids to amplify any DNA. I decided to try another pair of primers and continue working at CSU.

Primer Pair 3: cytbF_2 and cytbR_2 (228bp), gene=mitochondrial, Cytochrome B (cytb)

I decided to use another pair of primers that I had created with Myron Bruce from the same gene (cytb), but amplified a smaller fragment (228 bp) (F=cgaaaacgtggtaaaaatcca, R=tgatttttctgagggagaatctg). I used a new kit (Takara *Taq*), and I found these primers to amplify both *D. noxia* DNA and *D. noxia* DNA from spider guts (Figure 4.10).

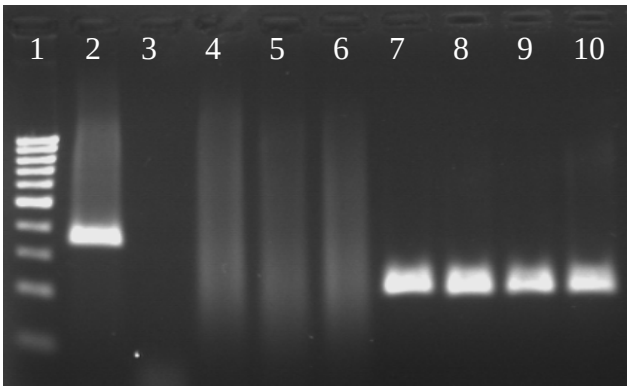

FIGURE 4.10. SUCCESSFUL PCR WITH NEW 228 CYTB PRIMERS (LAST FOUR LANES).

I continued to optimize these primers for several PCRs by doing gradient PCRs, testing different concentrations of primers and reagents, and trying to increase sensitivity. Since I tested several positive control spiders fed one *D. noxia* and frozen 0-24 hours post-feeding, I wanted to try to increase the sensitivity of these primers up to as many hours as possible.

I ran into a contamination issue after about 10 PCRs, which caused me to switch reagents, water, purchase new primers, and do a major clean-up. The contamination issue was off and on for several PCRs. I reduced the number of cycles along with several of the troubleshooting methods above, which appeared to help.

I continued to try to make the primers more sensitive, so I tried several PCRs with gradient magnesium chloride concentrations. I was able to detect *D. noxia* DNA from spider guts up to 12 hours post-feeding with additional $MgCl_2$ additions. I encountered a problem with multiple bands with all the spider positive controls (not as much with just *D. noxia* DNA), and it would not disappear (Figure 4.11). I tried gradient PCRs, reducing magnesium chloride concentrations, and cleaning. As I tried to use these primers again later, it may be that the DNA was very degraded, and these primers were amplifying fragmented DNA.

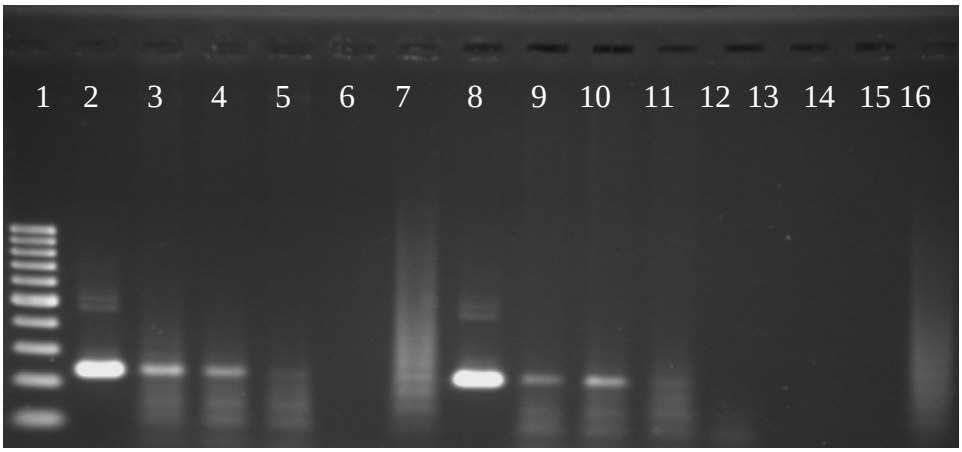

FIGURE 4.11. EXAMPLE OF PCR WITH MULTIPLE BANDS.

Primer Pair 4: COI_144F and COI_329R, gene=mitochondrial, cytochrome oxidase I (COI)

The ideal way to create primers for gut-content analysis is to sequence the target aphid and other non-targets that you specifically do not want to amplify with general

primers, align these sequences, and choose unique primer pairs (King et al. 2008). Therefore, I proceeded with this method. The universal primers C1-J-1718 and C1-N-2191 were used to amplify a partial fragment of the cytochrome oxidase I gene (COI) (Simon et al. 1994) (Figure 4.12). The extractions of three *D. noxia* and extractions of the following species were used for PCR design: *Diuraphis frequens*, *Diuraphis tritici*, *Sitobion avenae*, *Rhopalosiphum padi*, *Schizaphis graminum*, *Acyrthosiphum pisum*, *Sipha elegans*, *Pardosa sternalis*, *Tetragnatha laboriosa*. Sequences were aligned using Bioedit and CLUSTAL W (Thompson et al. 1997). Several pairs of primers were chosen using the primer design software Primer 3 (Rozen and Skaletsky 1998) and primer-design guidelines, and one pair of primers was selected and optimized (COI_144F-TCCATGATCAATTCTAATTACAGCTATTC, COI_329R-AAATATAAACTTCAGGATGTCCAAAAA). The primers produced for *D. noxia* were species-specific, which indicated that they did not amplify other non-target arthropods typically present with the agroecosystems sampled.

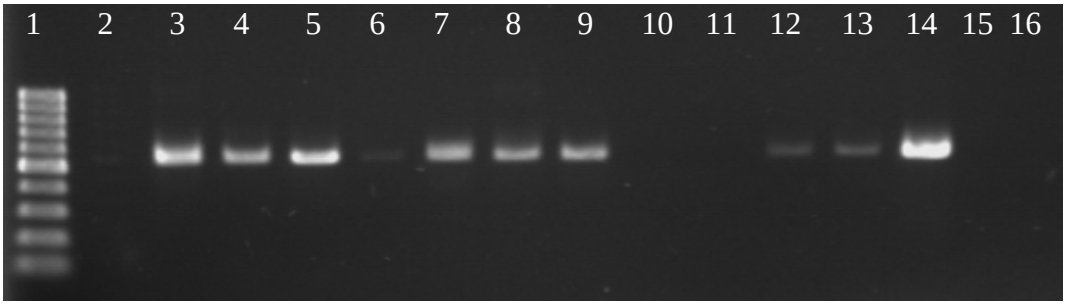

FIGURE 4.12. AMPLIFICATION OF *D. NOXIA* DNA AND OTHER NON-TARGET SPECIES USING GENERAL COI PRIMERS.

These primers were further optimized by testing them against other non-target aphid species and raising the annealing temperature to 60ºC (Figure 4.13). The primers did not amplify any non-target aphids at this temperature.

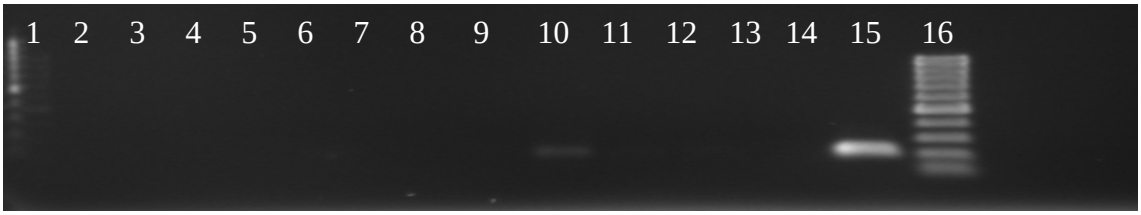

FIGURE 4.13. TESTING OF PRIMERS AGAINST NON-TARGET APHIDS

The primers effectively amplified *D. noxia* DNA from spider guts up to 16 hours post-digestion (Figure 4.14).

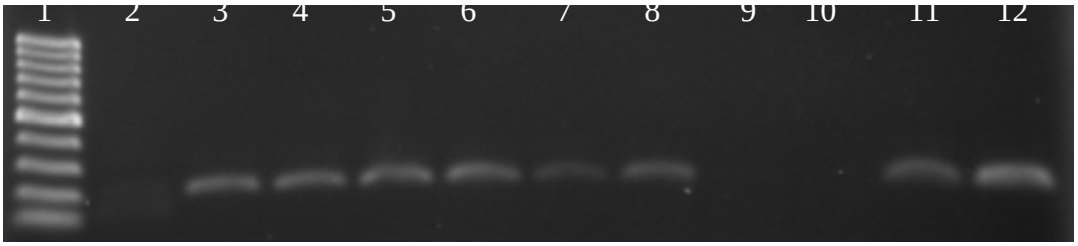

FIGURE 4.14. 16 HOUR POSITIVE *T. LABORIOSA* CONTROLS.

However, this was not repeatable. The negative control was inconsistently contaminated, but had a very bright, clear band (Figure 4.15-last lane). After several PCRs of thinking it was my mistake (many cleanings, reevaluating pipetting techniques, gel loading, glove use, etc.), I found a paper that indicated that sometimes *Taq* has impurities, and, thus, bacterial contaminations, such as *Escherichia coli* can be amplified in PCRs (Tonduer et al. 2004). *Taq* is often manufactured in *E. coli* as a recombinant protein, and bacterial contaminants can be common. I tried several different high fidelity DNA polymerases, but I still had the band in my negative control. I decided to try one last pair of primers, as this was beyond my control.

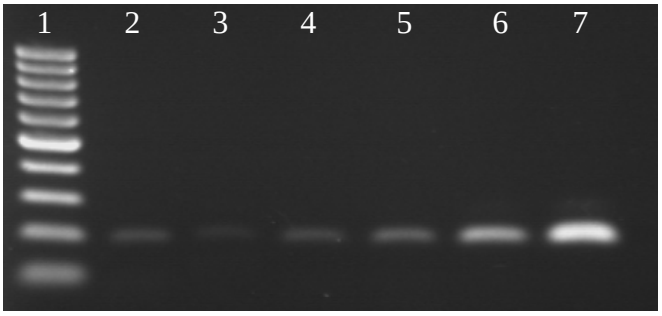

FIGURE 4.15. PCR INDICATING CONTAMINATED NEGATIVE CONTROL (LAST LANE).

Primer Pair 5: COI_809F and COI_1036R, gene=mitochondrial, cytochrome oxidase I (COI)

A partial 1146 bp sequence from the mitochondrial cytochrome oxidase I (COI) gene was retrieved from the GenBank database (Accession #FJ232620, *D. noxia*). See Chapter 4 for the remaining discussion with these primers.

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CHAPTER 5-CONCLUSION

A crop-intensified agricultural system had little effect on spider density or biodiversity when compared with a conventional system. The mean density of spiders for vacuum and pitfall sampling was low at all sites and years, with the exception of 2005 and 2006. When densities of spiders are low, the biological control potential generally is limited (Greenstone 2001). The years 2005 and 2006 showed increased mean spider densities and biodiversity, which can mainly be attributed to increased precipitation, weed, and vegetatative growth. Specifically, the density and biodiversity of linyphiid spiders increased during these years. Spider densities did not increase in the summer crop following wheat harvest, suggesting that this alternative crop did not act as a refuge for spiders following wheat harvest at any of the sites.

The faunal composition of spiders also can be suggestive of the biological control potential of pests. The number of spider individuals in this study was represented by over 70% of the cursorial hunting spider families, Gnaphosidae and Lycosidae, at all sites, with the majority of the fauna dominated by three or fewer species. Some Lycosidae, such as *Pardosa hortensis* Thorell, can contribute to reduced pest densities by both killing pests with and without consumption (Samu and Bíró 1993); however, the additional killing of pests through webs alone is not possible. The low density of spiders and the lack of predominately web-building spiders suggest that the biological control potential of eastern Colorado spiders is likely limited.

Because the spider composition was dominated by cursorial hunters, it was necessary to establish that pest prey were available for the dominant fauna present. *Diuraphis noxia* falling rates were greatest later in the wheat-growing season by the time of senescence. Falling rates were highest when *D. noxia* densities were lowest. When the predator-to-prey ratio is highest, this is when spiders can have a greater effect on suppressing pest densities (Edwards et al. 1979, Ekbom and Wiktelius 1985, Chiverton et al. 1986, Birkhofer et al. 2008). As this suggests that spiders will be more effective later in the season, this is after *D. noxia* has reached peak densities.

Increased biodiversity and biological control efficiency is typically case dependent (Straub et al. 2008). Furthermore, maintaining spider species that are consuming pests in the field is important. *Tetragnatha laboriosa* and *P. sternalis* consumed *D. noxia* DNA in the field, with a high percentage testing positive for the presence of aphid DNA within the gut, 32% and 48%, respectively. Interestingly, *T. laboriosa* appeared to track aphid densities, as 92% of *T. laboriosa* were collected at the 1x or 10x *D. noxia* infestation levels combined. Although *P. sternalis* did not appear to track aphid densities, it was present early in the wheat-growing season.

The hunting guild spp. and low mean density of spiders overall suggest a limited potential of spiders as biological control agents in these agroecosystems; however, the high percentage of *T. laboriosa* and *P. sternalis* testing positive for aphid consumption indicate that, when infestations of *D. noxia* are high, feeding on pest species is likely. It is, therefore, important to identify the dominant predators within an agricultural system and directly ascertain through molecular analyses whether these natural enemies are feeding on the target pest species.

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