Influence of Selective Insecticides and Cropping System on Arthropod Natural Enemies in Soybean

Rebecca Anne Whalen

Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Entomology

D. Ames Herbert, Chair
Carlyle C. Brewster
Thomas P. Kuhar
Doug G. Pfeiffer
Dominic D. Reisig

April 19, 2016
Blacksburg, VA

Keywords: Natural enemies, diamides, spiders, diversity, Carabidae

Influence of Selective Insecticides and Cropping System

on Arthropod Natural Enemies in Soybean

Rebecca Anne Whalen

Abstract

Arthropod natural enemies in soybean play a critical role in keeping potential pests under economic threshold. Soybean is one of the largest crops in Virginia in terms of acreage grown and soybean fields could be a rich source of natural enemies for other crops. Broad-spectrum insecticide application, which is a common practice in soybean, can decrease natural enemy densities and diversity and allow for secondary pest outbreaks. As an alternative, selective insecticides provide pest control while preserving natural enemies. Another factor that could affect the natural enemy community is cropping system, which determines when soybean is planted. My research objectives were to 1) determine the effects of a new class of selective insecticides on the natural enemy community; 2) compare the spider assemblages in two soybean cropping systems; and 3) compare the arthropod natural enemy communities in two soybean cropping systems. To address objective 1, we examined natural enemy diversity and abundance after exposure to selective diamide insecticides. By counting common natural enemies in soybean for three weeks after insecticide application, we found the diamides flubendiamide and chlorantraniliprole did not disrupt the natural enemy community. For objective 2 we identified 7,371 specimens and found 76 spider species in soybean fields over the course of two years. We found diversity of foliar-dwelling spiders in full season and double crop soybean to be similar, but in 2014 the ground-dwelling spider community in full season soybean had higher diversity. Double crop soybeans had higher abundance on the ground and in the foliage compared with full season soybean. To address objective 3 we examined the natural enemy community in double crop and full season soybeans and found more arthropod natural enemies in double crop fields, both on the ground and in the foliage. There were significant differences in some dominant predators and diversity of a family of predatory beetles was higher in full season soybean. The similarity

in spider and insect natural enemy diversity and abundance trends suggests that a greater number of species can co-exist in full season soybean, while in double crop soybeans a few dominant natural enemies thrive.

Influence of Selective Insecticides and Cropping System

on Arthropod Natural Enemies in Soybean

Rebecca Anne Whalen

General Audience Abstract

Arthropod natural enemies play a key role in controlling potentially damaging pest populations in agroecosystems. An abundant and diverse natural enemy community is associated with higher yields in a variety of crops. Certain aspects of soybean production can make a field more or less amenable to a robust natural enemy community. For instance, commonly used broad-spectrum insecticides which are highly toxic to most arthropods can decrease natural enemy densities and allow for secondary pest outbreaks. Selective insecticides that have less impact on natural enemy populations allow for pest control while preserving important predators. Another production decision that could alter natural enemy communities is the choice of cropping system, specifically planting early (full season) or late, after small grain harvest (double crop). My research objectives were to examine how 1) selective insecticides and 2) cropping system affect the density and diversity of natural enemies in Virginia soybean. To address the first objective I compared the natural enemy community in soybean plots that were exposed to selective insecticides, broad-spectrum insecticides or no insecticide. I sampled insects using three different techniques and found that the two selective insecticides I tested, both from a new class called diamides, did not reduce the natural enemy community compared to controls. To examine how cropping system affects the natural enemy community I sampled full season and double crop fields during the growing season for two years. In 2014 ground-dwelling spider diversity was higher in full season soybean. In both years, double crop soybeans had higher abundance of spiders and insect natural enemies on the ground and in the foliage compared with full season soybean. This was unexpected, since double crop soybeans are planted later than full season and arthropod populations would have less time to colonize and grow. When I compared diversity of a family of predatory beetles I found higher diversity in full season

soybean. The similarity in spider and insect natural enemy diversity and abundance trends suggests that a greater number of species can co-exist in full season soybean, while in double crop soybeans a few dominant natural enemies thrive.

Acknowledgements

I'd like to thank my advisor, Dr. Ames Herbert, for his guidance and patience, and Dr. Sean Malone, for his relentless help in the field and for giving me advice for this dissertation. I'd also like to thank Kenny Worrell, Bob Rogers, Scott McGee and Milton Dunn for allowing me to use their fields for my research. My spider research would not have been possible without the identification skills of Dr. Michael Draney of the University of Wisconsin, Green Bay. I also thank Pat Miller for her help with Pirata identification and Dr. Peter Messer for verification of Carabidae specimens. My dissertation is much improved thanks to comments by my committee, Drs. Doug Pfeiffer, Carlyle Brewster, Thomas Kuhar and Dominic Reisig (NCSU).

Finally I'd like to thank my parents, Deb and Bob, for their endless enthusiasm, support and kindness throughout my graduate career.

Table of Contents

CHAPTER ONE

Introduction and Literature Review

1.1 Arthropod Natural Enemies in Soybean

Arthropod natural enemies are a valuable asset to agroecosystems. Generalist natural enemies eat a wide range of species, compared with specialists that focus on one or a few types of prey. The generalist community can effectively reduce crop damage and increase yield in a variety of agricultural systems (Kajak 1978, Symondson et al. 2002). These natural enemies can keep potential herbivore pests under threshold levels. In soybean in particular, the natural enemy complex is considered to be of prime importance in preventing insects from attaining pest status (Turnipseed and Kogan 1976). Generalists are more versatile in their dietary habits than specialists, which can be highly advantageous in a fast changing environment such as an annual crop. Tillage and harvest could upset some species more than others, and generalists might be able to find prey after upheaval more easily than a predator with narrower preferences. A key benefit of generalists over specialists is their ability to arrive at and subsist in fields early in the growing season, or even live in a field year-round. This availability allows for populations to grow and achieve a high predator:pest ratio before herbivores can acquire pest status (Settle et al. 1996). Better pest control is often associated with a rich and even natural enemy assemblage (Gurr et al. 2012). Biological control with generalist predators is thought to work best when conservation efforts are targeted at species that work well together and aren't prone to intraguild predation, which refers to one predator eating another (Gurr et al. 2012). Determining compatible species requires a thorough knowledge of the natural enemy community in a given crop or area of the country. Knowledge of when natural enemies in a crop hit peak densities can be critical, since more efficient biological control is associated with high population densities early in the season (Edwards et al. 1979, Birkhofer et al. 2008).

1.2 Spiders as Natural Enemies

Spiders, order Araneae, are a ubiquitous generalist natural enemy and a lesser studied option for biological control. They are effective predators that tend to kill more insects than they consume (Maloney et al. 2002). The dietary plasticity associated with spiders could make them a valuable asset in crops (Howell and Pienkowski 1971, Young and Edwards 1990, Gurr et al. 2012, Royaute and Pruitt 2015). Spiders in eastern soybean tend to hit peak density in August and slowly decline through harvest (Culin and Yeargan 1983a). By living off prey such as detritivores spiders could potentially grow their populations well before pest species arrive. Research has proven spiders can lower pest densities in many crops, including wheat, apple, corn and rice (Laub and Luna 1992, Marc and Canard 1997, Fagan et al. 1998, Marc et al. 1999). Several factors can affect the identity and abundance of the spider community in a field. For instance, spiders can vary depending on the type of vegetation bordering a field. A study done in forests and hedgerows adjacent to soybean and corn found higher spider species diversity in deep forests compared with narrower forests (Buddle et al. 2004). The researchers also found that fields that border forests have different species compositions and diversity from fields that border streams and this can influence what spiders will colonize a field. However, bordering habitat doesn't always affect the spider community in a field (Schmidt et al. 2005, Fox et al. 2015, Horvath 2015). Information on species composition is important when determining if the spider species present in a field are effective at controlling a particular pest in a crop. In U.S. cropping systems, hunting spiders, like wolf spiders, Lycosidae, make up about half of any given community (Nyffler and Sunderland 2003). A comparison of wolf spiders in different crop rotations and tillage of cotton found certain species were more abundant in more complex crop rotations, while others were similar in density to those in simple crop rotations (Rendon et al. 2015). This sort of research can be informative when determining which spiders are more resilient or fast to recolonize after annual disturbance in fields. Ground disruption such as planting or harvesting could even lead to higher species richness (Culin and Yeargan 1983b).When attempting to optimize pest management, spider species evenness also needs to be considered. Evenness refers to how close the population number of each species is to each other. A very even community will have many species with similar numbers of individuals. Research in organic systems has found greater pest suppression in more even communities (Crowder et al. 2010). Species richness, or the number of species in a field, can affect prey suppression. High spider richness is associated with reduced pest densities (Riechert et al. 1999).

1.3 Insect Natural Enemies in Soybean

There are a few natural enemies that are frequently found in eastern United States soybean agroecosystems. These include ground beetles (Carabidae), bigeyed bugs (Geocoridae), and minute pirate bugs (Anthocoridae), specifically *Orius insidiosus* (Say) (Anderson and Yeargan 1998). Carabidae are mostly generalist predators and many species are predators of common agricultural pests (Sunderland et al. 1995, Kromp 1999). In a given agroecosystem it is common for a few species of beetles to dominate. Knowing the identity of dominant beetles could then give rise to research on the common prey of these species and if they coincide with the onset of major pests. Ground beetle communities are sometimes looked upon as ecological indicators in agroecosystem studies, in part because of ease of sampling and identification as well as their widespread nature (Clark et al. 2006). They also respond to change in microclimate and habitat (Wallin 1986, Hatten et al 2007). Researchers have examined the ground beetle community to gauge impact of farming practices such as organic and no-till cropping systems, transgenic crops, and intercropping (French et al. 2004, Clark et al. 2006, Hummel et al. 2012). By comparing species diversity and abundance of ground beetles researchers hope to gain insight on how management practices are affecting the generalist predator complex.

1.4 Natural Enemy Diversity in Agroecosystems

Generally, greater species richness and diversity in an agroecosystem is associated with greater herbivore suppression (Letourneau et al. 2009, Rusch et al. 2010, Gurr et al. 2012). Biodiversity acts to improve suppression of outbreaks in two ways: complementarity and facilitation. Two species may not share prey, leaving no overlap in function and allowing for complementary occupation of a field. An example of this is a large wolf spider preferring to eat live prey such as a caterpillar, and *O. insidiosus*, a tiny Hemipteran that is a valuable egg predator. The greater the number of species the more likely there will be predators that prefer different potential pests. Hunting space can also make two species complementary. A ground-dwelling centipede won't be fighting for the same food as a lady beetle that primarily forages in the foliage. Two species of predators can potentially aide each other in pest control, leading to facilitation. This happens when a pest is fleeing one predator and is therefore caught by a second predator. Additionally, more diverse natural enemy communities make fields more difficult for pests to invade (Hooper et al. 2005). More species means more hunting space or prey preference niches can be occupied, which can ensure a strong defensive from a variety of potential invaders.

1.5 Natural Enemy Sampling Techniques

A diverse generalist predator community can enhance pest control, but the effectiveness of the generalist community for biological control is system specific (Gurr et al. 2012). A survey of the natural enemy community to record identity and abundance is key for determining dominant species and population trends of common or especially useful natural enemies. Many research studies focused on the natural enemy community will employ more than one means of sampling. Pitfall traps placed in fields catch ground-dwelling arthropods both day and night but are considered to provide estimates of activity density, which depends on species population size, activity and catchability (Greenslade 1964). They are commonly used in soybean for spider and other natural enemy research (Ferguson et al. 1984, Witmer et al. 2003, French et al. 2004, Clark et al. 2006). Although pitfall traps don't estimate absolute density, it is considered valuable for estimating relative abundance of species in similar habitat. Sweep nets are a popular method for sampling natural enemies in soybean foliage (Lesar and Unzicker 1970, Ohnesorg et al. 2009, Varenhorst and O'Neal 2012).

1.6 Soybean Cropping Systems

Soybean, *Glycines max* (L. Merrill), is the second largest crop in terms of acreage grown in United States and one of the largest in Virginia (soystats.com); there were 600,000 acres in Virginia in 2014 mostly located in the eastern half of the state (Holshauser 2014). In 2014 the average yield was 39.5 bushels/acre in the state and crop value was $259 million (soystats.com).

Crop management can influence predator abundance and diversity. Crop rotation, tillage, and intercropping are each known to affect natural enemy assemblages (Clark et al. 2006, Hummel et al. 2012, Kerzicnik et al. 2014). A crop management technique popular in Virginia soybean is the double cropping method. By planting into harvested wheat or barley stubble in June or July, the soybean can better utilize available soil moisture among other benefits (McPherson et al. 1982). The other option is full season soybeans, which are planted in April or May in Virginia. There is some research on the natural enemy communities in the two systems. Two common natural enemies in soybean, *Geocoris punctipes* (Say) and Nabidae were found to be more common in full season soybean than double crop soybean (McPherson et al. 1982). The study by McPherson et al. (1982) was done in large soybean fields in Virginia, using sweep net sampling. They found no differences in spider abundance between full season and double crop soybeans. They concluded that predators in double crop soybean had low abundance until the beans were fully formed, and therefore predators couldn't be relied upon to provide adequate protection, compared with the enhanced predator build up that early planting allows for.

The stubble that is part of the double crop soybean field creates early season structural complexity. Habitat complexity in soybean has been associated with higher numbers of wolf spiders and higher spider diversity (Marshall and Rypstra 1999, Rypstra et al. 1999). Full season soybean is commonly planted into bare ground. The complexity of double crop fields before soybean even starts to grow could make it attractive to ground-dwelling predators. Ground spider diversity is higher with higher habitat complexity (Uetz et al. 1999).

1.7 Soybean Insecticides and Natural Enemies

Preservation of natural enemies is an integral part of an integrated pest management plan. One way to facilitate this goal is using selective insecticides. Selective insecticides are designed to be less harmful to some insects, often natural enemies. This is a different approach from that of broad-spectrum insecticides, which have historically been extremely popular and are generally less expensive. The use of broad-spectrum insecticides in fields that have several different pests and an effective natural enemy community can be detrimental to the natural enemy community (Croft and Brown 1975), induce secondary pest outbreaks (Ruberson et al. 2000) and result in increased Lepidoptera larvae in soybean (Morrison et al. 1979). Studying the effects of a given pesticide on the natural enemy community can help determine the IPM compatibility of the chemical control tool. Field trials are considered necessary to determine the effects of insecticides on natural enemy efficiency (Ruberson et al. 2000). To adequately gauge natural enemy community response to an insecticide, field trials must be performed in plots that are large enough so that between-plot movement of natural enemies and pesticide drift is minimized (Ruberson et al. 2000). Performing field trials is necessary to determine the effect of insecticides on the natural enemy community since many effects cannot be determined in lab settings. Generally to determine effect of selective insecticides to the natural enemy community, several arthropods are selected to screen before and after exposure. These should represent a cross section of exposure risk, resource guilds and those of high abundance or importance should be given priority (Ruberson et al. 2000). Natural enemies will likely respond in different ways to an insecticide. Different growth stages might react differently and exposure risk changes depending on foraging space or canopy cover. In cotton, Kilpatrick et al (2005) showed that some neonicotinoids, a class of selective insecticides, reduce populations of bigeyed bugs and red imported fire ants but not spiders. One neonicotinoid, thiamethoxam, was linked to higher bollworm populations due to the decrease in predators. Knowing that the spider abundance was unaffected could help with a management strategy that incorporates neonicotinoids to control sucking insects but also actively conserves or supplements the spider community. In another study, neonicotinoids applied to potatoes were found not to affect total ground dwelling predator densities (Koss et al. 2005).

Diamide insecticides are selective insecticides that are effective at killing a range of Lepidoptera larvae. These insecticides mimic the natural plant metabolite ryanodine, which is known to modify calcium channels (Lahm et al. 2009). Insecticides that target the ryanodine receptor were introduced commercially as flubendiamide and chlorantraniliprole. Both cause an immediate halt in feeding in Lepidoptera and death to the larvae within three days (Cordova et al. 2006). Flubendiamide and chlorantraniliprole are sprayed onto the crop and introduced into the plant via translaminar mobility and both are protected from ultraviolet degradation and precipitation (Lahm et al. 2009). These insecticides are reported to have low risk to predatory insects, mites and wasps, though field studies investigating this are scarce (Lahm et al. 2009). There is little published research on how natural enemies in soybean fields are affected by application of diamide insecticides.

1.8 Research Objectives

The objective of this research is to better understand the links between the natural enemy community and various farm management practices. First, we aim to determine how insecticides that are considered selective and soft on beneficial insects alter the natural enemy community in soybean. This will be one of the first studies that examine how the community in the field is affected by application of a specific class of insecticides, diamides. Data from this experiment can be used to affirm or dispute the claim that these insecticides are less detrimental to natural enemies than traditional broad-spectrum insecticides. Second, we will determine how two popular cropping systems in soybean affect the spider community in soybean. One system could harbor different spider diversities and abundance compared with the other, and this could mean different species should be targeted for conservation. Third, we will evaluate how the entire natural enemy community responds to these same two cropping systems. Soybeans are known to harbor many families and species of natural enemies and we will characterize how abundance and diversity change depending on when soybean is planted.

To address these objectives, we performed two experiments. The first experiment involved counting natural enemies in soybean before and after application of insecticides. Over two years a total of 40 soybean plots were exposed to the diamide insecticides flubendiamide or chlorantraniliprole; the pyrethroid lambda-cyhalothrin+chlorantraniliprole; or no insecticide. We sampled foliar dwelling natural enemies once prior to application and three times post-application using sweep nets, sticky cards and a beat cloth in 2013, and sweep nets in 2015. Our second experiment was conducted in commercial soybean fields and in fields at a Virginia research station. Large plots were established that were not sprayed with insecticides throughout the growing season. Half of the plots consisted of full season soybean and half double crop soybean. We used pitfall traps and sweep nets to survey predatory insects, spiders, and other arthropods throughout the growing season for two years. Spiders and ground beetles were identified to species and all other predatory arthropods were identified to the lowest taxonomic level possible.

References

Anderson, A. C. and K. V. Yeargan. 1998. Influence of soybean canopy closure on predator abundances and predation on Helicoverpa zea (Lepidoptera : Noctuidae) eggs. Environmental Entomology 27: 1488-1495.

Birkhofer, K., E. Gavish-Regev, K. Endlweber, Y. D. Lubin, K. von Berg, D. H. Wise and S. Scheu. 2008. Cursorial spiders retard initial aphid population growth at low densities in winter wheat. Bulletin of Entomological Research 98: 249-255.

Buddle, C. M., S. Higgins and A. L. Rypstra. 2004. Ground-dwelling spider assemblages inhabiting riparian forests and hedgerows in an agricultural landscape. American Midland Naturalist 151: 15-26.

Clark, S., K. Szlavecz, M. A. Cavigelli and F. Purrington. 2006. Ground beetle (Coleoptera : Carabidae) assemblages in organic, no-till, and chisel-till cropping systems in Maryland. Environmental Entomology 35: 1304-1312.

Cordova, D., E. A. Benner, M. D. Sacher, J. J. Rauh, J. S. Sopa, G. P. Lahm, T. P. Selby, T. M. Stevenson, L. Flexner, S. Gutteridge, D. F. Rhoades, L.Wu, R. M. Smith and Y. Tao. 2006. Anthranilic diamides: A new class of insecticides with a novel mode of action, ryanodine receptor activation. Pesticide Biochemistry and Physiology 84: 196-214.

Croft, B. A. and A. W. A. Brown. 1975. Responses of arthropod natural enemies to insecticides. Annual Review of Entomology 20: 285-335.

Crowder, D. W., T. D. Northfield, M. R. Strand and W. E. Snyder. 2010. Organic agriculture promotes evenness and natural pest control. Nature 466: 109-U123.

Culin, J. D. and K. V. Yeargan (a). 1983. Comparative study of spider communities in alfalfa and soybean ecosystems - foliage dwelling spiders. Annals of the Entomological Society of America 76: 825-831.

Culin, J. D. and K. V, Yeargan (b). 1983. Comparative study of spider communities in alfalfa and soybean ecosystems - ground-surface spiders. Annals of the Entomological Society of America 76: 832-838.

Denys, C. and T. Tscharntke. 2002. Plant-insect communities and predator-prey ratios in field margin strips, adjacent crop fields, and fallows. Oecologia 130: 315-324.

Edwards, C. A., K. D. Sunderland and K. S. George. 1979. Studies on polyphagous predators of cereal aphids. Journal of Applied Ecology 16: 811-823.

Fagan, W. F., A. L. Hakim and H. Ariawan. 1998. Interactions between biological control efforts and insecticide applications in tropical rice agroecosystems: The potential role of intraguild predation. Biological Control 13: 121-126.

Ferguson, H. J., R. M. McPherson and W. A. Allen. 1984. Effect of four soybean cropping systems on the abundance of foliage-inhabiting insect predators. Environmental Entomology 13: 1105-1112.

Fox, A. F., D. B. Orr and Y. J. Cardoza. 2015. The influence of habitat manipulations on beneficial ground-dwelling arthropods in a southeast US organic cropping system. Environmental Entomology 44: 114-121.

French, B. W., L. D. Chandler, M. M. Ellsbury, B. W. Fuller and M. West. 2004. Ground beetle (Coleoptera : Carabidae) assemblages in a transgenic corn-soybean cropping system. Environmental Entomology 33: 554-563.

Greenslade, P. J. M. 1965. Pitfall trapping as a method of studying populations of Carabidae (Coleoptera). Journal of Animal Ecology Oxford 33: 301-310.

Gurr, G., S. D. Wratten, W. E. Snyder and D. M. Y. Read. 2012. Biodiversity and pests : key issues for sustainable management. Chichester, West Sussex, UK ; Hoboken, NJ, John Wiley & Sons.

Hatten, T. D., N. A. Bosque-Perez, J. R. Labonte, S. O. Guy and S. D. Eigenbrode. 2007. Effects of tillage on the activity density and biological diversity of carabid beetles in spring and winter crops. Environmental Entomology 36: 356-368.

Holshouser, D. 2014. Double cropping soybeans in Virginia. Virginia Cooperative Extension publication CSES - 102NP.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setala, A. J. Symstad, J. Vandermeer and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75: 3-35.

Horvath, R., T. Magura, C. Szinetar, J. Eichardt, E. Kovacs and B. Tothmeresz. 2015. In stable, unmanaged grasslands local factors are more important than landscape-level factors in shaping spider assemblages. Agriculture Ecosystems & Environment 208: 106-113.

Howell, J. O. and Pienkowski, R. 1971. Spider populations in alfalfa, with notes on spider prey and effect of harvest. Journal of Economic Entomology 64: 163-&.

Hummel, J. D., L. M. Dosdall, G. W. Clayton, K. N. Harker and J. T. O'Donovan. 2012. Ground beetle (Coleoptera: Carabidae) diversity, activity density, and community structure in a diversified agroecosystem. Environmental Entomology 41: 72-80.

Kajak, A. 1978. Analysis of consumption by spiders under laboratory and field conditions. Ekologia Polska-Polish Journal of Ecology 26: 411-428.

Kromp, B. 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agriculture Ecosystems & Environment 74: 187-228.

Laub, C. A. and J. M. Luna. 1992. Winter cover crop suppression practices and natural enemies of armyworm (Lepidoptera, Noctuidae) in no-till corn. Environmental Entomology 21: 41-49.

LeSar, C. D. and J. D. Unzicker. 1978. Soybean spiders : species composition, population densities and vertical distribution. Biological notes 107.

Letourneau, D. K., J. A. Jedlicka, S.G. Bothwell and C.R. Moreno. 2009. Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annual Review of Ecology Evolution and Systematics. Palo Alto, Annual Reviews. 40: 573-592.

Maloney, D., F. A. Drummond and R. Alford. 2003. Spider predation in agroecosystems: Can spiders effectively control pest populations. Maine Agricultural and Forest Experiment Station, The University of Maine Technical Bulletin 190.

Marc, P. and A. Canard. 1997. Maintaining spider biodiversity in agroecosystems as a tool in pest control. Agriculture Ecosystems & Environment 62: 229-235.

Marc, P., A. Canard and F. Ysnel. 1999. Spiders (Araneae) useful for pest limitation and bioindication. Agriculture Ecosystems & Environment 74: 229-273.

Nyffeler, M. and K. D. Sunderland. 2003. Composition, abundance and pest control potential of spider communities in agroecosystems: a comparison of European and US studies. Agriculture Ecosystems & Environment 95: 579-612.

Ohnesorg, W. J., K. D. Johnson and M. E. O'Neal. 2009. Impact of reduced-risk insecticides on soybean aphid and associated natural enemies. Journal of Economic Entomology 102: 1816-1826.

Rendon, D., M. E. A. Whitehouse, N. R. Hulugalle and P. W. Taylor. 2015. Influence of crop management and environmental factors on wolf spider assemblages (Araneae: Lycosidae) in an Australian cotton cropping system. Environmental Entomology 44: 174-185.

Riechert, S. E., L. Provencher and K. Lawrence. 1999. The potential of spiders to exhibit stable equilibrium point control of prey: Tests of two criteria. Ecological Applications 9: 365-377.

Royaute, R. and J. N. Pruitt. 2015. Varying predator personalities generates contrasting prey communities in an agroecosystem. Ecology 96: 2902-2911.

Rusch, A., M. Valantin-Morison, J. P. Sarthou and J. Roger-Estrade, 2010. Biological control of insect pests in agroecosystems: Effects of crop management, farming systems, and seminatural habitats at the landscape scale: a review. Advances in Agronomy, Vol 109. D. L. Sparks. San Diego, Elsevier Academic Press Inc. 109: 219-259.

Schmidt, M. H., I. Roschewitz, C. Thies and T. Tscharntke. 2005. Differential effects of landscape and management on diversity and density of ground-dwelling farmland spiders. Journal of Applied Ecology 42: 281-287.

Settle, W. H., H. Ariawan, E. T. Astuti, W. Cahyana, A. L. Hakim, D. Hindayana, A. A. Lestari and Pajarningsih. 1996. Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. Ecology 77: 1975-1988.

soystats.com. 2016. American Soybean Association.

Sunderland, K. D., G. L. Lovei and J. Fenlon. 1995. Diets and reproductive phenologies of the introduced ground beetles Harpalus-affinis and Clivina-australasiae (Coleoptera, Carabidae) in New Zealand. Australian Journal of Zoology 43: 39-50.

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

Turnipseed, S. G. and M. Kogan. 1976. Soybean entomology. Annual Review of Entomology 21: 247-282.

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

Varenhorst, A. J. and M. E. O'Neal. 2012. The response of natural enemies to selective insecticides applied to soybean. Environmental Entomology 41: 1565-1574.

Wallin, H. 1986. Habitat choice of some field-inhabiting carabid beetles (Coleoptera, Carabidae) studied by recapture of marked individuals. Ecological Entomology 11: 457-466.

Witmer, J. E., J. A. Hough-Goldstein and J. D. Pesek. 2003. Ground-dwelling and foliar arthropods in four cropping systems. Environmental Entomology 32: 366-376.

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

CHAPTER TWO

Effects of diamide insecticides on abundance and diversity of natural enemies in soybean

Abstract

Natural enemies can be important for preventing insect pests from reaching damaging levels in soybean. However, the natural enemy community can be compromised when pest control strategies include the application of broad-spectrum insecticides. The use of selective insecticides such as diamides could conserve natural enemies while still providing necessary pest control. In 2013 and 2015 we evaluated two selective diamide insecticides, chlorantraniliprole and flubendiamide, and a broad-spectrum insecticide, lambda-cyhalothrin in combination with chlorantraniliprole, for impact on the abundance and diversity of natural enemies in soybean. We applied insecticides to field plots and documented natural enemy abundance prior to and up to three weeks post application using sticky card, beat sheet, and sweep net sampling methods.

A total of 10,023 natural enemy specimens were collected and identified—5,015 from sweep nets, 2,900 from beat sheets, and 2,108 from sticky cards—representing eight orders and eight families of insects and spiders. Anthocoridae and Araneae were dominant, followed by Geocoridae and Nabidae.

For sweep net samples, in both 2013 and 2015 total natural enemy abundance in plots treated with the selective insecticides was not significantly different from untreated control plots.

For beat sheet samples, there were no significant differences in the abundance of total natural enemies on any day post-application between the selective diamide insecticides or the untreated control, but numbers decreased after application of lambda-cyhalothrin + chlorantraniliprole and did not rebound. For sticky cards, there were no differences in natural enemy abundance among treatments on any day post-application.

Over all, results showed that there were no significant differences in the abundance of total natural enemies, Anthocoridae, Araneae, or Geocoridae after application of flubendiamide or chlorantraniliprole compared with the untreated control for up to three weeks after application in either year, sample date, or with any sampling method. All insecticides significantly decreased populations of lepidopteran pests compared with the untreated control, but only lambda-cyhalothrin + chlorantraniliprole reduced natural enemy abundance. These results suggest a good fit of these selective insecticides in soybean IPM programs where lepidopterans are the primary pests of concern.

2.1 Introduction

Arthropod generalist predators, often termed 'natural enemies', are common to most agroecosystems where they play a key role in suppressing a variety of pest populations (Sunderland et al. 1997, Symondson 2002). Specific predators including anthocorids and carabids can reduce herbivore populations (Glen 1977, Sunderland 2002, Symondson et al. 2002) and can be effective at keeping insect pests below threshold (Hutchison and Pitre 1983, Ruberson and Greenstone 1998, Kilpatrick et al. 2005). Gurr et al. (2012) stated that a diverse predator community can suppress herbivores more effectively than a community with less biodiversity; a more diverse community results in predators occupying different microhabitats in the plant canopy or ground surface, or eating different prey species, sizes, or life stages.

Application of broad-spectrum insecticides normally decreases predator abundance in agroecosystems including soybean fields (Ohnesorg et al. 2009, Varenhorst and O'Neal 2012). This can lead to secondary pest outbreaks later in the growing season (Dutcher 2007, Gross and Rosenheim 2011). Applications of broad-spectrum insecticides which are generally harmful to the natural enemy community have been shown to increase certain lepidopteran pest densities in soybean (Shepard et al. 1977, Morrison et al. 1979). These insecticides have the ability to drastically decrease the entire arthropod community. When new pests populate a sprayed field, there are few natural enemies available for biological control. The generally slower colonization and relatively slow reproductive rate of natural enemies amplifies the problem.

Use of more selective insecticides could conserve natural enemies and help prevent resurgence of both primary and secondary pests. As such, natural enemies in IPM programs can be effective to decrease the amount of chemical control used in crops (Ruberson and Greenstone 1998). The integration of selective insecticides that are effective against specific pests but preserve the natural enemy community by not disrupting the longevity or feeding behavior of natural enemies has been examined for a variety of insecticide classes including neonicotinoids (Gentz et al. 2010).

Anthranilic diamides are a relatively new group of selective insecticides that stimulate ryanodine receptors in muscle tissue to release calcium stores from the sarcoplasmic reticulum of insect muscle cells causing lethal paralysis in sensitive species (Lahm et al. 2009). In 2002 DuPont Corporation (E.I. du Pont de Nemours and Company, 1007 Market Street, Wilmington, DE) introduced the diamide chlorantraniliprole. Chlorantraniliprole is an anthranilic diamide which claims to be selective for pests including Lepidoptera larvae while being less toxic to many beneficial insects (DuPont 2008). Research in turf grass has shown little impact of chlorantraniliprole on predatory arthropods (Larson et al. 2012). Chlorantraniliprole targets receptors in Lepidoptera and select species from orders including Coleoptera, Diptera and Hemiptera (Dupont 2008). The mode of entry is primarily by ingestion and secondarily by contact. It has been shown to be highly efficacious to corn earworm, Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae) (Kuhar et al. 2010). Anthranilic diamides have high selectivity for insect ryanodine receptors when compared to mammalian ryanodine receptors (Cordova et al. 2006, Wang et al. 2012). Differences in target site sensitivity between, and in some cases within, insect orders may account for its activity against some insects but not others (Wang et al. 2012). Flubendiamide, introduced by Bayer Crop Science (Research Triangle Park, NC) is a phthalic diamide that has a similar mechanism of action as chlorantraniliprole, acting on the ryanodine receptors of muscle tissues leading to calcium release and death. Both insecticides are highly effective against Lepidoptera larvae (Seo et al. 2007, Lahm et al. 2009).

Many of the insect pests in Virginia soybean, for example corn earworm, are common pests throughout the U.S. The common method in Virginia and elsewhere for combating insect pest problems in soybean is the application of broad-spectrum insecticides. A survey in 2011 showed that producers in Virginia sprayed more than 70,000 ha (30%) of soybean with a broad-spectrum insecticide at least once, primarily targeting corn earworm (Herbert 2011). In 2013, 37% of soybean planted in Virginia was treated with insecticide (USDA NASS) 14% of which was with the broad-spectrum insecticide lambda-cyhalothrin.

Spiders (Araneae), minute pirate bugs, (Anthocoridae) and bigeyed bugs (Geocoridae), are known to be effective predators of corn earworm (Bell and Whitcomb 1964, Ruberson and Greenstone 1998). Broad-spectrum insecticides such as lambda-cyhalothrin can be detrimental to these natural enemy species (Croft and Brown 1975, Elzin 2001, Studebaker and Kring 2003a, b, Chapman et al. 2009). An alternative pest control method is to use selective insecticides that are less harmful to natural enemies. Chlorantraniliprole and flubendiamide are listed as options for lepidopteran pest control in soybean in most states (e.g., Virginia, Herbert 2014). The objective of this project was to use Virginia soybean fields as a case study to assess the impact of two selective diamide insecticides on the abundance and diversity of natural enemy populations in soybean compared with the broad-spectrum pyrethroid, lambda-cyhalothrin.

2.2 Materials and Methods

2.2.1 Experimental design

In 2013, a randomized complete block design was used with three insecticide treatments and one untreated control; each treatment was replicated once within six blocks. Field was treated as the block and treatments were randomized within each field. Six fields were used, five at the Virginia Tech Tidewater

Agricultural Research and Extension Center (TAREC) near Suffolk, VA, (36.684879°, -76.767067°), and one at Dayton Farm in Plymouth, NC (35.6443, -76.7320). Blocks were divided into four plots that ranged in size from 0.04 to 0.19 ha (Table 1). Plots within a block were of equal size. A second experiment was conducted in 2015 at the TAREC location using a randomized complete block design, with three insecticide treatments and one untreated control; each treatment was replicated once within four blocks. Due to field sizes and configurations, plots sizes varied among experiments, and standard agronomic practices for Virginia were used with commercially available soybean lines (Table 2.1).

Table 2.1 Description of experimental plot planting date, soybean cultivar, row spacing, and plot size, 2013 and 2015.

Year	Field	Planting date	Cultivar	Row spacing	Plot size
2013	A	13 Jun	H58-10*	91.44 cm	0.04 ha
	B	12 Jun	H58-10R2	91.44 cm	0.07 ha
	C	12 Jun	H58-10R2	91.44 cm	0.19 ha
	D	15 Jun	H58-10RR	91.44 cm	0.02 ha
	E	13 Jun	H58-10R2	91.44 cm	0.15 ha
	F	15 Jun	Pioneer 94Y50	76.2 cm	0.04 ha
2015	G	16 Jun	S56-G6*	19.05 cm	0.07 ha

* "H" refers to Hubner. "S" refers to Syngenta

2.2.2 Insecticide treatments

Insecticide treatments included an untreated control and three foliar-applied products: chlorantraniliprole (Prevathon, E.I. du Pont de Nemours and Company, Wilmington, DE) at 0.068 kg ai ha^{-1} ; flubendiamide (Belt 480SC, Bayer CropScience, Research Triangle Park, NC) at 0.07 kg ai ha^{-1} ; and lambda-cyhalothrin + chlorantraniliprole (Besiege, Syngenta Crop Protection, LLC, Greensboro, NC) at 0.067 kg ai ha^{-1}. In 2013 and 2015, insecticides were applied when soybean reached the R3 growth stage, beginning pod formation (Fehr and Caviness 1977). In 2013 treatments were applied with a Spider Spray Trac-mounted

CO_2-pressurized sprayer at 186 liters/ha and 262 kPa through 8002VS nozzles spaced 45.72 cm apart on the spray boom. In 2015 treatments were applied with a Ridick Co. 3-point hitch sprayer at 187 liters/ha and 165 kPa through 8003VS nozzles spaced 45.72 cm apart on the spray boom.

2.2.3 Natural enemy sampling and evaluation

Natural enemies were sampled in each plot during July, one week prior to insecticide application which coincided with the R2 growth stage (Fehr and Caviness 1977) and weekly for three weeks afterward (twice in August and once in late August-early September). Three sampling methods were employed in each plot in 2013 and one sampling method was employed in 2015. In 2013, four 7.6 × 12.7 cm yellow sticky cards (Olson Products Inc., Medina, OH) placed slightly higher than the plant canopy were used to capture aerial species. On each of four sampling dates, four sticky cards were placed in the central rows of each plot and were collected after 48 hours. The second method involved using sweep nets to collect aerial species and those residing in the mid and upper plant canopy. Five sweep net samples consisting of 15 pendulum sweeps with a 38-cm-diameter net were taken in each plot on each sampling date. Samples were placed in plastic bags, transferred to a cooler and transported to the laboratory for identification. Arthropods were sorted and identified to the lowest taxonomic level possible. Beat sheets were used to collect stem and leaf dwelling species. Five samples with a 71.12 × 91.44 cm beat sheet were taken per plot per week. The sheet was placed between two rows of soybean, and plants on both sides were shaken vigorously over the sheet for five seconds. Abundance of natural enemies and caterpillars were recorded in the field. In 2015 only sweep net samples were taken. The other two sampling methods were dropped due to time restrictions and previous research has shown that sweep nets are the most effective collection method for predators in soybean (Varenhorst and O’Neal 2012).

2.2.4 Analysis

To determine the impact of insecticides on natural enemy abundance, count data were square-root + 0.5 transformed to meet assumptions of normality and analyzed using a repeated measure analysis of variance (ANOVA) using PROC MIXED (SAS Institute 2001) and means were separated using F-protected least-squares means test at $\alpha = 0.05$. A first-order autoregressive was used for covariance structure. A separate ANOVA was conducted for each sampling method and each of the following: total natural enemies, Anthocoridae, Geocoridae, Araneae, and all Lepidoptera. To determine if abundance of natural enemies was different in each year, we compared the densities of natural enemies in control plots for both years.

2.3 Results

2.3.1 Natural enemies in sweep net samples

We observed natural enemies in eight orders and eight families (Table 2.2) across the two years of the experiment. A total of 1,925 and 3,090 natural enemies were identified in 2013 and 2015 sweep net samples, respectively. *Orius insidiosus* (Say) and spiders predominated in 2013 and 2015 (Figs. 2.1 and 2.2). Natural enemy abundance in sweep net samples was higher in untreated controls in 2015 (787 total natural enemies in all plots throughout the season) compared to 2013 (483 total natural enemies in all plots throughout the season).

Table 2.2 Natural enemies and Lepidoptera taxa collected in soybean in southeastern Virginia pre- and post-insecticide application in 2013 and 2015.

Order	**Family**	**Species**
Coleoptera	Coccinellidae	*Harmonia axyridis*
		Coleomegilla maculata
		Hippodamia convergens
Neuroptera	Hemorobiidae	
	Chrysopidae	
Hemiptera	Anthocoridae	*Orius insidiosus*
	Geocoridae	
	Nabidae	
	Reduviidae	
Diptera	Dolichopodidae	
Lepidoptera		
		Helicoverpa zea
		Hypena scabra
		Spodoptera ornithogalli
		Chrysodeixis includens
Araneae		

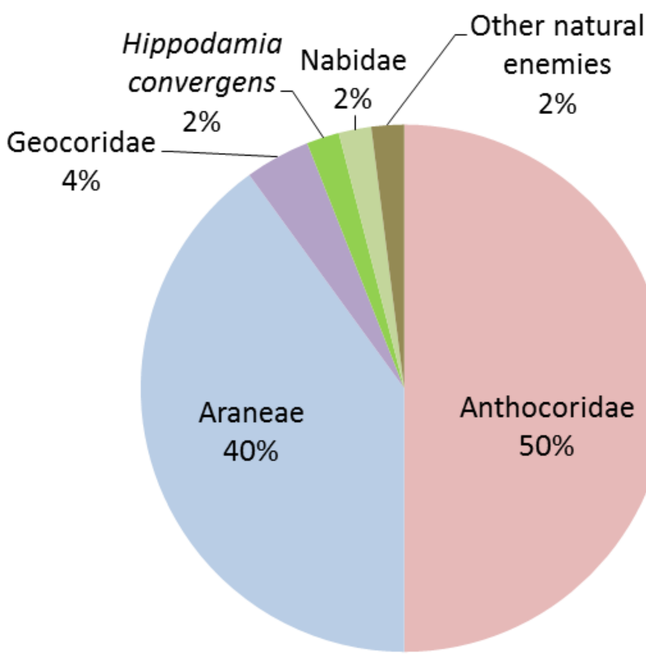


Figure 2.1. Natural enemy diversity collected with a sweep net in insecticide free plots of soybean in southeastern Virginia in 2013.

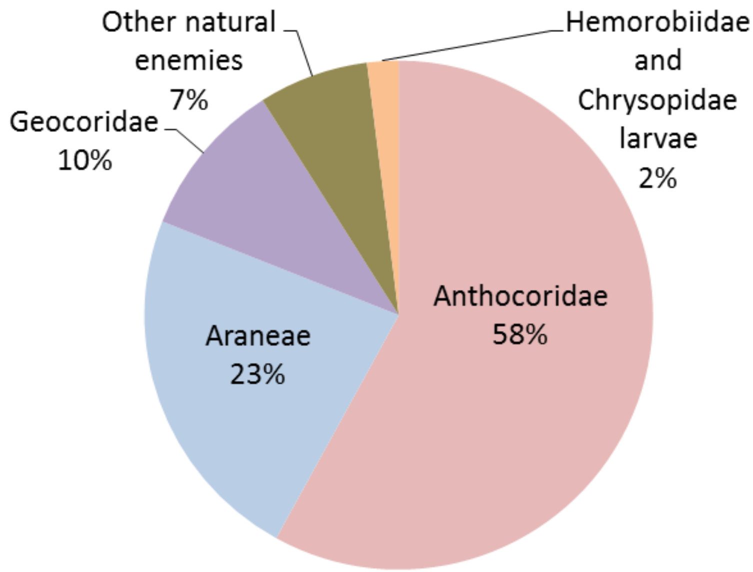


Figure 2.2. Natural enemy diversity collected with a sweep net in insecticide free plots of soybean in southeastern Virginia in 2015.

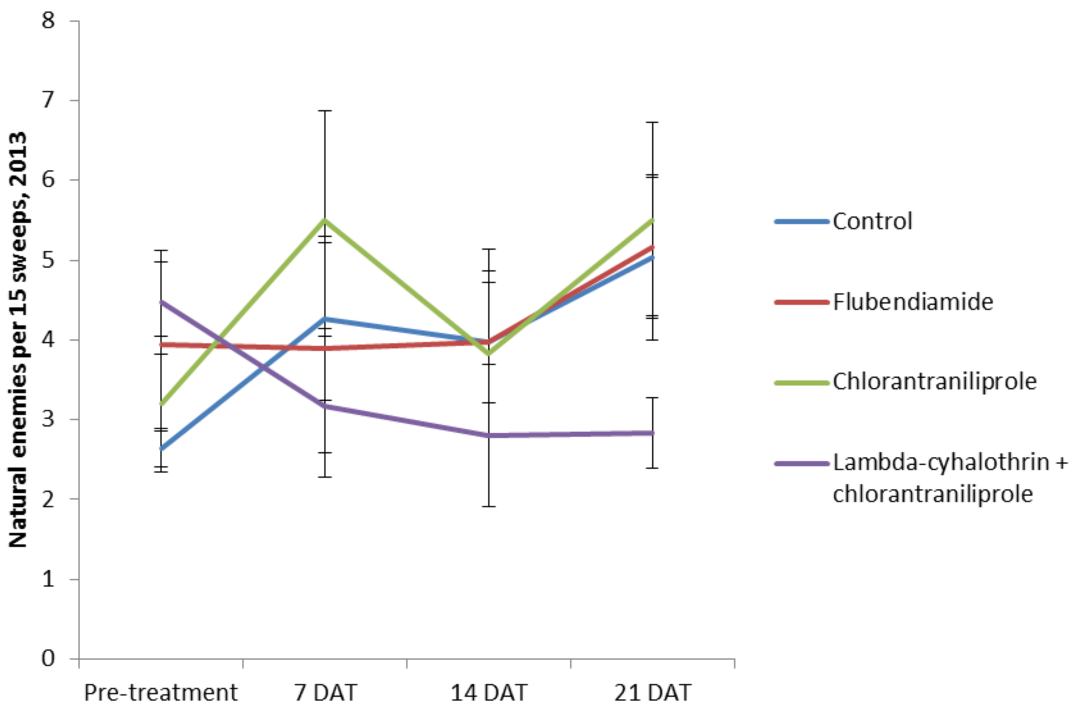
In 2013, natural enemy abundance in plots treated with either chlorantraniliprole or flubendiamide was not significantly different from untreated control plots (Fig. 2.3, Table 2.3, $F_{3, 75} = 0.45$, $P = 0.24$). Plots treated with lambda-cyhalothrin + chlorantraniliprole had fewer natural enemies compared to plots treated with selective insecticides or the untreated control on all days post-application, though differences were not significant (Table 2.3). Sweep net data confirmed that abundance of Anthocoridae increased throughout the season in untreated control and selective insecticide treated plots. Anthocoridae abundance decreased after application of lambda-cyhalothrin + chlorantraniliprole and did not rebound. However, there were no significant treatment differences in Anthocoridae abundance at any date post-application (Fig. 2.4, $F_{3, 75} = 0.12$, $P = 0.95$). Araneae abundance decreased at 7 DAT in the lambda-cyhalothrin + chlorantraniliprole treated plots but not in the selective insecticide treated plots, though there was no significant difference (Fig. 2.5, $F_{3\ ,\ 75} = 1.46$, $P = 0.23$). Each of the insecticide treatments reduced Lepidoptera populations on all sampling days post-application (Table 2.3, $F_{3\ 75} = 6.72$, P <0.001).

Table 2.3. Analysis of variance showing response of natural enemy abundance to insecticide treatments in soybean in 2013 and 2015.

Year	Method	Natural enemy	Variable	F	DF*	*P*
2013	Sweep net	Total natural enemies	Treatment	1.43	3, 75	*0.2399*
			Day	1.26	3. 75	*0.2933*
			Treatment x Day	0.99	9, 75	*0.452*
		Anthocoridae	Treatment	0.12	3, 75	*0.9508*
			Day	0.94	3. 75	*0.4236*
			Treatment x Day	0.84	9, 75	*0.5808*
		Araneae	Treatment	1.46	3, 75	*0.233*
			Day	0.68	3. 75	*0.5661*
			Treatment x Day	1.28	9, 75	*0.2612*
		Geocoridae	Treatment	0.97	3, 75	*0.4096*
			Day	2.97	3. 75	*0.0373*
			Treatment x Day	0.56	9, 75	*0.8276*
		Lepidoptera	Treatment	6.72	3, 75	*0.0004*
			Day	2.06	3. 75	*0.113*
			Treatment x Day	2.33	9, 75	*0.0228*
2015	Sweep net	Total natural enemies	Treatment	2.1	3, 45	*0.114*
			Day	10.46	3, 45	*<.0001*
			Treatment x Day	0.5	9, 45	*0.8693*
		Anthocoridae	Treatment	2.22	3, 45	*0.099*
			Day	14.1	3, 45	*<.0001*
			Treatment x Day	0.62	9, 45	*0.7771*
		Araneae	Treatment	2.04	3, 45	*0.1219*
			Day	2.7	3, 45	*0.0569*
			Treatment x Day	0.81	9, 45	*0.6076*
		Geocoridae	Treatment	1.5	3, 45	*0.2261*
			Day	2.43	3, 45	*0.0776*
			Treatment x Day	1.25	9, 45	*0.2913*
		Lepidoptera	Treatment	18.55	3, 45	*<.0001*
			Day	15.54	3, 45	*<.0001*
			Treatment x Day	6.94	9, 45	*<.0001*
2013	Beat sheet	Total natural enemies	Treatment	2.97	3, 75	*0.037*
			Day	11.25	3. 75	*<.0001*
			Treatment x Day	0.73	9, 75	*0.6777*
		Anthocoridae	Treatment	0.48	3, 75	*0.6985*
			Day	0.43	3. 75	*0.732*
			Treatment x Day	0.47	9, 75	*0.8927*
		Araneae	Treatment	2.22	3, 75	*0.0925*
			Day	3.07	3. 75	*0.0327*

			Treatment x Day	0.65	9, 75	*0.7511*
		Geocoridae	Treatment	1.89	3, 75	*0.1388*
			Day	6.26	3. 75	*0.0008*
			Treatment x Day	1.36	9, 75	*0.2202*
		Lepidoptera	Treatment	5.79	3, 75	*0.0013*
			Day	6.13	3. 75	*0.0009*
			Treatment x Day	2.96	9, 75	*0.0046*
2013	Sticky cards	Total natural enemies	Treatment	1.39	3, 75	*0.2537*
			Day	33.43	3. 75	*<.0001*
			Treatment x Day	0.22	9, 75	*0.9912*
		Anthocoridae	Treatment	1.8	3, 75	*0.1542*
			Day	37.95	3. 75	*<.0001*
			Treatment x Day	0.25	9, 75	*0.9861*
		Araneae	Treatment	0.29	3, 75	*0.8359*
			Day	7.26	3. 75	*0.0002*
			Treatment x Day	0.52	9, 75	*0.8568*

*Degrees of freedom, numerator followed by denominator degrees of freedom.



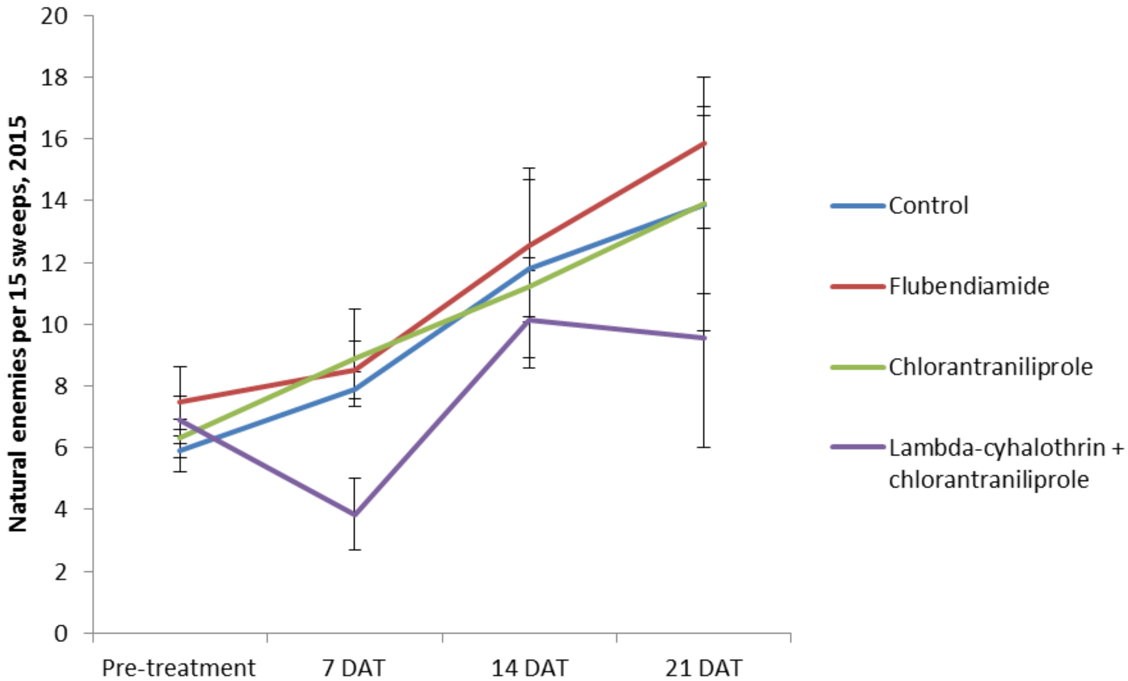
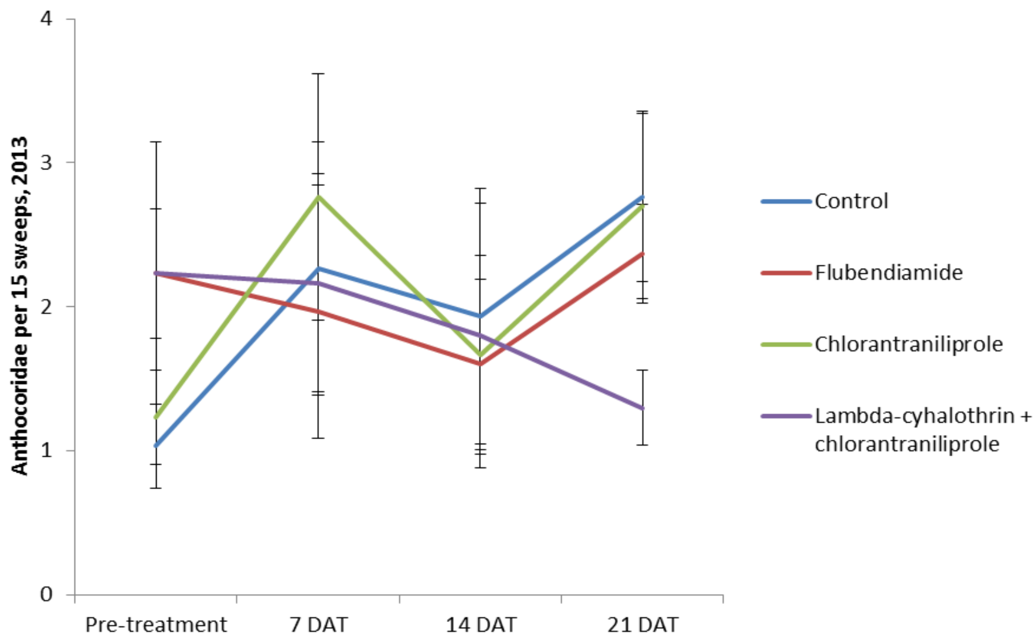


Figure 2.3. Abundance of the natural enemy community (mean ± SE) in soybean collected with a sweep net in 2013 and 2015. Soybean were planted in 13-15 June 2013 and 16 June 2015. Foliar treatments of flubendiamide, chlorantraniliprole and lambda-cyhalothrin+chlorantraniliprole were applied when soybeans reached R3 growth stage. DAT stands for days after treatment with foliar insecticide.



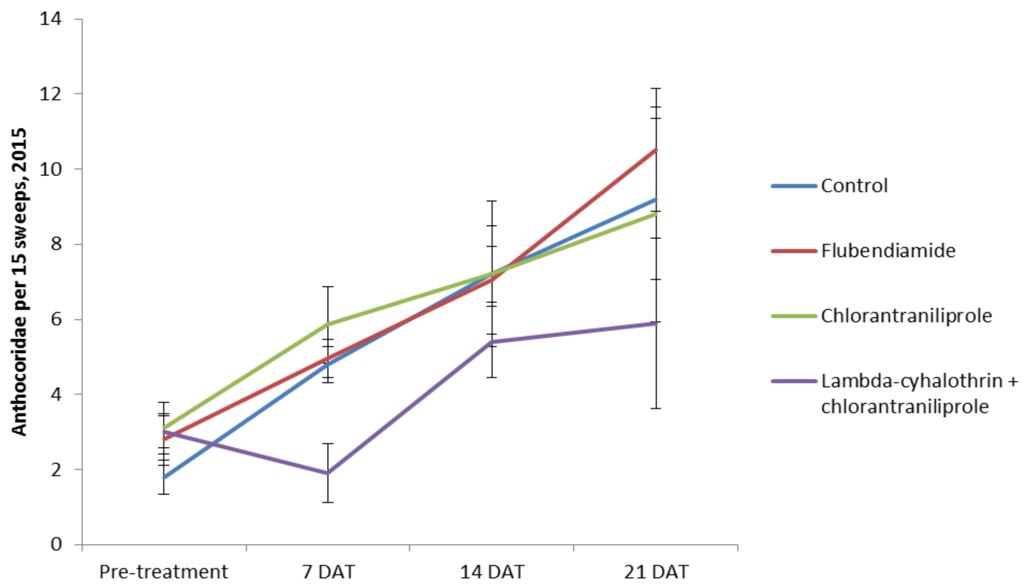
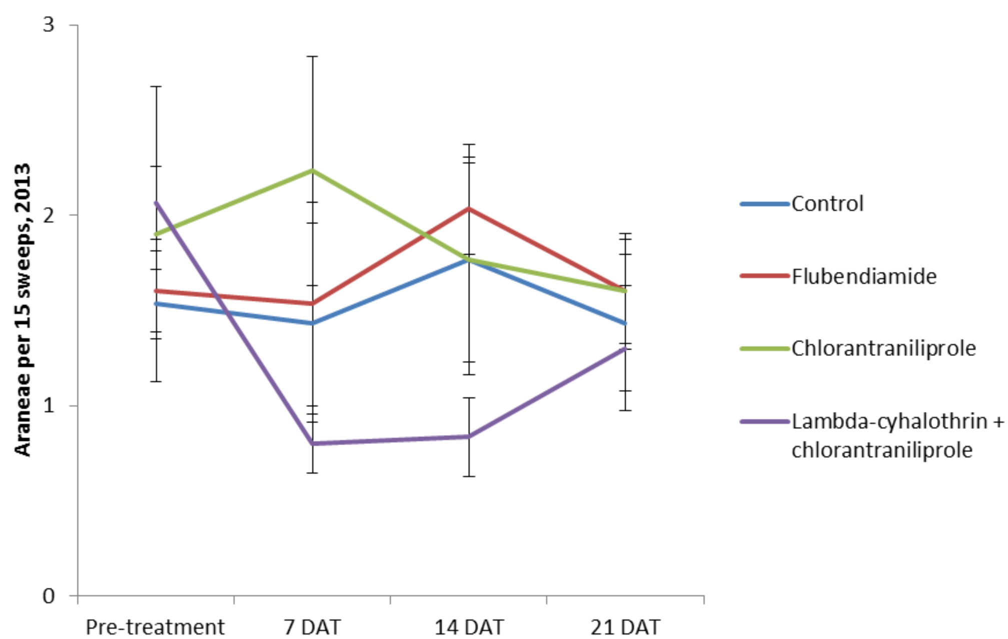


Figure 2.4. Abundance of Anthocoridae (mean ± SE) collected with a sweep net in soybean in 2013 and 2015. Soybean were planted in 13-15 June 2013 and 16 June 2015. Foliar treatments of flubendiamide, chlorantraniliprole and lambda-cyhalothrin+chlorantraniliprole were applied when soybeans reached R3 growth stage. DAT stands for days after treatment with foliar insecticide.



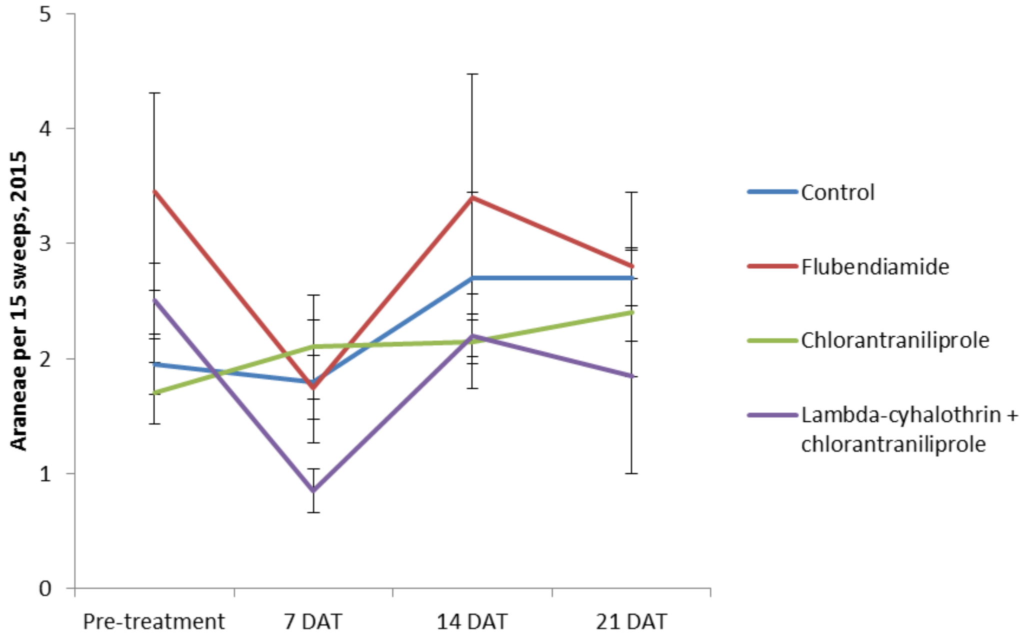


Figure 2.5. Abundance of Araneae (mean ± SE) in soybean collected with a sweep net in 2013 and 2015. Soybean were planted in 13-15 June 2013 and 16 June 2015. Foliar treatments of flubendiamide, chlorantraniliprole and lambda-cyhalothrin+chlorantraniliprole were applied when soybeans reached R3 growth stage. DAT stands for days after treatment with foliar insecticide.

In 2015, natural enemy abundance in plots treated with the selective insecticide was not significantly different from untreated control plots (Fig. 2.3, Table 2.3, $F_{3\ 45} = 2.1$, $P = 0.11$). Natural enemy abundance in plots treated with the broad-spectrum insecticide decreased one week after application but rebounded after 14 days. Spiders followed the same trend (Fig. 2.5). We observed a significant effect of insecticides on Lepidoptera abundance (Table 2.3, $F_{3,\ 45} = 18.55$, $P < 0.0001$). Untreated control plots had a significantly higher abundance of Lepidoptera on each date post-application compared to treated plots. As in 2013, all insecticide treatments reduced Lepidoptera populations compared to untreated controls on all dates post-application.

2.3.2 Natural enemies in beat sheet samples

In 2013, 2,900 natural enemies were identified with the beat sheet sampling method. Total natural enemies collected by beat sheet decreased after lambda-cyhalothrin + chlorantraniliprole application and did not rebound (Fig. 2.6, Table 2.4, $F_{3, 75}$= 2.97, *P* = 0.04). Araneae were the most abundant natural enemies in untreated control plots (44%) followed by Anthocoridae (27%) (Fig. 2.7). There was a trend toward fewer Araneae in plots treated with lambda-cyhalothrin + chlorantraniliprole though differences were not significant (Fig. 2.8, Table 3, $F_{3, 75}$ = 2.22, *P* = 0.09). There were no significant differences in the abundance of natural enemies on any date post-application between the untreated control plots and the selective insecticide treatments. .

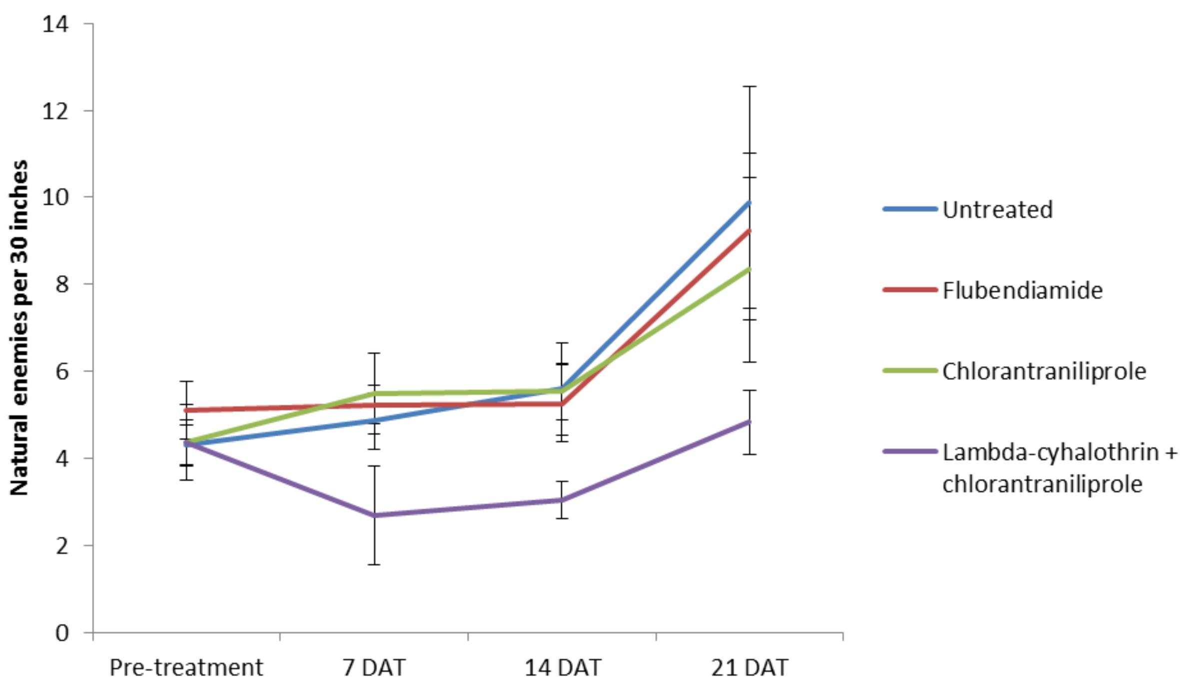


Figure 2.6. Abundance of natural enemies (mean ± SE) in soybean collected with a beat sheet in 2013. Soybean were planted in 13-15 June 2013 and 16 June 2015. Foliar treatments of flubendiamide, chlorantraniliprole and lambda-cyhalothrin+chlorantraniliprole were applied when soybeans reached R3 growth stage. DAT stands for days after treatment with foliar insecticide.

Table 2.4. Mean +/- SEM of total natural enemies collected in soybean with a beat sheet before and after application of foliar insecticides, 2013

Treatment	**Pretreatment**	**7 DAT***	**14 DAT**	**21 DAT**
Untreated	4.3 a$^{\Delta}$ A	4.87 a A	5.6 a A	9.87 b A
Flubendiamide	5.1 a A	5.23 a A	5.27 a A	9.23 b A
Lambda-cyhalothrin+chlorantraniliprole	4.37 a A	2.7 a A	3.03 a A	4.83 a B
Chlorantraniliprole	4.37 a A	5.5 a A	5.53 a A	8.33 a A

*DAT, days after treatment of foliar insecticides.

$^{\Delta}$Capital letters indicate significance in columns, lowercase letters indicate significance in rows ($p < 0.05$).

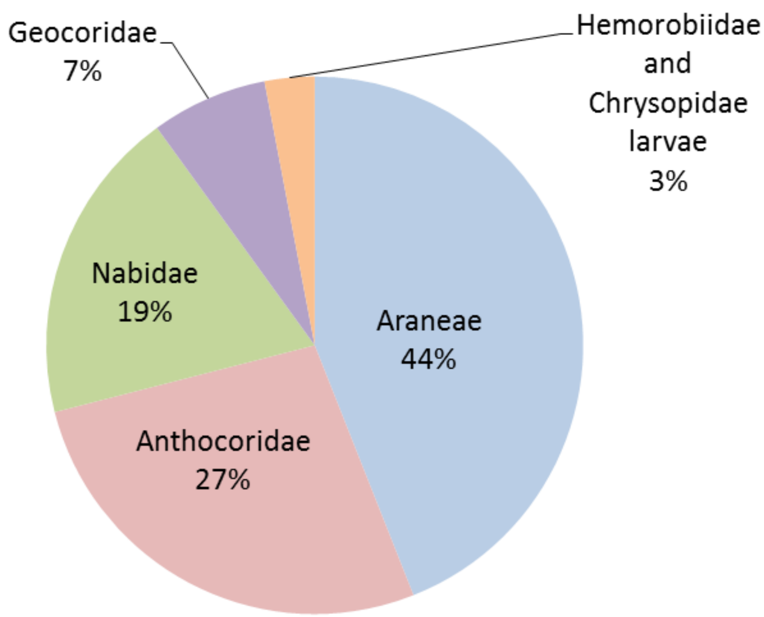


Figure 2.7. Natural enemy diversity collected with a beat sheet in insecticide free plots of soybean in southeastern Virginia in 2013.

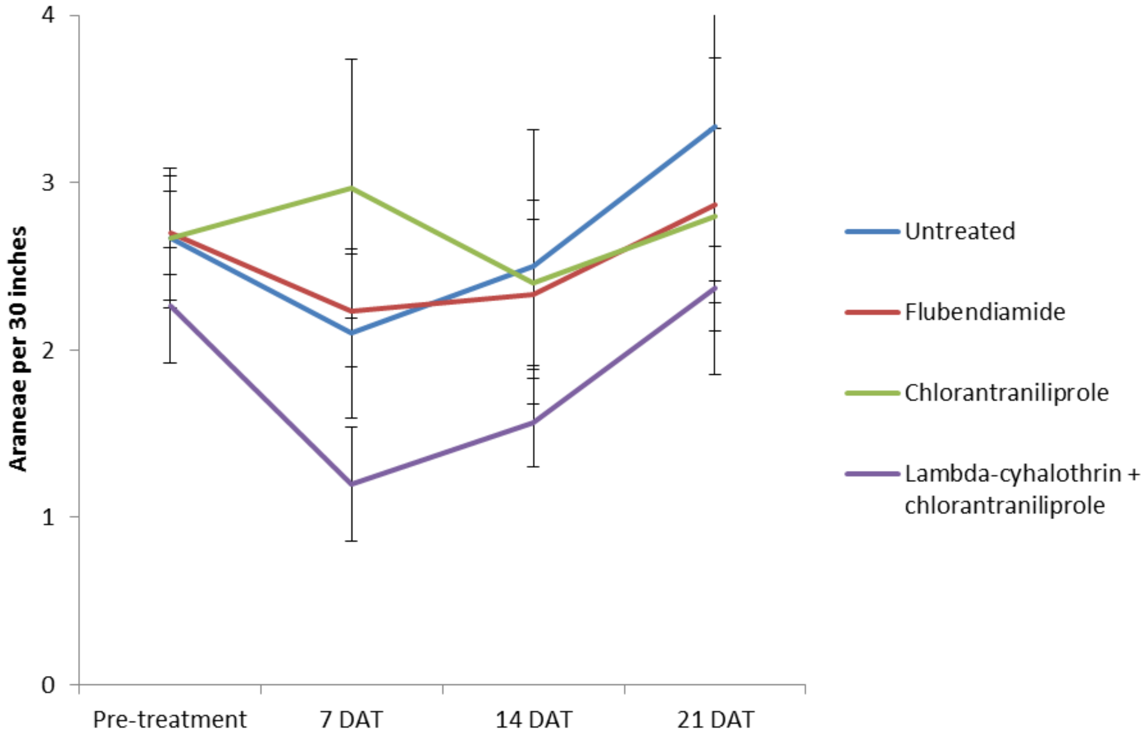


Figure 2.8. Abundance of Araneae (mean ± SE) collected with a beat sheet in soybean in 2013. Soybean were planted in 13-15 June 2013 and 16 June 2015. Foliar treatments of flubendiamide, chlorantraniliprole and lambda-cyhalothrin+chlorantraniliprole were applied when soybeans reached R3 growth stage. DAT stands for days after treatment with foliar insecticide.

2.3.3 Natural enemies in sticky card samples

In 2013, a total of 2,108 natural enemies were identified from 320 sticky cards collected on the four sampling dates. Anthocoridae were the most abundant natural enemy caught in untreated control plots (87%) followed by Araneae (8%) (Fig. 2.9). Two lady beetles, *Coleomegilla maculata* (DeGeer) and *Hippodamia convergens* (Guerin) made up the remainder of the specimens. There was no significant difference in natural enemy abundance among treatments on any day post-application (Table 2.3, $F_{3, 75} = 1.39$, $P = 0.25$).

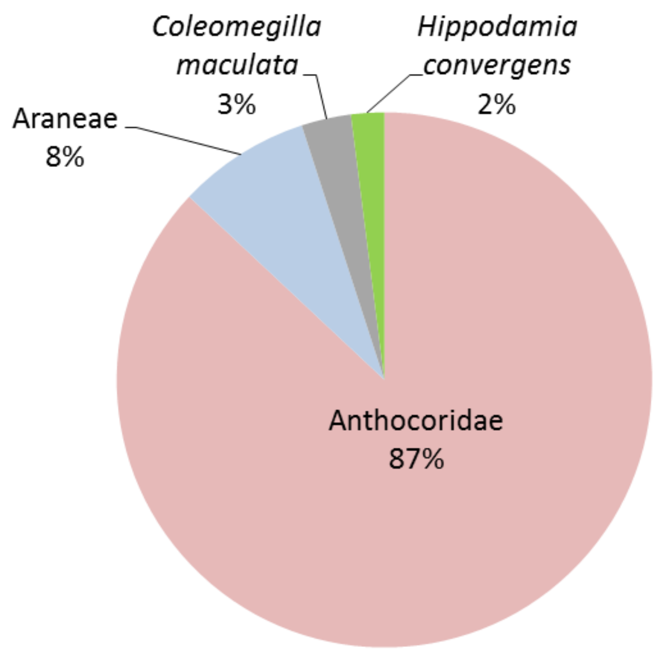


Figure 2.9. Natural enemy diversity collected with sticky cards in insecticide free plots of soybean in southeastern Virginia in 2013.

2.4 Discussion

The impact of diamide insecticides on common arthropod natural enemies in Virginia soybean has not been previously reported. This study documents the effects of chlorantraniliprole and flubendiamide compared to lambda-cyhalothrin in combination with chlorantraniliprole on natural enemy abundance in soybean. Chlorantraniliprole and flubendiamide are marketed as having reduced impact on beneficial insects while providing a high degree of activity against Lepidoptera; however, there has been relatively little published field research to support the claim regarding impact on non-target species. Data from our field experiments showed that there were no significant differences in the abundance of three types of arthropod predators, Anthocoridae, Araneae and Geocoridae after application of these selective diamide insecticides compared with the untreated control in either year, sample date, or with any sampling method. In 2013 we observed a negative impact of lambda-cyhalothrin + chlorantraniliprole on the natural enemy community with beat sheet sampling. Natural enemy abundance increased throughout the season in control and selective insecticide plots sampled with sweep nets in 2013, while lambda-cyhalothrin + chlorantraniliprole plots had decreasing abundance. In 2015, natural enemy abundance sampled with sweep nets in lambda-cyhalothrin + chlorantraniliprole plots decreased at 7 days post-application, though not significantly. This was not the case in control and selective insecticide plots, where natural enemy abundance increased throughout the season. We observed no negative impact on the natural enemy community from the selective insecticides on any date, in either year, using sweep net data.

There was a difference in the abundance and composition of the natural enemy community between the two study years. The most abundant natural enemy species collected by sweep net in both years was *O. insidiosus*, an important egg predator, but spiders made up 23% of total specimens in 2015 compared to 40% in 2013. Yearly variation of the natural enemy community has been reported previously and could be due to factors including weather and prey availability. In a study examining predator response to selective insecticides in Iowa soybean, the ladybeetle *Harmonia axyridis* (Pallas) was the dominant predator surveyed in 2009, while the following year *O. insidiosus* was the dominant predator (Varenhorst and O'Neal 2012). As with our experiments, the authors concluded that natural enemy response to insecticides depends on interactions with variables that change between years, including weather. During our research, several days of heavy rainfall in June and early July 2015 could have washed out existing predator populations, discouraged migrating predators from inhabiting fields until later in the season, and slowed the early season colonization process. Rain can be especially detrimental to the establishment of ground-dwelling predators such as many spider species.

There was also a large variation in numbers of natural enemies among plots within treatments which likely contributed to the lack of significant differences seen in repeated measures analysis between the broad-spectrum insecticide and selective insecticides and untreated plots. Reasons for these differences in abundance among plots could include factors like variation in plant nutrition and subsequent prey populations, differences in canopy closure and temperature making certain plots more desirable than others, and edge effects from surrounding woods or neighboring crops (Welch et al. 2012, Zhao et al. 2013, Puentes et al. 2015).

In this two-year study, 10,023 natural enemy specimens were collected before and after exposure to field applied insecticides and identified. Overall, results suggest that flubendiamide and chlorantraniliprole have minimal effect on the natural enemy abundance up to three weeks post application. This in-depth research should provide better insight regarding the effects of these selective insecticides on natural enemy abundance and diversity—and therefore, their potential role in soybean insect pest management programs.

Further research should examine whether ingesting or coming in contact with either insecticide alters rate of feeding, fecundity, interaction with other predators and other parameters that can alter a predator's effectiveness at controlling pests.

References

Bell, K. O. and W. H. Whitcomb. 1964. Field studies on egg predators of the bollworm, *Heliothis zea* (Boddie). The Florida Entomologist 47:171-180.

Chapman, A., T. Kuhar, P. Schultz, T. Leslie, S. Fleischer, G. Dively, and J. Whalen. 2009. Integrating chemical and biological control of European corn borer in bell pepper. Journal of Economic Entomology 102 : 287-295.

Cordova, D., E. A. Benner, M. D. Sacher, J. J. Rauh, J. S. Sopa, and G. P. Lahm. 2006. Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. Pesticide Biochemistry and Physiology 84:196-214.

Croft, B. A. and A. W. A. Brown. 1975. Response of arthropod natural enemies to insecticides. Annual Review of Entomology 20: 285-335.

Elzen, G. W. 2001. Lethal and sublethal effects of insecticide residues on *Orius insidiosus* (Hemiptera: Anthocoridae) and *Geocoris punctipes* (Hemiptera: Geocoridae). Journal of Economic Entomology 94: 55-59.

Dutcher, J. D. 2007. A review of resurgence and replacement causing pest outbreaks in IPM. In General Concepts in Integrated Pest and Disease Management, edited by A. Ciancio and K.G. Mukerji, 27-43. Springer, Netherlands.

Dupont. 2008. Coragen Technical Bulletin. Reorder No.: K-14833.

Fehr, W. R. and C. E. Caviness. 1977. Stages of soybean development. Iowa State University Cooperative Extension Service, Agriculture and Home Economics Experiment Station, Iowa State University, Ames, Iowa. Special Report 80.

Gentz, M. C., G. Murdoch, and G. F. King. 2010. Tandem use of selective insecticides and natural enemies for effective, reduced-risk pest management. Biological Control 52:208-215.

Glen, D. M. 1977. Predation of codling moth eggs, *Cydia pomonella,* the predators responsible and their alternative prey. Journal of Applied Ecology 14:445-56.

Gross, K. and J. A. Rosenheim. 2011. Quantifying secondary pest outbreaks in cotton and their monetary cost with causal-inference statistics. Ecological Applications 21:2770-2780.

Gurr, G. M., S. D. Wratten, and W. E. Snyder. 2012. Biodiversity and insect pests. In Biodiversity and Insect Pests: Key Issues for Sustainable Management, edited by G.M. Gurr, S.D. Wratten, and W.E. Snyder, 3-20. Oxford:Wiley-Blackwell.

Herbert, D. A., 2011. Insect Pest Management in Virginia Cotton, Peanut and Soybean. Virginia Cooperative Extension publication VT/1211/AREC-7.

Herbert, D. A. 2014. Insect Pest Management in Virginia Cotton and Peanut. Virginia Cooperative Extension publication. ENTO-109NP.

Hutchinson, W. D. and H. N. Pitre. 1983. Predation of *Heliothis virescens* (Lepidoptera: Noctuidae) eggs by *Geocoris punctipes* (Hemiptera: Lygaeidae) adults on cotton. Environmental Entomology 12:1652-1656.

Kilpatrick, A. L., A. M. Hagerty, S. G. Turnipseed, M. J. Sullivan, and W. C. Bridges. 2005. Activity of selected neonicotinoids and dicrotophos on nontarget arthropods in cotton: Implications in insect management. Journal of Economic Entomology 98:814-820.

Kuhar, T., Walgenbach, J.F., and H. B. Doughty. 2010. Control of *Helicoverpa zea* in tomatoes with chlorantraniliprole applied through drip chemigation. Online. Plant Health Progress doi:10.1094/PHP-2009-0407-01-RS.

Lahm, G. P., D. Cordova, and J. D. Barry. 2009. New and selective ryanodine receptor activators for insect control. Bioorganic & Medicinal Chemistry 12:4127-33.

Larson, J. L., C. T. Redmond, and D. A. Potter. 2012. Comparative impact of an anthranilic diamide and other insecticidal chemistries on beneficial invertebrates and ecosystem services in turfgrass. Pest Management Science 68:740-748.

Morrison, D. E., J. R. Bradley, and J. W. Vanduyn. 1979. Populations of corn-earworm (Lepidoptera: Noctuidae) and associated predators after applications of certain soil-applied pesticides to soybeans. Journal of Economic Entomology 72:97-100.

NASS/USDA National Agricultural Statistics Service/U.S. Department of Agriculture. 1=2013. Agricultural chemical usage 2013 field crops summary. http://quickstats.nass.usda.gov/#E794C299-4B61-3F88-A1C7-597ECCC14E38.

Ohnesorg, W. J., K. D. Johnson, and M. E. O'Neal. 2009. Impact of reduced-risk insecticides on soybean aphid and associated natural enemies. Journal of Economic Entomology 102:1816-1826.

Puentes, A., M. Torp, M. Weih, and C. Bjorkman. 2015. Direct effects of elevated temperature on a tri-trophic system: Salix, leaf beetles and predatory bugs. Arthropod-Plant Interactions 9:567-575.

Ruberson, J. R. and M. H. Greenstone. 1998. Predators of budworm/bollworm eggs in cotton: an immunological study, pp. 1095-1098. In Proceedings, Beltwide Cotton Conference, National Cotton Council. Memphis, TN.

Seo, A., M. Tohnishi, H. Nakao, T. Furuya, H. Kodama, K. Tsubata, S. Fujioka, H. Kodama, T. Nishimatsu, and T. Hirooka. 2007. Pesticide Chemistry: Crop Protection, Public Health and Environmental Safety, edited by H. Ohkawa, H. Miyagawa, and P.W. Lee. 127-135. Weinheim, Germany: Wiley-VCH. 2007.

Shepard, M., G. R. Carner, and S. G. Turnipseed. 1977. Colonization and resurgence of insect pests of soybean in response to insecticide and field isolation. Environmental Entomology 6:501-506.

Studebaker, G. E., and T. J. Kring. 2003a. Effects of insecticides on *Orius insidiosus* (Hemiptera: Anthocoridae) measured by field, greenhouse and Petri dish bioassays. Florida Entomologist 86: 178-185.

Studebaker, G. E., and T. J. Kring. 2003b. Effects of various insecticide residues in cotton on gender and developmental stage of the insidious flower bug (Hemiptera: Anthocoridae). Journal of Entomological Science 38: 409-419.

Sunderland, K. D., J. A. Axelsen, K. Dromph, B. Freier, and J. L. Hemptinne. 1997. Pest control by a community of natural enemies. Acta Jutlandica 72:271-326.

Sunderland, K. D. 2002. Invertebrate pest control by carabids. In The Agroecology of Carabid Beetles, edited by J.M. Holland, 165-214. Andover: Intercept Ltd.

Symondson, W. O. C., K. D. Sunderland, and M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? Annual Review of Entomology 47:561-94.

Varenhorst, A. J. and M. E. O'Neal. 2012. The response of natural enemies to selective insecticides applied to soybean. Environmental Entomology 41:1565-1574.

Wang, J., Y. Li, Z. Han, Y. Zhu, Z. Xie, J. Wang, Y. Liu, and X. Li. Molecular characterization of a ryanodine receptor gene in the rice leaffolder, *Cnaphalocrocis medinalis* (Guenee). PLOS ONE 7:e36623.

Welch, K. D., R. S. Pfannenstiel, and J. D. Harwood. 2012. The role of generalist predators in terrestrial food webs: lessons for agricultural pest management. In Biodiversity and Insect Pests: Key Issues for Sustainable Management.

Zhao, Z. H., C. Hui, D. H. He, and F. Ge. 2013. Effects of position within wheat field and adjacent habitats on the density and diversity of cereal aphids and their natural enemies. Biocontrol 58:765-776.

CHAPTER THREE

Abundance and diversity of foliar and ground-dwelling spiders in two soybean cropping systems

Abstract

Spiders are some of the most abundant natural enemies in soybean and have the potential to keep pests under economic threshold. Spiders are common in two of the cropping systems used in Virginia, full season soybean, which is planted in April or May and double crop soybean, planted in June or July. There is little recent information on spiders in Virginia soybean and how cropping systems affect species abundance and diversity. This study was carried out in 2014 and 2015 in southeastern Virginia to determine how cropping systems influence the foliar and ground-dwelling spider assemblages. To accomplish this we collected spiders using sweep nets and pitfall traps from 20 soybean fields. A total of 7,371 spiders were identified from 16 families and 76 species. Spider abundance was higher both in the foliage and on the ground in double crop soybean compared with full season soybean in 2014. This was also the case in 2015 but results were not significant. Foliage-dwelling spider diversity, as measured by the Shannon Index and the Simpson Index, was similar in the two cropping systems. Ground-dwelling spiders in full season soybean had higher diversity indices in 2014, but spiders in double crop soybean had a higher Shannon Index in 2015. The dominant ground-dwelling spider in both systems, *Pardosa milvina*, was more abundant in double crop soybean in 2014. The results of the study indicate that the two cropping systems have different spider communities that aren't necessarily consistent from year to year, but both have relatively high diversity and a dominant spider that was common both in the plant canopy and on the ground. Conservation efforts should target this dominant spider in both systems, since it is a known predator of many common soybean pests.

3.1 Introduction

In agroecosystems a diverse natural enemy community can suppress herbivores more effectively than a community with less biodiversity (Letourneau et al. 2009). A diverse community of predatory arthropods such as spiders can occupy more niches in the crop habitat and exploit more prey species, sizes, and life stages and is associated with lower plant damage and higher pest suppression in some crops (Gurr et al. 2012). Spiders are proven to reduce prey densities in wheat, apple orchards, corn and rice (Laub and Luna 1992, Marc and Canard 1997, Fagan et al. 1998, Marc et al. 1999). Information on spider abundance and identity in an agroecosystem can indicate which predatory species might be targeted for conservation practices.

Natural enemies in soybean are of prime importance for preventing herbivores from attaining pest status in fields (Turnipseed and Kogan 1980, Anderson and Yeargan 1998). Generalist predators such as spiders can reduce pest populations and maintain pests at low densities (Kajak 1978, Post and Travis 1979, Symondson et al. 2002), and high spider richness has been linked to reduced pest densities in other systems (Riechert et al. 1999).

In soybean as in other crops, plant architectural complexity increases throughout the growing season as the plant canopy grows (Lesar and Unzicker 1978). This increases the number of living niches available for arthropod pests, as well as spiders. In Virginia and much of the eastern US, soybean is grown using two distinct cropping systems, full season and double crop. The full season system is generally planted in April or May, earlier than the double crop system, and into fields with less plant residue on the soil surface. Double crop fields are planted later in the season, in June or July after winter small grain harvest, and in most cases, the residue from the preceding grain crop is left on the soil surface. This increased residue level benefits spiders by creating an early season habitat that is significantly more complex than relatively barren soil that full season soybean is planted into. This habitat complexity is especially attractive to spiders (Marshall and Rypstra 1999).

In annual crops like soybean, spiders will have few resources in bare fields when not in the growing season. Spiders might emigrate from soybean fields to surrounding vegetation until food becomes available in the fields. Winter small grains, which are a component of the double-crop soybean rotations, could provide a year-round refuge for spiders to hunt and reproduce in. Many foliar-dwelling spiders overwinter in plant debris on the ground (Turnbull 1973, Culin and Yeargan 1983a). Plant cover and litter cover are both associated with higher species richness and overall abundance in spider communities (Horvath et al. 2015). Both of these features provide hiding places and moderate temperature and humidity extremes, and they are also associated with higher spider diversity (Rypstra et al. 1999). If double cropped soybeans have a pre-existing spider community that is established when planting occurs, this could result in a greater abundance of spiders available for biological control earlier in the season, and throughout the season.

I conducted a 2-year study to determine how the full season and double crop soybean cropping systems may affect spider communities in southeast Virginia.

3.2 Materials and Methods

3.2.1 Study sites

Research was conducted from July through October of 2014 and 2015 in several soybean fields in southeastern Virginia. Experimental plots were located in both full season and double crop planted fields at the Tidewater Agricultural Research and Extension Center in Suffolk, VA (36.68N, 76.76W.), and in producers' fields in Sussex County, VA (36.84N, 77.29W). The two cropping systems each had six replicates in 2014 and four replicates in 2015. Plots were 0.12 ha in area and were inset 25 m from the field edge (Table 3.1). Standard weed management practices were used. Plots were located in fields bordered by woods, soybean, road, dirt path or grass (Table 3.2).

Table 3.1. Description of experimental plot location, cropping system, row spacing, soybean variety and planting date, southeastern Virginia, 2014 and 2015.

Year	Location	Cropping system	Row spaci	Variety	Planting dat
2014	Sussex County	Full season	17.78 cm	Liberty Link$^{\Delta}$	29-Apr-14
	Sussex County	Full season	17.78 cm	Liberty Link$^{\Delta}$	30-Apr-14
	Sussex County	Full season	17.78 cm	Liberty Link$^{\Delta}$	30-Apr-14
	Sussex County	Full season	17.78 cm	Liberty Link$^{\Delta}$	30-Apr-14
	Sussex County	Full season	17.78 cm	Liberty Link$^{\Delta}$	1-May-14
	Sussex County	Full season	17.78 cm	Liberty Link$^{\Delta}$	1-May-14
	Suffolk County	Double crop	17.78 cm	58-12 R2$^{\Psi}$	30-Jun-14
	Suffolk County	Double crop	17.78 cm	58-12 R2$^{\Psi}$	1-Jul-14
	Suffolk County	Double crop	17.78 cm	58-12 R2$^{\Psi}$	1-Jul-14
	Suffolk County	Double crop	17.78 cm	58-12 R2$^{\Psi}$	2-Jul-14
	Suffolk County	Double crop	17.78 cm	58-12 R2$^{\Psi}$	2-Jul-14
	Suffolk County	Double crop	17.78 cm	58-12 R2$^{\Psi}$	2-Jul-14
2015	Suffolk County	Full season	91.44 cm	58-12 R2$^{\Psi}$	13-May-15
	Suffolk County	Full season	91.44 cm	58-12 R2$^{\Psi}$	13-May-15
	Suffolk County	Full season	91.44 cm	58-12 R2$^{\Psi}$	13-May-15
	Suffolk County	Full season	91.44 cm	58-12 R2$^{\Psi}$	13-May-15
	Suffolk County	Double crop	19.05 cm	S56-G6Δ	16-Jun-15
	Suffolk County	Double crop	19.05 cm	S56-G6Δ	16-Jun-15
	Suffolk County	Double crop	91.44 cm	SS5911N R2$^{\Omega}$	25-Jun-15
	Suffolk County	Double crop	91.44 cm	AG5732$^{\Sigma}$	25-Jun-15

Δ Syngenta
Ψ Hubner
Ω Genuity
Σ Asgrow

Table 3.2. Description of vegetation surrounding experimental soybean plots in southeastern Virginia, 2014 and 2015.

Year	Cropping system	Field	Bordering vegetation			
			Border 1	Border 2	Border 3	Border 4
2014	Full season	1	Woods	Soybean	Soybean	Path
	Full season	2	Woods	soybean	Soybean	Path
	Full season	3	Woods	Soybean	Soybean	Path
	Full season	4	Woods	soybean	soybean	path
	Full season	5	Soybean	Soybean	Road	path
	Full season	6	Soybean	Soybean	Road	path
	Double crop	7	Road	Soybean	Soybean	Path
	Double crop	8	Soybean	Soybean	Soybean	Grass
	Double crop	9	Soybean	Soybean	Soybean	Grass
	Double crop	10	Woods	Path	Soybean	Soybean
	Double crop	11	Woods	Soybean	Soybean	Soybean
	Double crop	12	Woods	Soybean	Soybean	Soybean
2015	Full season	13	Soybean	Soybean	Soybean	Road
	Full season	14	Soybean	Soybean	Path	Grass
	Full season	15	Woods	Soybean	Soybean	Path
	Full season	16	Woods	Soybean	Soybean	Road
	Double crop	17	Path	Path	Soybean	Soybean
	Double crop	18	Grass	Path	Soybean	Soybean
	Double crop	19	Soybean	Soybean	Soybean	Woods
	Double crop	20	Soybean	Soybean	Road	Woods

3.2.2 Sweep net sampling

Sweep nets were used to sample spiders in the plant canopy, 29 July to 2 October 2014, and 5 August to 30 September 2015. Sampling was conducted at 2-week intervals with six samples in 2014 and five in 2015. Five sweep net samples consisting of 15 pendulum sweeps with a 38-cm diameter sweep net were taken in each plot on each sampling date. Samples were placed into plastic bags, transferred to a cooler and transported to the laboratory for enumeration and identification. Spiders from the five sweep net samples for each plot were combined for analysis.

3.2.3 Pitfall trapping

To sample ground dwelling spiders, five pitfall traps were placed into each plot from 29 July to 2 October 2014, and 5 August to 30 September 2015. Traps were inset at least 7 m from the plot edge and were placed randomly throughout the plot. Traps consisted of a 473-ml plastic cup 11.5-cm in diameter, filled with 120 ml of propylene glycol and 60 ml of water. The cup was set inside an identical 473-ml plastic cup with holes in the bottom for drainage. To protect traps from precipitation, a 30-cm plastic plate was suspended 5 cm above each trap and held in place by metal flags. Traps were collected one week after placement and propylene glycol was replaced. Samples were returned to the laboratory and specimens were rinsed, enumerated, and prepared for identification. Spiders from the five pitfall traps in each plot on each date were combined in the analysis.

3.2.4 Spider identification

Spiders collected were fixed in 70% ethanol and adults were identified to species level under a dissecting microscope. Immatures were identified to family level. The species names and the order of families follow the taxonomic order of Platnick's catalog version 15 (Platnick 2016). Voucher specimens have been placed in the Virginia Museum of Insects at Virginia Tech, Blacksburg, VA.

3.2.5 Analysis

Spider communities in cropping systems were compared by examining abundance, diversity and similarity coefficients. Abundance was calculated as the total number of individual spiders collected in each full season or double crop plot on each sampling date. Spider biodiversity was estimated using the Shannon Index (H'), which gives equal weight to rare and abundant species, as well as the Simpson Index (D), which is more sensitive to change in common species. The Shannon Index is calculated as follows: $H' = -\Sigma p_i \times \ln p_i$, where p_i is the proportion of individuals found in the ith species (Shannon and Weaver 1949). The Simpson Index is calculated as follows: $D = \Sigma n_i(n_i - 1)/N(N-1)$, where n is the number of individuals of the ith species, and N the total number of individuals (Simpson 1949). The values for the Simpson Index were modified (1/D), so that the increase in the index reflects an increase in diversity. Diversity indices were calculated for each plot using season-long total number of individual spiders.

Spider communities were compared using the Jaccard similarity coefficient, which is used to determine the similarity of communities in each plot. The Jaccard similarity coefficient deals with presence/absence data and is calculated as follows: Sj = a/a + b + c, where *a* is the number of species shared between two plots, *b* is the number of species in the second but not the first plot, and *c* is the number of species in the first but not the second plot. The coefficient ranges from 0 for complete dissimilarity to 1 for total similarity between two plots. A matrix of similarity coefficients was calculated for each sampling method and year. An average clustering technique was used to detect simple divisions of the data sets into a small number of distinct groups. Dendrograms were constructed for each year and sampling method to illustrate species shared between plots based on hierarchical clustering of Jaccard index values. The program MATLAB (MATLAB 2012) was used to construct dendrograms showing clustering patterns. The biodiversity and Jaccard indices were calculated using EstimateS (Colwell 2013), and all spiders that could not be identified to species were excluded from the similarity and biodiversity index analyses.

Species accumulation curves were generated to assess species richness. The curves show the rate at which added species are encountered as more individuals are collected. Species accumulation curves were constructed using EstimateS (Colwell 2013).

A repeated-measures analysis of variance (ANOVA) with cropping system as between-subjects factor was used to test whether spider abundance differed between full season and double crop soybean plots across the growing season. A first-order autoregressive was used for covariance structure. Data were log(n+1)-transformed for statistical analysis in SAS (SAS Institute 2009). An ANOVA was also used to analyze the effect of cropping system on the Shannon Index and the Simpson Index using PROC MIXED. For both analyses, means were separated using F-protected least squares means test at $\alpha = 0.05$.

3.3 Results

3.3.1 Spider communities

A total of 7,371 individual spiders were captured and identified. In 2014, Lycosidae (33%), Salticidae (31%), Linyphiidae (10%), Oxyopidae (9%), and Thomisidae (9%) made up 92% of 1,448 specimens caught by sweep net. Lycosidae (77%) and Linyphiidae (16%) made up 93% of 2,477 specimens caught by pitfall trap.

In 2015, Salticidae (31%), Oxyopidae (29%), Lycosidae (16%) and Thomisidae (11%) made up 87% of the 1,372 specimens caught by sweep net. Lycosidae (86%) and Linyphiidae (7%) made up 93% of the 2,034 specimens caught by pitfall trap.

Overall, a total of 76 species in 16 families and 55 genera were found (Table 3.3). The five dominant spider species in 2014 sweep net samples were *Pardosa milvina* (Hentz) (32%), *Pelegrina galathea* Walckenaer) (12%), *Habronattus coecatus* (Hentz) (7%), *Oxyopes salticus* (Hentz) (7%), and *Agyneta unimaculata* (Banks) (7%). In 2015 sweep net samples the dominant species were *O. salticus* (30%), *P. milvina* (21%), *Thymoites expulse* (Gertsch & Mulaik) (14%), *P. galathea* (12%), and *H. coecatus* (5%). The five dominant spider species in 2014 pitfall trap samples were *P. milvina* (64%), *A. unimaculata* (9%), *Grammonota inornata* (Emerton) (6%), *Allocosa funerea* (Hentz) (3%), and *Erigone autumnalis* (Emerton) (3%). In 2015 pitfall trap samples the dominant species were *P. milvina* (77%), *A. unimaculata* (4%), *E. autumnalis* (3%), *G. inornata* (2%), and *H. coecatus* (2%).

Table 3.3. Spider species collected in experimental soybean plots using pitfall traps and sweep nets in southeastern Virginia, 2014 and 2015.

Family	Species	Family	Species
Agelenidae	*Agelenopsis kastoni* (Chamberlin & Ivie)	Mysmenidae	*Microdipoena guttata (Banks)*
Araneidae	*Acanthepeira stellata* (Walckenaer)	Oxyopidae	*Oxyopes salticus* (Hentz)
	Araneus cingulatus (Walckenaer)	Philodromidae	*Tibellus duttoni* (Hentz)
	Mangora gibberosa (Hentz)	Salticidae	*Eris flava* (Peckham & Peckham)
	Neoscona arabesca (Walckenaer)		*Habronattus coecatus* (Hentz)
Clubionidae	*Clubiona abboti* (L. Koch)		*Hentzia palmarum* (Hentz)
	Clubiona bishopi (Edwards)		*Peckhamia picata* (Hentz)
	Clubiona kastoni (Gertsch)		*Pelegrina galathea* (Walckenaer)
Corinnidae	*Castianeira amoena* (C.L. Koch)		*Phidippus audax* (Hentz)
	Castianeira cingulata (C.L. Koch)		*Phidippus clarus* (Keyserling)
	Castianeira gertschi (Kaston)		*Zygoballus sexpunctatus* (Hentz)
	Castianeira longipalpa (Hentz)	Tetragnathidae	*Pachygnatha autumnalis* (Marx)
Eutichuridae	*Cheiracanthium inclusum* (Hentz)		*Tetragnatha laboriosa* (Hentz)
Gnaphosidae	*Drassyllus dixinus* (Chamberlin)	Theridiidae	*Asagena americana* (Emerton)
	Gnaphosa sericata (L. Koch)		*Episinus amoenus* (Banks)
	Sergiolus minutus (Banks)		*Euryopis argentea* (Emerton)
	Zelotes pullus (Bryant)		*Faiditus cancellatus* (Hentz)
Linyphiidae	*Agyneta regina* (Chamberlin & Ivie)		*Latrodectus mactans* (Fabricius)
	Agyneta semipallida (Chamberlin & Ivie)		*Theridion differens* (Emerton)
	Agyneta serrata (Emerton)		*Thymoites expulsus* (Gertsch & Mulaik)
	Agyneta unimaculata (Banks)	Thomisidae	*Mecaphesa celer* (Hentz)
	Bathyphantes pallidus (Banks)		*Misumenoides formosipes* (Walckenaer
	Ceraticelus emertoni (O. Pickard-Cambridge)		*Misumessus oblongus* (Keyserling)
	Ceraticelus limnologicus (Crosby & Bishop)		*Xysticus banksi* (Bryant)
	Ceratinopsis nigriceps (Emerton)		*Xysticus gulosus* (Keyserling)
	Eridantes erigonoides (Emerton)		*Xysticus texanus* (Banks)
	Erigone autumnalis (Emerton)	Uloboridae	*Uloborus glomosus* (Walckenaer)
	Florinda coccinea (Hentz)		
	Grammanota inornata (Emerton)		
	Idionella formosa (Banks)		
	Mermessus fradeorum (Berland)		
	Mermessus tridentatus (Emerton)		
	Tennesseellum formica (Emerton)		
	Walckenaeria dixiana (Chamberlin & Ivie)		
	Walckenaeria spiralis (Emerton)		
Lycosidae	*Allocosa funerea* (Hentz)		
	Hogna carolinensis (Walckenaer)		
	Hogna helluo (Walckenaer)		
	Hogna lenta (Hentz)		
	Pardosa atlantica (Emerton)		
	Pardosa milvina (Hentz)		
	Pardosa pauxilla (Montgomery)		
	Pirata seminolus (Gertsch & Wallace)		
	Pirata suwaneus (Gertsch)		
	Pirata sylvanus (Chamberlin & Ivie)		
	Rabidosa punctulata (Hentz)		
	Rabidosa rabida (Walckenaer)		
	Schizocosa avida (Walckenaer)		
	Tigrosa annexa (Chamberlin & Ivie)		

3.3.2 Effect of cropping system on spider communities

In the 2014 sweep net samples, 463 spiders were caught in full season soybean representing 12 families, 24 genera, and 25 species; also in 2014 sweep net samples, 718 spiders were caught in double crop soybean representing 12 families, 23 genera, and 25 species. In 2015, 315 spiders were caught in full season soybean representing 9 families, 10 genera, and 10 species; and 371 spiders were caught in double crop soybean representing 12 families, 11 genera, and 11 species. In 2014 pitfall traps, 555 spiders were caught in full season soybean representing 12 families, 26 genera, and 31 species; and 1,599 spiders were caught in double crop soybean representing 10 families, 22 genera, and 32 species. In 2015 pitfall traps, 284 spiders were caught in full season soybean representing five families, seven genera, and eight species; and 394 spiders were caught in double crop soybean representing seven families, 18 genera, and 19 species.

Sweep net samples in 2014 and 2015 showed no significant differences in diversity with either the Shannon Index or the Simpson Index (Table 3.4). When examining species diversity of total spiders caught per plot throughout the entire season, in 2014 pitfall trap samples in full season soybean had greater diversity, with a Shannon Index of 1.67 and Simpson Index of 3.5, compared with 1.10 and 1.88 in double crop soybean (Shannon Index: $F = 16.19$; $df = 1,10$; $P = 0.002$; Simpson Index: $F = 7.25$; $df = 1,10$; $P = 0.02$; Table 3.4). In 2015 pitfall traps, double crop soybean had greater diversity with a Shannon Index of 1.31 compared with 0.38 in full season soybean, but there was no significant difference in Simpson Index between the two cropping systems (Shannon Index: $F = 7.01$; $df = 1,10$; $P = 0.04$; Simpson Index: $F = 3.16$; $df = 1,10$; $P = 0.13$).

Table 3.4. Biodiversity (mean+-SE) of spider community in double crop and full season soybean throughout the soybean growing season. Each index was calculated using the total species counts over the growing season from each plot. There were six double crop plots and six full season plots in 2014, and four double crop plots and four full season plots in 2015.

	Year	Diversity index	Full season	Double crop	F-ratio	df	P value
Sweep net	2014						
		Shannon Index	1.898 ± 0.11	1.558 ± 0.241	1.64	1,10	0.229
		Simpson Index	6.095 ± 0.617	3.788 ± 0.843	4.88	1,10	0.052
	2015						
		Shannon Index	1.43 ± 0.233	1.523 ± 0.052	0.15	1,6	0.711
		Simpson Index	4.118 ± 0.949	3.925 ± 0.411	0.03	1,6	0.859
Pitfall trap	2014						
		Shannon Index	1.672 ± 0.132	1.098 ± 1.877	16.19	1,10	0.002
		Simpson Index	3.502 ± 0.599	0.05 ± 0.075	7.25	1,10	0.023
	2015						
		Shannon Index	0.375 ± 0.155	1.308 ± 0.316	7.01	1,6	0.038
		Simpson Index	1.24 ± 0.128	3.07 ± 1.021	3.16	1,6	0.126

Overall, the abundance of spiders in each family differed significantly depending on cropping system. Mean numbers of spiders caught with a sweep net and with pitfall traps were significantly more abundant in double crop soybean in 2014 (treatment × day interaction, sweep net: F = 5.29, df = 5,50; P = 0.0006; treatment × day interaction, pitfall trap: F = 10.35, df = 5,50; P < 0.0001; Tables 3.5 and 3.6, Figs. 3.1 and 3.2). In sweep net samples, the difference in abundance was most evident on the final counting date, while in pitfall trap samples, differences in abundance decreased through time. While mean spiders per plot was higher in double crop soybean in 2015 in both sweep net and pitfall trap samples, the differences were not significant (treatment, sweep net: F = 0.35; df = 1,4; P = 0.588; treatment, pitfall trap: F = 5.12; df = 1,4; P = 0.086, Figs. 3.3 and 3.4). In 2014 sweep net and pitfall trap samples, Lycosidae and Linyphiidae were more abundant in double crop soybean (treatment × day interaction, sweep net Lycosidae: F = 11.7; df = 5,50; p<0.001; treatment, sweep net Linyphiidae: F = 10.49; df = 1,10; P = 0.009; treatment × day interaction, pitfall trap Lycosidae: F = 12.38; df = 5,50; P < 0.0001; treatment × day interaction, pitfall trap Linyphiidae: F 8.9; df = 5, 50; P<0.0001; Figs. 3.5-3.8). In 2015 pitfall trap samples Linyphiidae and Thomisidae spiders were more abundant in double crop soybean (treatment, Linyphiidae: F = 20.68; df = 1,4; P = 0.01; treatment, Thomisidae: F = 9.54; df = 1,4; P = 0.031).

Table 3.5. Mean number of spiders in each common spider family per plot caught with a sweep net in full season and double crop soybeans in 2014 and 2015. Numbers in the full season and double crop columns represent the mean numbers of spiders (± SE) caught with 75 sweeps of a sweep net in six full season plots or six double crop plots. Counts were taken biweekly from 29 July 2014 to 2 October 2014, and 5 August 2015 to 30 September 2015.

	Treatment		RM ANOVA F-ratio (p-value)								
	Full season	Double crop	Treatment			Day			Interaction (T x D)		
Total spiders			F-ratio	df	p-value	F-ratio	df	p-value	F-ratio	df	p-value
2014	12.86 ± 2.29	19.75 ± 7.21	1.66	1,10	0.2271	14.78	5,50	<.00001	5.29	5,50	0.0006
2015	15.75 ± 5.0	18.55 ± 5.85	0.35	1,4	0.5878	1.07	4,16	0.4029	4.44	4,16	0.0132
Lycosidae immatures											
2014	0.72 ± 0.55	7.33 ± 5.39	24.12	1,10	0.0006	37.731	5,50	<0.0001	12.03	5,50	<0.0001
2015	2.9 ± 1.33	1.7 ± 01.09	6.89	1,4	0.0586	0.59	4,16	0.6753	1.8	4,16	0.1789
Lycosidae total											
2014	0.78 ± 0.54	9.53 ± 5.92	25.52	1,10	0.0005	35.44	5,50	<.0001	11.7	5,50	<.0001
2015	3.35 ± 1.47	2.05 ± 1.16	7.56	1,4	0.0514	0.69	4,16	0.6094	2.53	4,16	0.0815
Linyphiidae immatures											
2014	0.47 ± 0.28	0.94 ± 0.45	4.53	1,10	0.0592	1.06	5,50	0.3947	0.93	5,50	0.4695
2015	0.25 ± 0.28	1.05 ± 1.01	3.69	1,4	0.1271	8.59	4,16	0.0007	8.02	4,16	0.001
Linyphiidae total											
2014	0.64 ± 0.31	2 ± 0.76	10.49	1,10	0.0089	1.58	5,50	0.1843	1.49	5,50	0.2085
2015	0.45 ± 0.30	1.25 ± 1.21	1.39	1,4	0.3043	10.4	4,16	0.0002	9.1	4,16	0.0005
Salticidae immatures											
2014	4.94 ± 1.32	4.17 ± 1.15	0.48	1,10	0.5031	10.17	5,50	<.0001	1.95	5,50	0.103
2015	4.25 ± 1.76	5.5 ± 2.22	1.64	1,4	0.2697	0.74	4,16	0.5768	1.26	4,16	0.3247
Salticidae total											
2014	5.92 ± 1.35	4.78 ± 1.33	1.58	1,10	0.238	6.33	5,50	0.0001	2.6	5,50	0.0363
2015	4.5 ± 1.70	5.95 ± 2.23	0.96	1,4	0.3822	0.77	4,16	0.5605	1.06	4,16	0.4087

Table 3.6. Mean number of spiders in each common spider family per plot caught with a pitfall traps in full season and double crop soybeans in 2014 and 2015. Numbers in the full season and double crop columns represent the mean numbers of spiders (± SE) caught in pitfall traps in six full season plots or six double crop plots. In each plot there were five pitfall traps, which were combined after collection. Traps were open for seven days. Counts were taken biweekly from 29 July 2014 to 2 October 2014, and 5 August 2015 to 30 September 2015.

	Treatment		RM ANOVA F-ratio (p-value)								
	Full season	Double crop	Treatment			Day			Interaction (T x D)		
Total spiders			F-ratio	df	p-value	F-ratio	df	p-value	F-ratio	df	p-value
2014	15.47 ± 3.30	44.53 ± 16.56	21.22	1,10	0.001	14.32	5,50	<.0001	10.35	5,50	<.0001
2015	16.69 ± 7.25	21.56 ± 10.17	5.12	1,4	0.0864	4.26	4,16	0.0156	2.22	4,16	0.1131
Lycosidae immatures											
2014	6.08 ± 1.89	7.47 ± 3.55	0.39	1,10	0.5441	1.18	5,50	0.3342	1.82	5,50	0.1268
2015	6.31 ± 6.07	10.38 ± 6.87	3.56	1,4	0.1324	3.01	4,16	0.0496	0.11	4,16	0.9767
Lycosidae total											
2014	11.58 ± 2.95	34.22 ± 13.83	16.53	1,10	0.0023	22.17	5,50	<.0001	12.38	5,50	<.0001
2015	15.56 ± 7.14	17.56 ± 9.91	0.21	1,4	0.6723	6.59	4,16	0.0025	1.61	4,16	0.2191
Linyphiidae immatures											
2014	0.47 ± 0.25	0.97 ± 0.38	6.05	1,10	0.0337	2.01	5,50	0.0931	1.74	5,50	0.1437
2015	0.13 ± 0.17	0.50 ± 0.52	3.53	1,4	0.1334	1.89	4,16	0.1609	1.42	4,16	0.271
Linyphiidae total											
2014	1.14 ± 0.60	8.92 ± 3.25	55.65	1,10	<.0001	5.33	5,50	0.0005	8.9	5,50	<.0001
2015	0.81 ± 0.49	1.75 ± 0.81	20.68	1,4	0.0104	1.34	4,16	0.2974	1.28	4,16	0.3181
Thomisidae immatures											
2014	1.03 ± 0.50	0.66 ± 0.35	1.55	1,10	0.2419	0.75	5,50	0.5896	1.55	5,50	0.192
2015	0.19 ± 0.27	0.94 ± 0.67	9.54	1,4	0.0366	2.77	4,16	0.0634	1.79	4,16	0.1795
Thomisidae total											
2014	1.08 ± 0.50	0.67 ± 0.35	3.36	1,10	0.0966	0.52	5,50	0.7629	1.05	5,50	0.3991
2015	0.19 ± 0.27	0.94 ± 0.67	9.54	1,4	0.0366	2.77	4,16	0.0634	1.79	4,16	0.1795

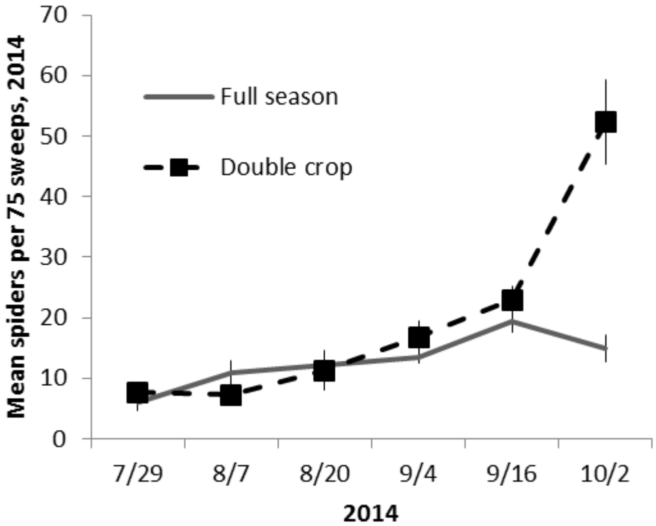


Figure 3.1. Mean spiders (± SE; *, P < 0.05) caught per 75 sweeps with a sweep net from six double crop and six full season fields in southeastern Virginia in 2014. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

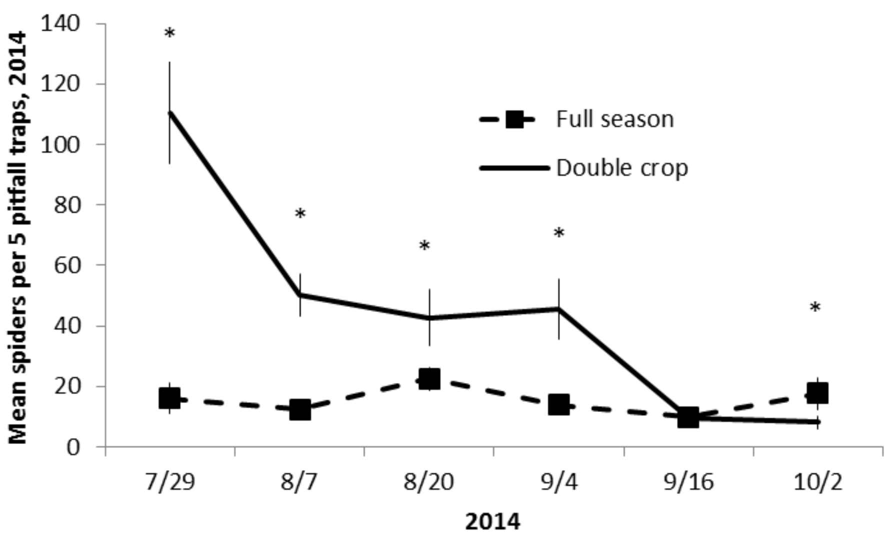


Figure 3.2. Mean spiders (± SE; *, P < 0.05) caught per five pitfall traps from six double crop and six full season fields in southeastern Virginia in 2014. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

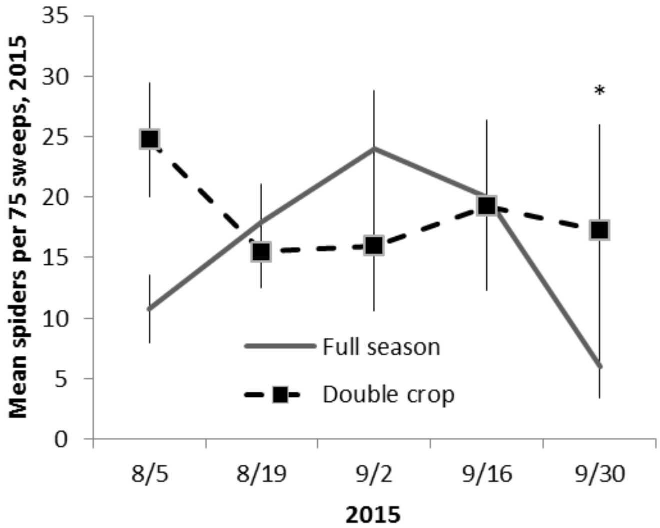


Figure 3.3. Mean spiders (± SE; *, $P < 0.05$) caught per 75 sweeps with a sweep net from four double crop and four full season fields in southeastern Virginia in 2015. Counts were taken biweekly from 5 August 2015 to 30 September 2015.

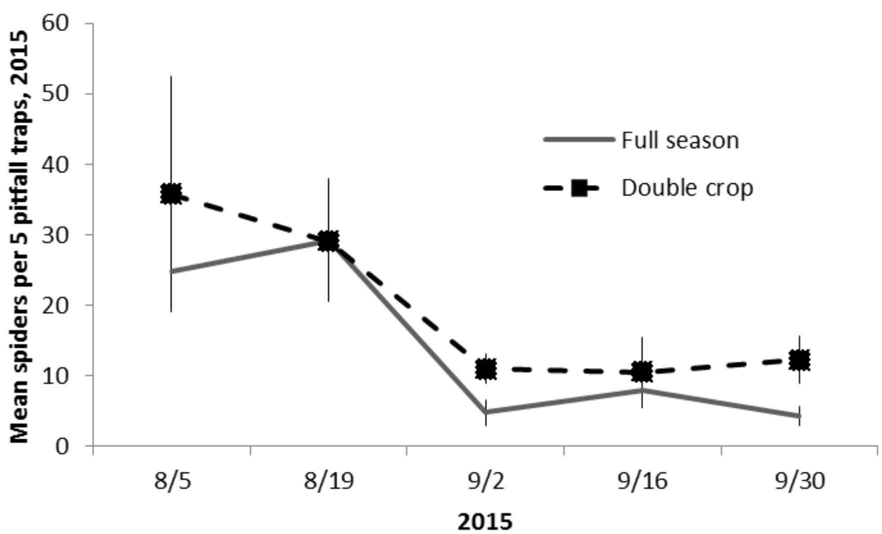


Figure 3.4. Mean spiders (± SE) caught per five pitfall traps from four double crop and four full season fields in southeastern Virginia in 2015. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 5 August 2015 to 30 September 2015.

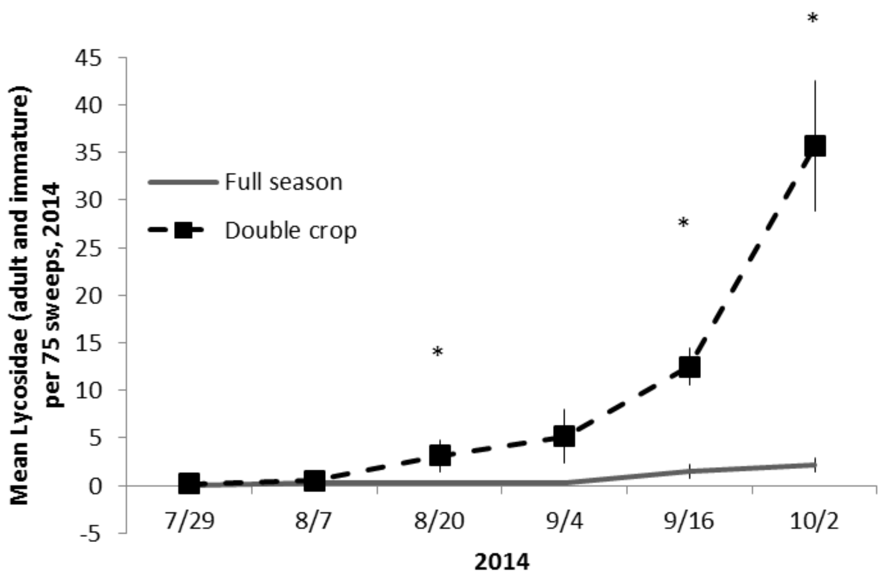


Figure 3.5. Mean Lycosidae (± SE; *, P < 0.05) caught per 75 sweeps with a sweep net from six double crop and six full season fields in southeastern Virginia in 2014. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

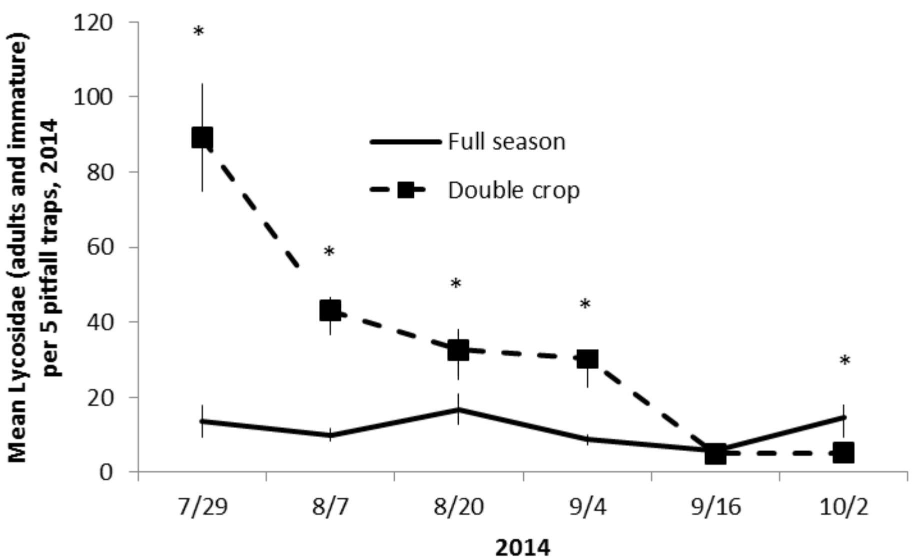


Figure 3.6. Mean Lycosidae (± SE; *, P < 0.05) caught per five pitfall traps from six double crop and six full season fields in southeastern Virginia in 2014. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

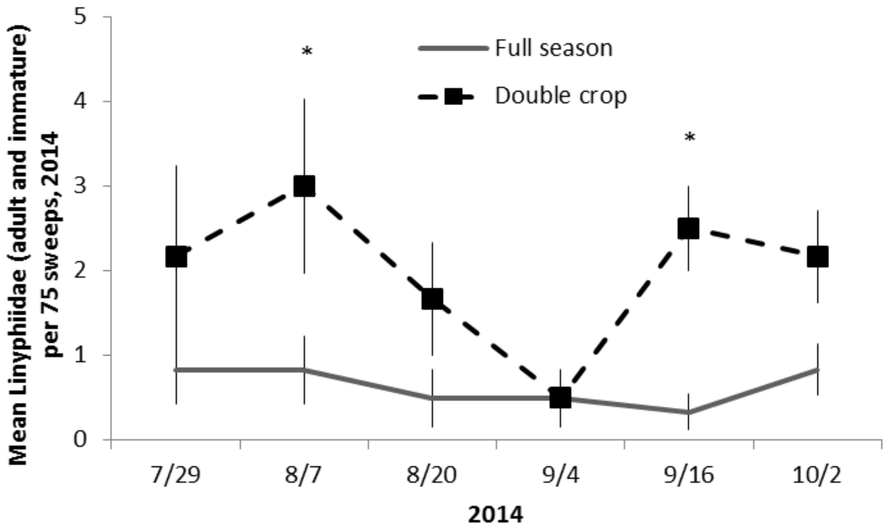


Figure 3.7. Mean Linyphiidae (± SE; *, P < 0.05) caught per 75 sweeps with a sweep net from six double crop and six full season fields in southeastern Virginia in 2014. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

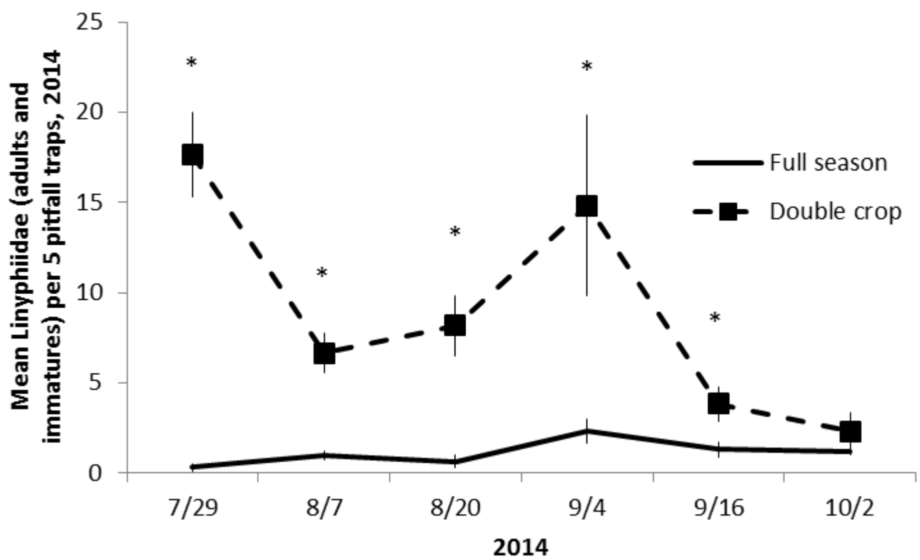


Figure 3.8. Mean Linyphiidae (± SE; *, P < 0.05) caught per five pitfall traps from six double crop and six full season fields in southeastern Virginia in 2014. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

A total of 76 adult spiders were identified from full season sweep net samples and 153 from double crop sweep net samples in 2014. *O. salticus* was the dominant species in full season soybean and *P. milvina* was the dominant species in double crop soybean (Fig. 3.9). A total of 28 adult spiders were identified from full season sweep net samples and 51 from double crop sweep net samples in 2015. *P. milvina* was the dominant species in full season soybean and *O. salticus* was the dominant species in double crop (Fig. 3.10). A total of 259 adult spiders were identified from full season pitfall traps and 1,260 from double crop pitfall traps in 2014. *P. milvina* was the dominant species in both systems (55% and 71% and respectively; Fig. 3.11). A total of 164 adult spiders were identified from full season pitfall traps and 162 from double crop pitfall traps in 2015. *P. milvina* was the dominant species in both systems (90% and 62% respectively; Fig. 3.12). There were significantly more *P. milvina* in double crop soybean than full season caught with pitfall traps in 2014, but not 2015 (Figs. 3.13 and 3.14).

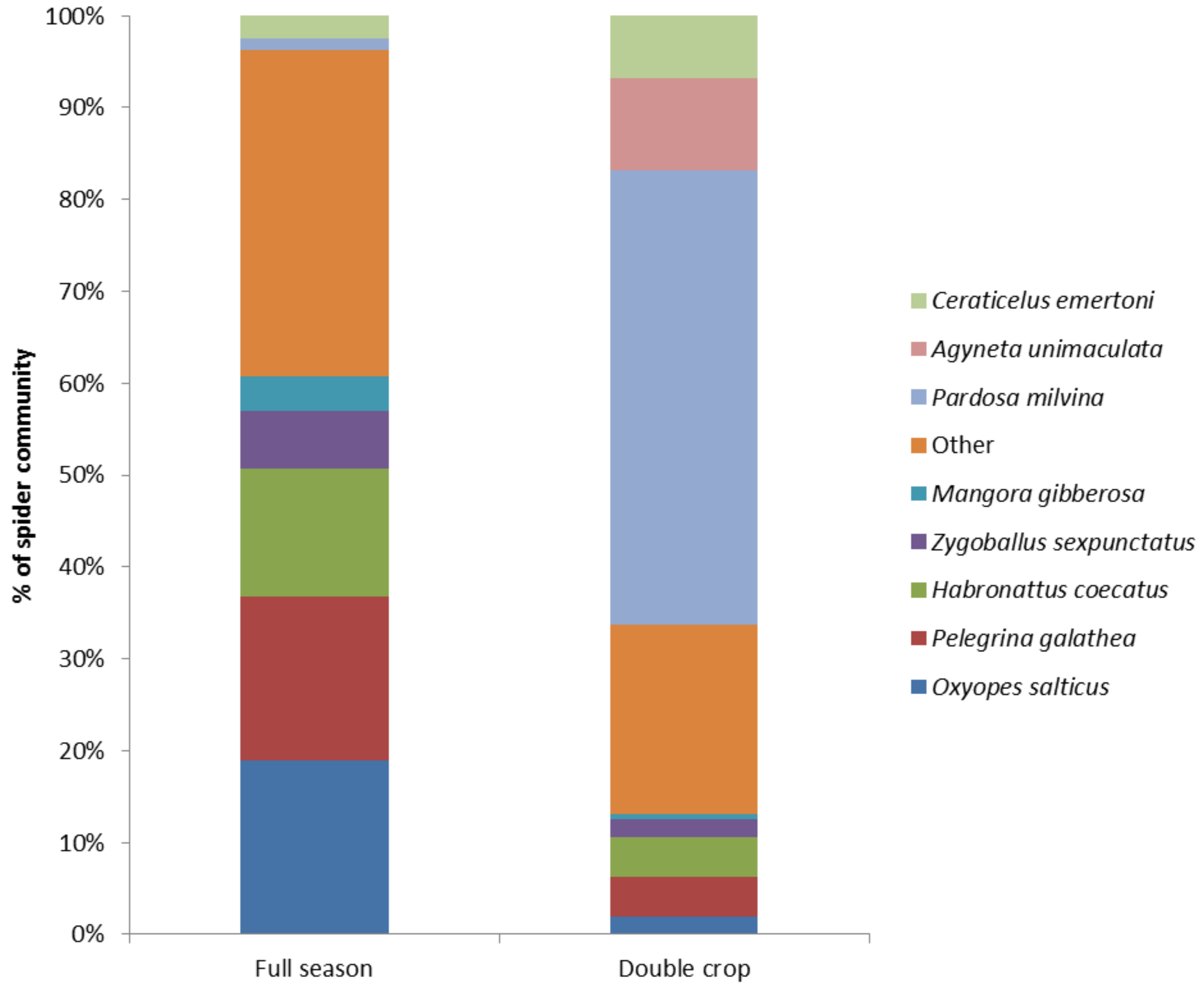


Figure 3.9. Composition of dominant spiders caught with sweep nets in full season and double crop soybean throughout the 2014 growing season, southeastern Virginia.

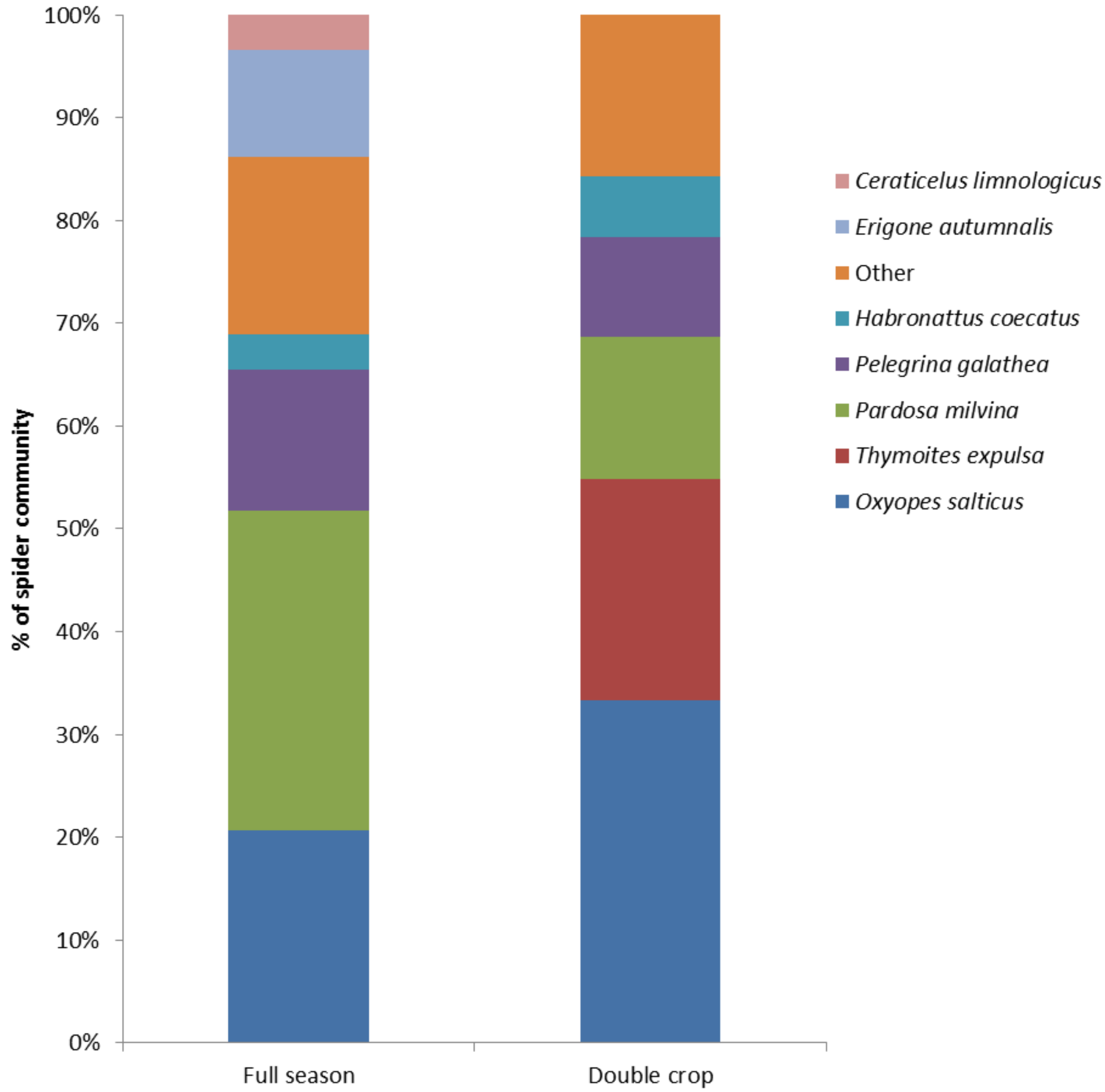


Figure 3.10. Composition of dominant spiders caught with sweep nets in full season and double crop soybean throughout the 2015 growing season, southeastern Virginia.

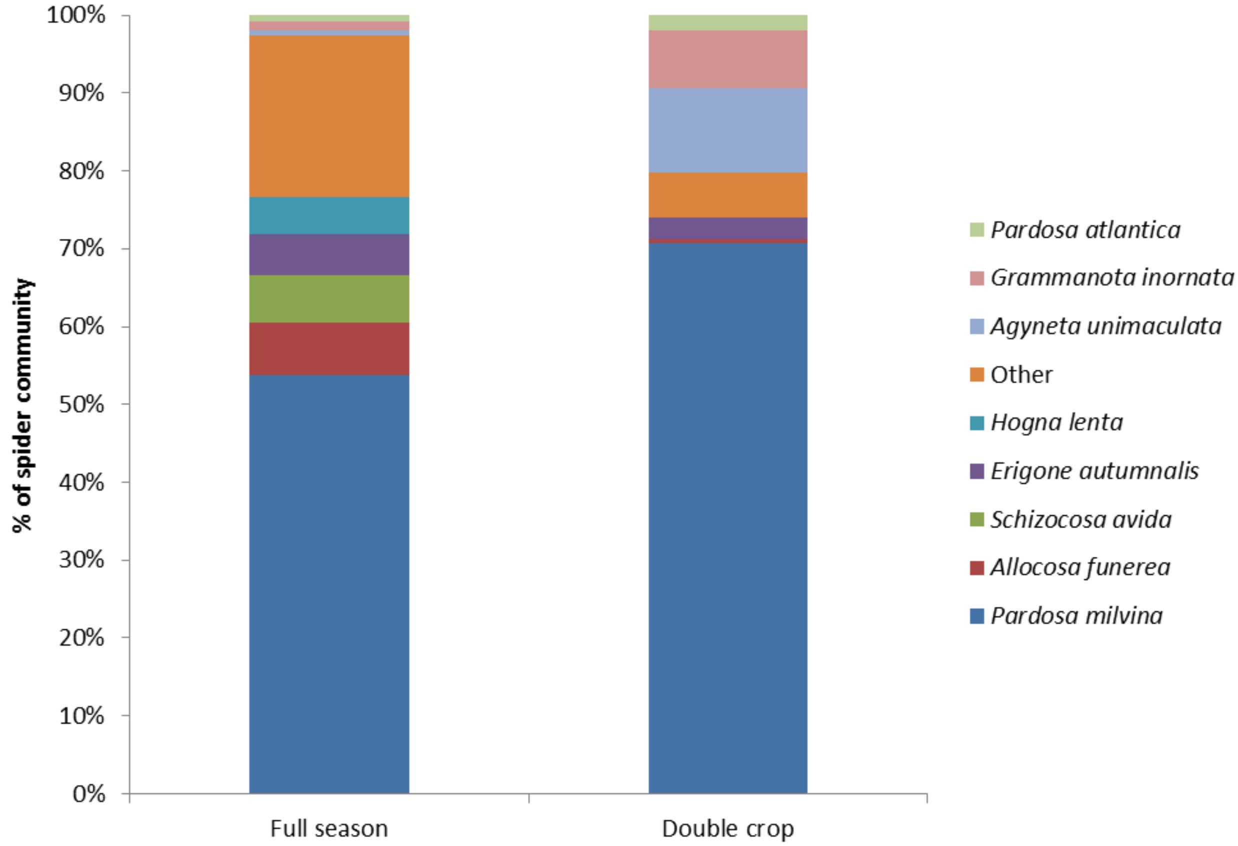


Figure 3.11. Composition of dominant spiders caught with pitfall traps in full season and double crop soybean throughout the 2014 growing season, southeastern Virginia.

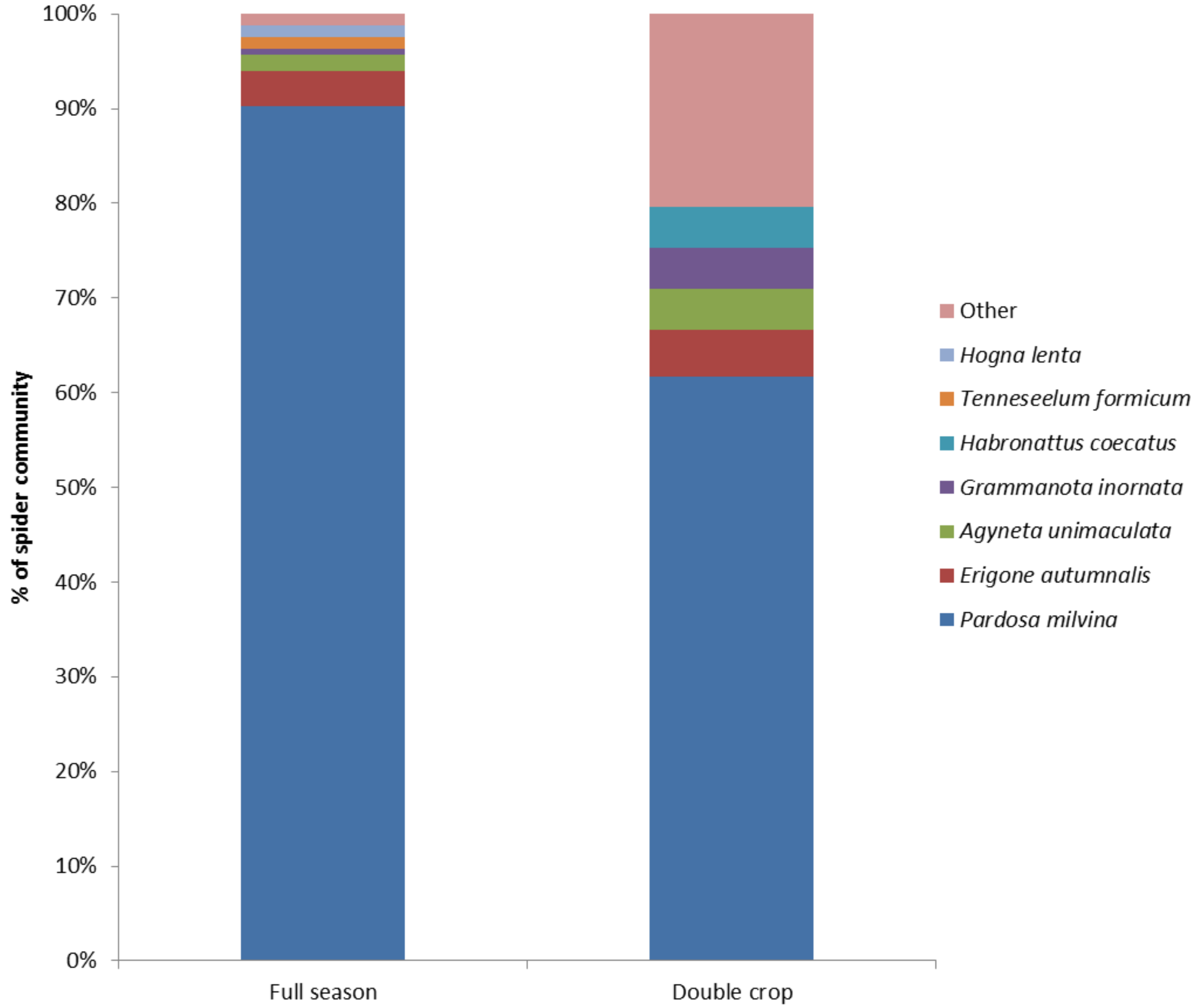


Figure 3.12. Composition of dominant spiders caught with pitfall traps in full season and double crop soybean throughout the 2015 growing season, southeastern Virginia.

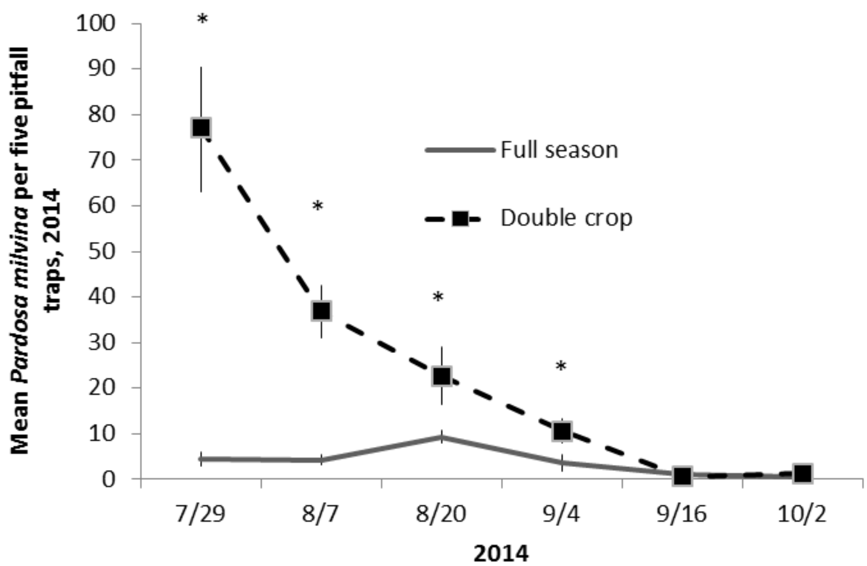


Figure 3.13. Mean *Pardosa milvina* (± SE; *, $P < 0.05$) caught per five pitfall traps from six double crop and six full season fields in southeastern Virginia in 2014. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

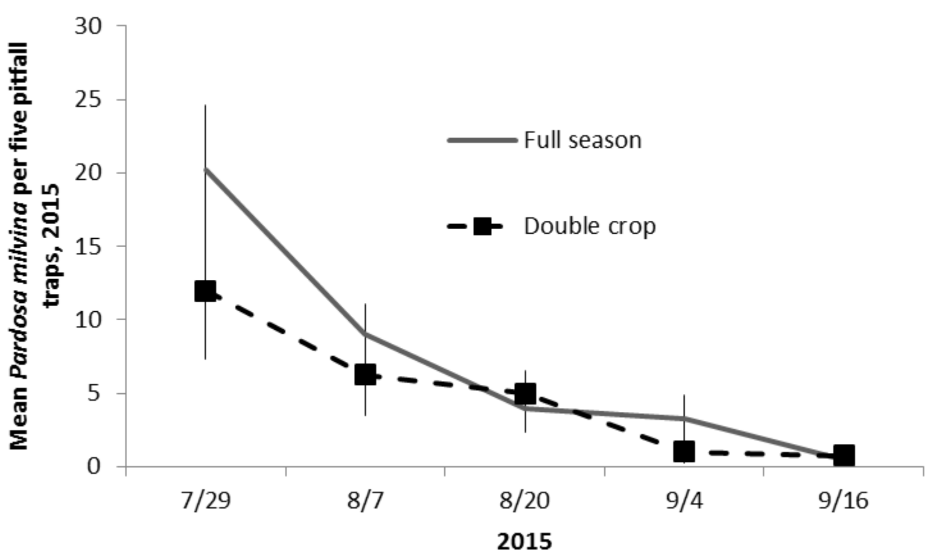
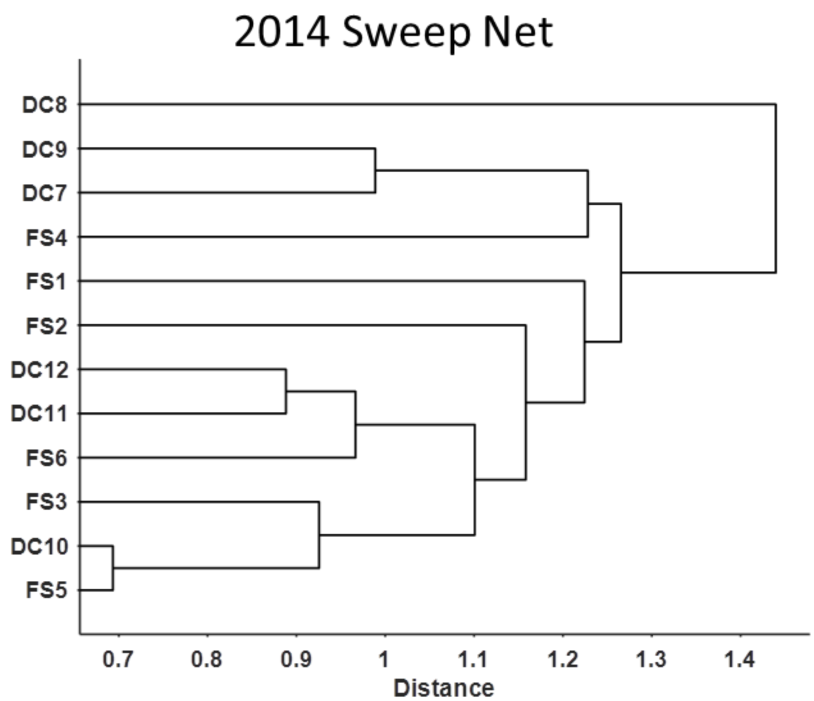
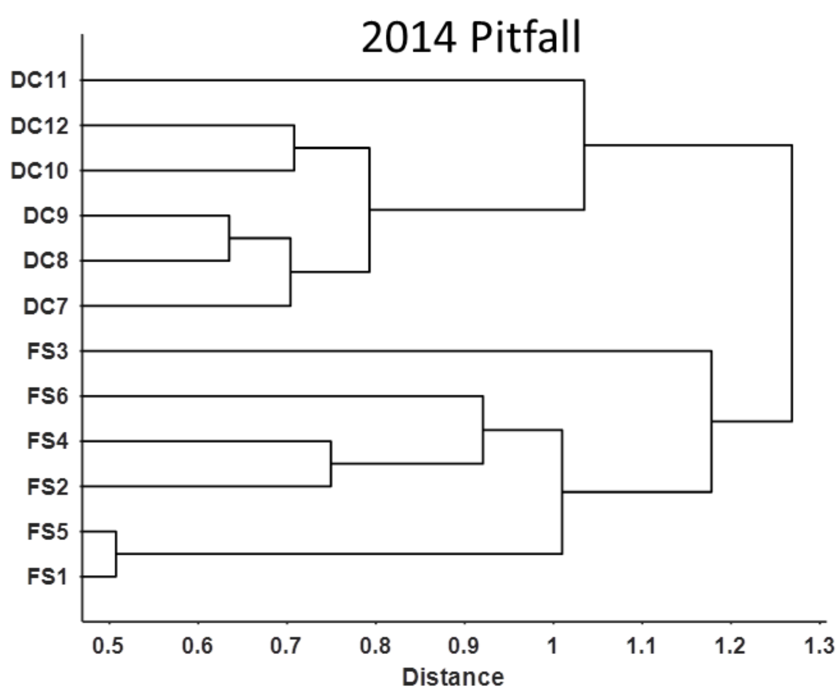


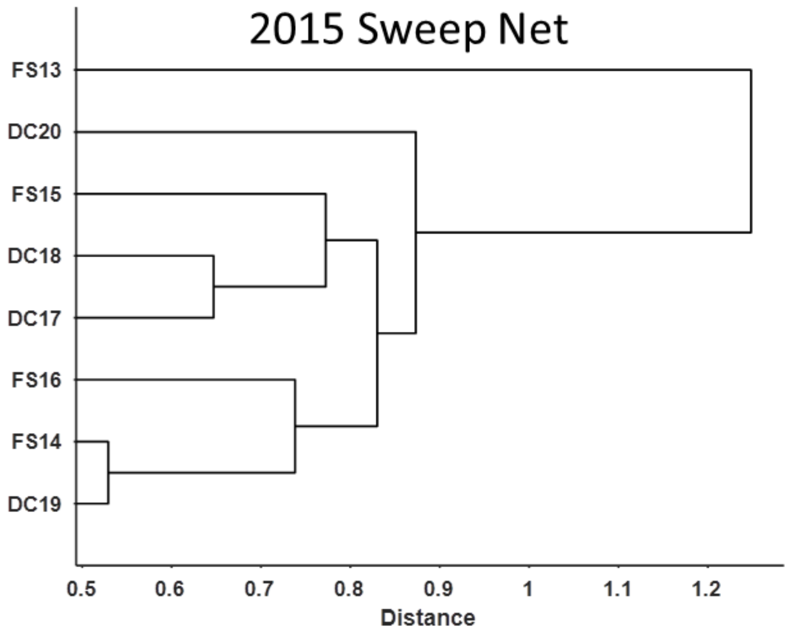
Figure 3.14. Mean *Pardosa milvina* (± SE) caught per five pitfall traps from four double crop and four full season fields in southeastern Virginia in 2015. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 7 July 2015 to 7 October 2015.

In 2014, full season sweep net samples had similar populations of *O. salticus*, *P. galathea* and *H. coecatus* as the dominant species caught (Fig. 3.9). *P. milvina* made up 50% of adults caught in double crop soybean sweep net samples in 2014. The dominant spider in pitfall traps samples in both cropping systems in 2014 and 2015 was *P. milvina* (Figs. 3.11 and 3.12). There were significantly more *P. milvina* in double crop soybean in 2014 but not 2015 (2014: $F = 62.31$, $df = 1,10$, $p < 0.0001$, 2015: $F = 1$; $df = 1,6$; $p = 0.36$). In full season soybeans, the second and third most abundant spiders in 2014 pitfall traps were lycosids, *A. funerea* and *Schizocosa avida* (Walckenaer). That same year the second and third most abundant spiders were linyphiids, *A. unimaculata* and *G. inornata*, in double crop pitfall traps. In 2015 sweep net samples, *P. milvina* made up 30% of adults caught in full season soybean, while *H. coecatus* was the dominant species in double crop soybean (Fig. 3.10). In 2015 pitfall trap samples, *P. milvina* made up more than 90% of adult spiders in full season soybeans, while in double crop soybeans, *P. milvina* made up 60% of samples, and the second and third most abundant spiders were *A. unimaculata* and *G. inornata.*

The dendrograms of the Jaccard similarity matrix for 2014 pitfall trap samples show very distinctly that fields of the same cropping system were more similar in species composition than fields in a different cropping system (Fig. 3.15). The dendrogram for 2014 sweep net samples shows a less clear pattern, with a double crop field, DC10, and a full season field, FS5, with the highest degree of similarity, followed by two double crop fields (DC12 and DC11). In 2015, the dendrogram for pitfall trap samples shows two full season fields as most similar (FS13 and FS15), and all of the double crop fields more similar to each other than to full season fields. As in 2014, the sweep net samples for 2015 showed no clear similarities between cropping systems, with a full season and a double crop field showing the highest similarity (FS14 and DC19).


2014 Sweep Net
DC8
DC9
DC7
FS4
FS1
FS2
DC12
DC11
FS6
FS3
DC10
FS5
0.7
0.8
0.9
1
1.1
1.2
1.3
1.4
Distance


2014 Pitfall
DC11
DC12
DC10
DC9
DC8
DC7
FS3
FS6
FS4
FS2
FS5
FS1
0.5
0.6
0.7
0.8
0.9
1
1.1
1.2
1.3
Distance



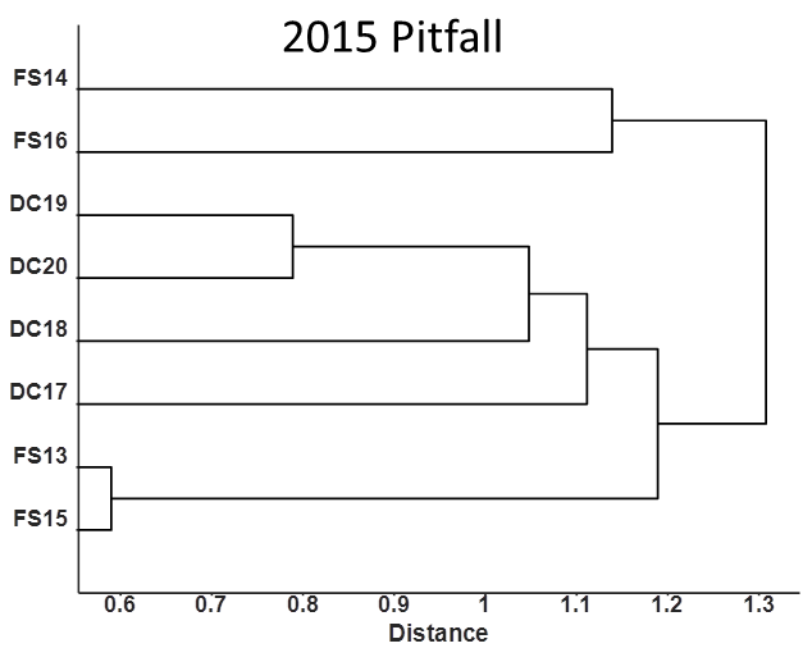


Figure 3.15. Cluster analysis dendrograms for spiders collected by pitfall trap and sweep net in full season and double crop soybean fields in southeastern Virginia in 2014 and 2015. Distance represents Euclidean distance. (FS = Full season; DC = Double crop).

The species-accumulation curves for sweep net and pitfall trap samples in 2014 indicates that full season soybean plots contained more species than double crop plots (Figs. 3.16 and 3.17). The opposite trend was seen in sweep net and pitfall trap samples in 2015, with double crop curves indicating more species in those soybean plots than in full season plots (Figs. 3.18 and 3.19). However, the lack of asymptote in the curves of both cropping systems suggests that there are more species present that have not yet been collected.

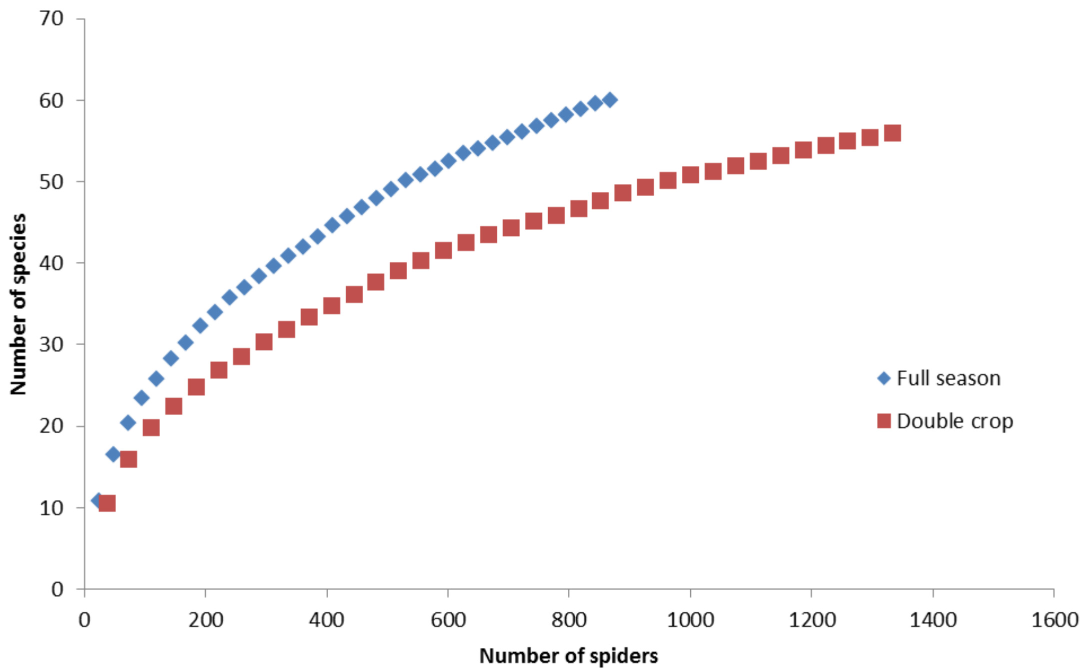


Figure 3.16. Species accumulation curve of adult spiders caught with a sweep net in full season and double crop soybeans in southeastern Virginia in 2014.

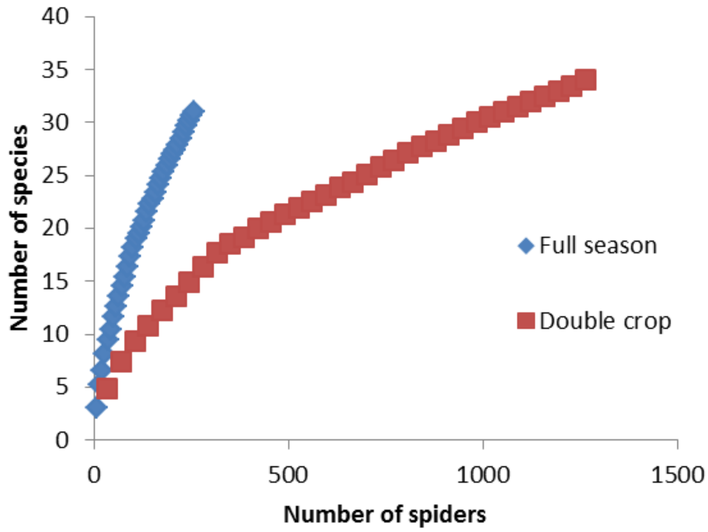


Figure 3.17. Species accumulation curve of adult spiders caught with pitfall traps in full season and double crop soybeans in southeastern Virginia in 2014.

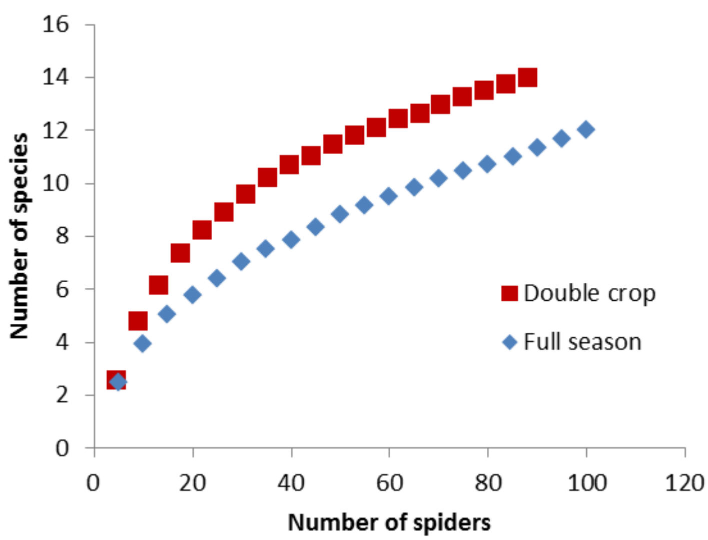


Figure 3.18. Species accumulation curve of adult spiders caught with a sweep net in full season and double crop soybeans in southeastern Virginia in 2015.

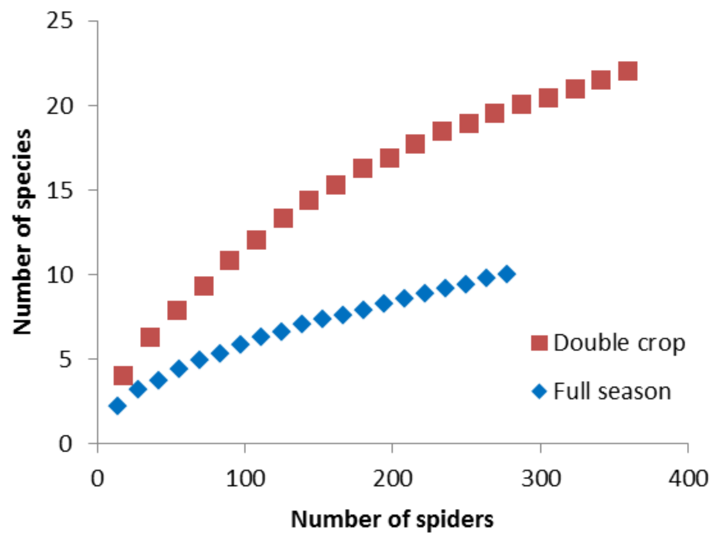


Figure 3.19. Species-accumulation curve of adult spiders caught using a pitfall trap in full season and double crop soybeans in southeastern Virginia in 2015.

3.4 Discussion

Spiders caught with a sweep net were significantly more abundant in double crop fields in 2014. This was also the case in 2015 but results were not significant. Previous research showed no difference in spider abundance between the two cropping systems in Virginia soybean (McPherson et al. 1982). A different study examined spider family abundance in Virginia soybean and found more ground-dwelling spiders in double crop soybean and foliage dwelling species were similar in abundance for full season and double crop soybean (Ferguson et al. 1984). Double crop soybeans develop more rapidly than full season (Holshouser 2014). The higher abundance of spiders in double crop compared to full season soybean could in part be because in the double crop system there is rapid canopy closure creating a cooler microhabitat for spiders, which could benefit spider survival (Sprenkel et al. 1979, Ferguson et al. 1984). Year-round litter and detritus could allow for continuous habitation of spiders in a double crop soybean field; however, harvesting and planting would cause disturbances in both full season and double crop soybean. The pre-existing spider assemblage in double crop soybean could allow for rapid population growth once soybeans are planted, which creates an even more complex habitat for living and hunting and might encourage overall higher populations than full season soybean that was planted earlier but in relatively bare ground.

The common spider families in the foliage and on the ground were similar in some respects to a previous survey of spider families in soybean. Ferguson et al. (1984) found Oxyopidae, Thomisidae and Salticidae to be the most abundant foliage dwelling spider families. We also found these to be among the dominant spider families in foliage, but Lycosidae was by far the most abundant family in 2014 and was third-most abundant in 2015. Immatures made up the majority of lycosids found in the foliage, though they are usually classified as a ground-dwelling family. Though immatures cannot be identified to species, it is likely that the majority of the specimens were *P. milvina*, which was the dominant adult lycosid species identified. Lycosids were found to be fourth-most dominant foliage dwelling spiders in Kentucky soybean (Culin and Yeargan 1980a). Lycosidae and Linyphiidae were the most abundant ground-dwelling families in both years of our study, which Ferguson et al. (1984) also reported. They reported lycosids were more abundant in pitfall traps in double crop fields, which we also found in 2014 but not 2015.

The temporal patterns in spider abundance in each cropping system were different for some families. In 2014, spiders caught with a sweep net reached peak numbers in double crop soybean in October, with Lycosidae making up the majority of the community that late in the season. Spider numbers in full season soybean increased gradually throughout the season, and had begun to decline by October. We found a different dynamic in 2015, with double crop spider abundance peaking in July, while full season spider abundance peaked in early September. Initial counts in both cropping systems were higher in 2015 than 2014. This suggests that spiders moved from plant debris or via immigration early in the season more successfully in 2015 but survival or fecundity was higher in 2014 double crop soybean.

Diversity indexes suggest similar biodiversity in sweep net samples between full season and double crop soybean. There were opposing results in pitfall trap samples in different years, with higher diversity indexes in full season pitfall trap samples in 2014, but a higher Shannon Index in double crop soybean in 2015. The higher diversity in double crop soybean in 2015 can be explained by the fact that *P. milvina was* 90% of the identified adults in full season pitfall traps that year. Double crop soybean is planted into wheat stubble from the previous crop and generally has more litter cover than full season soybean, which is associated with higher spider diversity. In spite of this, 2014 pitfall trap samples had higher diversity indexes in full season soybean. This could be due to more spider species moving into full season soybean soon after they are planted in April or May, allowing for populations to increase sufficiently by the first sampling date in July. Double crop soybeans had only one month for species to move into the field, and so lower diversity might have resulted. In 2015 there were many fewer species present in both systems, with only eight species in full season and 19 species in double crop soybean, compared with 31 and 32 species respectively in 2014. It is unclear why full season plots were less attractive to a diverse community in 2015. Although research has suggested row spacing does not influence natural enemy abundance, wider rows have more bare ground, which is associated with lower spider diversity (Anderson and Yeargan 1971, Horvath et al. 2015). Two of four full season fields had 91-cm rows, and this could have discouraged spider colonization early in the season and led to lower overall diversity.

Another possibility for the reduced species richness in 2015 could be attributable to surrounding landscape factors. Surrounding habitat allows for spider refuge during the winter and during harvest and tillage. It also can serve as an alternative food resource early in the growing season. A recent study in North Carolina found no effect of surrounding habitat on spider density within organic soybean (Fox et al. 2015). Although not organic, the fields in our study were not sprayed with any insecticides. The lack of influence of surrounding landscapes on organic fields has been attributed to spider populations that are self-sustaining (Schmidt et al. 2005, Fox et al. 2015). This suggests that the varying field borders in both years of our experiments might not have had much effect on the spider densities, although individual species could have been influenced.

The spider community in both cropping systems had significant species overlap with a survey done in Kentucky soybean in 1983. Culin and Yeargan (1983b) reported the linyphiid spiders *E. autumnalis, Tennesseelum formicum* (Emerton), *G. inornata* and *A. unimaculata* as the dominant ground dwelling spiders. With the exception of *T. formicum,* which we saw infrequently, these spiders were common in our plots. However, the dominant spider in our research, *P. milvina,* made up less than 6% of their community. Our spider family abundances were similar to reported spider compositions in U.S. cropping systems, where hunting spiders like lycosids made up more than 50% of the community and linyphiids approximately 17% (Nyffler and Sunderland 2003).

In both years of this study and in both cropping systems, overall ground dwelling spider populations caught with pitfall traps were highest early in the season. This is encouraging if one is attempting to plan an IPM program with spider conservation in mind. A large assemblage of generalist predators early in the season is associated with more efficient biological control (Edwards et al. 1979, Birkhofer et al. 2008). In 2014, the early season high abundance of ground dwelling spiders was complimented by increasing foliage-dwelling spiders as the season progressed in both systems. This increase in spiders can be especially helpful when *Helicoverpa zea* (Boddie) arrives in soybean, which is early August in Virginia (Herbert et al. 2003). While 2015 populations in the foliage did not follow this trend, there were still on average 15 to 25 spiders per 75 sweeps in both cropping systems.

This is the first spider survey done in eastern Virginia soybean for many years. The dominance of the foliage dwelling lynx spider *O. salticus* in both years of this study is encouraging, as this spider is a known predator of *H. zea* and at least 33 other insect species (Young and Edwards 1990, Nyffeler et al. 1992). *P. milvina,* was the most common ground dwelling spider both years in both cropping systems. Although immatures cannot be identified to species, it is likely that most of the immature lycosids found in the foliage were *P. milvina*. This spider is known to feed on common soybean pests, including spotted cucumber beetle, three-cornered alfalfa hopper, leafhoppers, and fall armyworms (Howell and

Pienkowski 1971, Royaute and Pruitt 2015). With the decrease in species richness in 2015 pitfall trap samples, *P. milvina* made up 90% of the community. This indicates that factors decreasing many species densities had less of an effect on *P. milvina* in full season soybean. The resilience of this dominant spider is encouraging, since spiders such as *P. milvina* that forage on the plant are considered the most efficient sort of spider for capturing pests (Mahoney et al. 2003).

Spiders can reduce pest populations in agroecosystems and have been used as biological control agents in various crops (Mahoney et al. 2003). Biological control using generalist predators works best when conservation focuses on species that complement each other and discourages species that primarily feed on other natural enemies. Future research can investigate how the dominant spider in Virginia soybean, *P. milvina*, interacts with other natural enemies to maximize their utility as a biological control agent.

References

Anderson, A. C. and K. V. Yeargan. 1998. Influence of soybean canopy closure on predator abundances and predation on *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs. Environmental Entomology 27: 1488-1495.

Birkhofer, K., E. Gavish-Regev, K. Endlweber, Y. D. Lubin, K. von Berg, D. H. Wise, and S. Scheu. 2008. Cursorial spiders retard initial aphid population growth at low densities in winter wheat. Bulletin of Entomological Research 98: 249-255.

Colwell, R. K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. Persistent URL <purl.oclc.org/estimates>.

Culin, J. D. and K. V. Yeargan. 1983a. Comparative study of spider communities in alfalfa and soybean ecosystems - foliage dwelling spiders. Annals of the Entomological Society of America 76: 825-831.

Culin, J. D. and K. V. Yeargan. 1983b. Comparative study of spider communities in alfalfa and soybean ecosystems - ground-surface spiders. Annals of the Entomological Society of America 76: 832-838.

Edwards, C. A., K. D. Sunderland, and K. S. George. 1979. Studies on polyphagous predators of cereal aphids. Journal of Applied Ecology 16: 811-823.

Fagan, W. F., A. L. Hakim, H. Ariawan and S. Yuliyantiningsih. 1998. Interactions between biological control efforts and insecticide applications in tropical rice agroecosystems: The potential role of intraguild predation. Biological Control 13: 121-126.

Ferguson, H. J., R. M. McPherson and W. A. Allen. 1984. Ground-dwelling and foliage-dwelling spiders in 4 soybean cropping systems. Environmental Entomology 13: 975-980.

Fox, A. F., D. B. Orr and Y. J. Cardoza. 2015. the influence of habitat manipulations on beneficial ground-dwelling arthropods in a southeast UW organic cropping system. Environmental Entomology 44: 114-121.

Gurr, G., S. D. Wratten, W. E. Snyder and D. M. Y. Read. 2012. Biodiversity and pests: key issues for sustainable management. Chichester, West Sussex, UK; Hoboken, NJ, John Wiley & Sons.

Herbert, A., C. Hull and E. Day. 2003. Corn earworm biology and management in soybeans. Virginia Cooperative Extension publication 444-770.

Holshouser, D. 2014. Double cropping soybeans in Virginia. Virginia Cooperative Extension publication CSES - 102NP.

Horvath, R., T. Magura, C. Szinetar, J. Eichardt, E. Kovacs and B. Tothmeresz. 2015. In stable, unmanaged grasslands local factors are more important than landscape-level factors in shaping spider assemblages. Agriculture Ecosystems & Environment 208: 106-113.

Howell, J. O. and R. I. Pienkowski. 1971. Spider populations in alfalfa, with notes on spider prey and effect of harvest. Journal of Economic Entomology 64: 163-&.

Kajak, A. 1978. Analysis of consumption by spiders under laboratory and field conditions. Ekologia Polska-Polish Journal of Ecology 26: 411-428.

Kogan, M. and S. G. Turnipseed. 1987. Ecology and management of soybean arthropods. Annual Review of Entomology 32: 507-538.

Laub, C. A. and J. M. Luna. 1992. Winter cover crop suppression practices and natural enemies of armyworm (Lepidoptera, Noctuidae) in no-till corn. Environmental Entomology 21: 41-49.

LeSar, C. D. and J. D. Unzicker. 1978. Soybean spiders: species composition, population densities and vertical distribution. Biological notes 107.

Letourneau, D. K., J. A. Jedlicka, S. G. Bothwell and C. R. Moreno. 2009. Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annual Review of Ecology Evolution and Systematics. Palo Alto, Annual Reviews. 40: 573-592.

Maloney, D., F. A. Drummond and R. Alford. 2003. Spider predation in agroecosystems: Can spiders effectively control pest populations. Maine Agricultural and Forest Experiment Station, The University of Maine Technical Bulletin 190.

Marc, P. and A. Canard. 1997. Maintaining spider biodiversity in agroecosystems as a tool in pest control. Agriculture Ecosystems & Environment 62: 229-235.

Marc, P., A. Canard and F. Ysnel. 1999. Spiders (Araneae) useful for pest limitation and bioindication. Agriculture Ecosystems & Environment 74: 229-273.

Marshall, S. D. and A. L. Rypstra. 1999. Patterns in the distribution of two wolf spiders (Araneae : Lycosidae) in two soybean agroecosytems. Environmental Entomology 28: 1052-1059.

MATLAB. 2012. The Mathworks, Inc., Natick, Massachusetts, U.S.

McPherson, R. M., J. C. Smith and W. A. Allen. 1982. Incidence of arthropod predators in different soybean cropping systems. Environmental Entomology 11: 685-689.

Nyffeler, M., D. A. Dean and W. L. Sterling. 1992. Diets, feeding specialization, and predatory role of 2 lynx spiders, *Oxyopes salticus* and *Peucetia viridans* (Araneae, Oxyopidae) in a Texas cotton agroecosystem. Environmental Entomology 21: 1457-1465.

Nyffeler, M. and K. D. Sunderland. 2003. Composition, abundance and pest control potential of spider communities in agroecosystems: a comparison of European and US studies. Agriculture Ecosystems & Environment 95: 579-612.

Platnick, N. I. 2016. The world spider catalog, version 15. http://research.amnh.org/iz/spiders/catalog/.

Post, W. M. and C. C. Travis. 1979. Quantitative stability in models of ecological communities. Journal of Theoretical Biology 79: 547-553.

Rendon, D., M. E. A. Whitehouse, N.R. Hulugalle and P.W. Taylor. 2015. Influence of crop management and environmental factors on wolf spider assemblages (Araneae: Lycosidae) in an Australian cotton cropping system. Environmental Entomology 44: 174-185.

Riechert, S. E., L. Provencher and K. Lawrence. 1999. The potential of spiders to exhibit stable equilibrium point control of prey: Tests of two criteria. Ecological Applications 9: 365-377.

Royaute, R. and J. N. Pruitt. 2015. Varying predator personalities generates contrasting prey communities in an agroecosystem. Ecology 96: 2902-2911.

Rypstra, A. L., P. E. Carter, R. A. Balfour and S. D. Marshall. 1999. Architectural features of agricultural habitats and their impact on the spider inhabitants. Journal of Arachnology 27: 371-377.

SAS Institute Inc. 2009. SAS 9.2 reference, 2nd ed. SAS Institute, Cary, NC.SAS Institute Inc. (2010) Using JMP 9. SAS Institute, Cary, NC.

Schmidt, M. H., I. Roschewitz, C. Thies and T. Tscharntke. 2005. Differential effects of landscape and management on diversity and density of ground-dwelling farmland spiders. Journal of Applied Ecology 42: 281-287.

Shannon, C. E. and W. Weaver. 1949. The mathematical theory of communication, University of Illinois Press, Urbana.

Simpson, E. H. 1949. Measurement of diversity. Nature 163: 688.

Sprenkel, R. K., W. M. Brooks, J. W. Vanduyn and L. L. Deitz. 1979. Effects of 3 cultural variables on the incidence of *Nomuraea rileyi*, phytophagous Lepidoptera, and their predators on soybeans. Environmental Entomology 8: 334-339.

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

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

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

CHAPTER FOUR

Influence of cropping system on foliar and ground-dwelling natural enemy assemblages in soybean

Abstract

Natural enemies in soybean help keep potential pests under economic threshold. Natural enemy abundance and biodiversity can influence the effectiveness of a pest management strategy. Details on how soybean cropping systems affect natural enemy species identity and seasonal occurrence can help determine which natural enemy to target for conservation or enhancement. We surveyed plots in full season and double crop soybean in 2014 and 2015 to assess the abundance and composition of natural enemies in Virginia soybean and how cropping system can alter this community. We collected natural enemies using sweep nets and pitfall traps from late July through early October. Over 9,800 natural enemies were captured and identified. Double crop soybean had a higher abundance of natural enemies in both years, though this was only significant in 2014. Full season soybean had higher diversity in 2014. The two systems had different dominant natural enemies. In both years of the study *Geocoris punctipes* (Say) was a dominant predator in full season soybean, but had low densities in double crop soybean. *Orius insidiosus* (Say) was a dominant predator in both systems in both years, with significantly higher populations in double crop soybean in 2014. The ground-dwelling natural enemy community consisted mostly of spiders, Carabidae beetles and Staphylinidae beetles in both years and in both systems. The two systems had different species as the dominant Carabidae. The natural enemy community was much smaller in 2015 in both systems, possibly due to heavy rainfall early in the season. Generally, the two systems had different dominant natural enemies on the ground and in the foliage; future conservation efforts should consider cropping system when planning which species or families to focus on for possible biological control.

4.1 Introduction

Natural enemies in soybean are of prime importance for preventing herbivores from attaining pest status in fields (Turnipseed and Kogan 1980, Anderson and Yeargan 1998). Natural enemies such as spiders and ground beetles specifically can reduce pest populations and maintain pests at low densities (Kajak 1978, Post and Travis 1979, Symondson et al. 2002). Information on natural enemy biodiversity can be helpful when developing a management plan for a given crop in a given area. First, density information can indicate potential natural enemies to target for conservation. Second, species identity can be used to research whether a given dominant predator is known to eat and have a preference for a dominant pest in the area or crop. Third, a diverse natural enemy community is associated with higher yield and bigger plants in many crops (Gurr et al. 2012). If a community is found to have low biodiversity steps can be taken to improve species richness and evenness.

Natural enemies that are generalist predators have the ability to occupy a field prior to significant pest populations since they can subsist on non-pest prey items including detritivores. The ability to establish populations that can start growing soon after planting could mean higher predator populations on earlier planted crops.

Among the most abundant natural enemies reported in soybean are Nabidae, *Geocoris* spp., *Orius insidiosus* (Say) and spiders (Anderson and Yeargan 1998). Some research on natural enemies in the U.S. Mid-Atlantic States has shown a difference in abundance between early and late planted soybean (Ferguson et al. 1984) while some have not (Anderson and Yeargan 1998). Natural enemy density in soybean can be affected by tillage and pesticide usage (Witmer et al. 2003). The study of how different cropping systems affect this community can help when determining factors such as insecticide application, cultivation or other production practices.

One of the most serious pests of soybean in Virginia is corn earworm (*Helicoverpa zea* (Boddie)), which moves from corn to soybean in their third generation (Kogan 1987, Swenson et al. 2013). Cultural controls have been used in soybean to control corn earworm and up-to-date information on the natural enemy community in early and late planted soybean can ensure these controls are effective in preventing crop losses (Herbert 2003). Natural enemies can be extremely important to keep corn earworm populations in check when moth activity peaks in early August, since they can eat up to 75% of corn earworm eggs present in fields (Anderson and Yeargan 1998)

Our research was conducted in 2014 and 2015 in two soybean cropping systems: full season and double crop. Both are popular cropping systems in Virginia. The main difference in the systems is the planting date. Full season soybean in Virginia is normally planted in May to June while double crop soybean is planted in June or July after the previous crop such as wheat or barely has been harvested. This study examines the effect of the two cropping systems on foliar and ground-dwelling natural enemy community in soybean.

4.2 Materials and Methods

4.2.1 Study sites

Research was conducted from July through October of 2014 and 2015 in several soybean fields in southeastern Virginia. Experimental plots were located in both full season and double crop planted fields at the Tidewater Agricultural Research and Extension Center in Suffolk, VA (36.68N, 76.76W.), and in producers' fields in Sussex County, VA (36.84N, 77.29W). The two cropping systems each had six replicates in 2014 and four replicates in 2015. Plots were 0.12 ha in area and were inset 25 m from the field edge (Table 4.1). Standard weed management practices were used. Plots were located in fields bordered by woods, soybean, road, dirt path and/or grass.

Table 4.1. Description of experimental plot location, cropping system, row spacing, soybean variety and planting date, southeastern Virginia, 2014 and 2015.

Year	Location	Field	Cropping system	Row spacing	Variety	Planting date
2014	Sussex County	FS1	Full season	17.78 cm	Liberty Link$^{\Delta}$	29-Apr-14
	Sussex County	FS2	Full season	17.78 cm	Liberty Link$^{\Delta}$	30-Apr-14
	Sussex County	FS3	Full season	17.78 cm	Liberty Link$^{\Delta}$	30-Apr-14
	Sussex County	FS4	Full season	17.78 cm	Liberty Link$^{\Delta}$	30-Apr-14
	Sussex County	FS5	Full season	17.78 cm	Liberty Link$^{\Delta}$	1-May-14
	Sussex County	FS6	Full season	17.78 cm	Liberty Link$^{\Delta}$	1-May-14
	Suffolk County	DC7	Double crop	17.78 cm	58-12 R2$^{\Psi}$	30-Jun-14
	Suffolk County	DC8	Double crop	17.78 cm	58-12 R2$^{\Psi}$	1-Jul-14
	Suffolk County	DC9	Double crop	17.78 cm	58-12 R2$^{\Psi}$	1-Jul-14
	Suffolk County	DC10	Double crop	17.78 cm	58-12 R2$^{\Psi}$	2-Jul-14
	Suffolk County	DC11	Double crop	17.78 cm	58-12 R2$^{\Psi}$	2-Jul-14
	Suffolk County	DC12	Double crop	17.78 cm	58-12 R2$^{\Psi}$	2-Jul-14
2015	Suffolk County	FS13	Full season	91.44 cm	58-12 R2$^{\Psi}$	13-May-15
	Suffolk County	FS14	Full season	91.44 cm	58-12 R2$^{\Psi}$	13-May-15
	Suffolk County	FS15	Full season	91.44 cm	58-12 R2$^{\Psi}$	13-May-15
	Suffolk County	FS16	Full season	91.44 cm	58-12 R2$^{\Psi}$	13-May-15
	Suffolk County	DC17	Double crop	19.05 cm	S56-G6Δ	16-Jun-15
	Suffolk County	DC18	Double crop	19.05 cm	S56-G6Δ	16-Jun-15
	Suffolk County	DC19	Double crop	91.44 cm	SS5911N R2$^{\Omega}$	25-Jun-15
	Suffolk County	DC20	Double crop	91.44 cm	AG5732$^{\Sigma}$	25-Jun-15

Δ Syngenta
Ψ Hubner
Ω Genuity
Σ Asgrow

4.2.2 Sweep net sampling

Sweep nets were used to sample natural enemies in the plant canopy, 29 July 2014 to 2 October 2014, and 5 August 2015 to 30 September 2015. Sampling was conducted at 2-week intervals with six samples in 2014 and five in 2015. Five sweep net samples consisting of 15 pendulum sweeps with a 38-cm diameter sweep net were taken in each plot on each sampling date. Samples were placed into plastic bags, transferred to a cooler and transported to the laboratory for enumeration and identification. Natural enemies from the five sweep net samples for each plot were combined for analysis.

4.2.3 Pitfall trapping

To sample ground-dwelling natural enemies, five pitfall traps were placed into each plot from 29 July 2014 to 2 October 2014, and 5 August 2015 to 30 September 2015. Traps were inset at least 7 m from the plot edge and were placed randomly throughout the plot. Traps consisted of a 473-ml plastic cup 11.5-cm in diameter, filled with 120 ml of propylene glycol and 60 ml of water. The cup was set inside an identical 473-ml plastic cup with holes in the bottom for drainage. To protect traps from precipitation, a 30-cm plastic plate was suspended 5 cm above each trap and held in place by metal flags. Traps were collected one week after placement and propylene glycol was replaced. Samples were returned to the laboratory and specimens were rinsed, enumerated, and prepared for identification. Natural enemies from the five pitfall traps in each plot on each date were combined in the analysis.

4.2.4 Identification

Insect natural enemies collected were identified to family or species under a dissecting microscope. Araneae and Opiliones were identified to order and Chilopoda were identified to class. All Carabidae were identified to species since almost all members of this family are predatory or omnivorous and could be used as a proxy to look at species diversity. Species-level identification of Carabidae allowed for diversity indexes to be calculated and compared, which was important to this research. Carabidae were identified to species using Cieglar (2000) and the reference collection at the Virginia Natural History Museum, Martinsville, VA. Voucher specimens have been placed in the Virginia Museum of Insects at Virginia Tech, Blacksburg, VA.

4.2.5 Analysis

Natural enemy communities in cropping systems were compared by examining abundance, diversity and similarity coefficients. Abundance was calculated as the total number of individual natural enemies collected in each full season or double crop plot on each sampling date. Carabidae biodiversity was estimated using the Shannon Index (H'), which gives equal weight to rare and abundant species, as well as the Simpson Index (D), which is more sensitive to change in common species. The Shannon Index is calculated as follows: $H' = -\Sigma p_i \times \ln p_i$, where p_i is the proportion of individuals found in the ith species (Shannon and Weaver 1949). The Simpson Index is calculated as follows: $D = \Sigma n_i(n_i - 1)/N(N-1)$, where n is the number of individuals of the ith species, and N the total number of individuals (Simpson 1949). The values for the Simpson Index were modified (1/D), so that the increase in the index reflects an increase in diversity. Diversity indices were calculated for each plot using season-long total number of individual Carabidae species.

Carabidae communities were compared using the Jaccard similarity coefficient, which is used to determine the similarity of communities in each plot. The Jaccard similarity coefficient deals with presence/absence data and is calculated as follows: $Sj = a/a + b + c$, where a is the number of species shared between two plots, b is the number of species in the second but not the first plot, and c is the number of species in the first but not the second plot. The coefficient ranges from 0 for complete dissimilarity to 1 for total similarity between two plots. A matrix of similarity coefficients was calculated for each sampling method and year. An average clustering technique was used to detect simple divisions of the data sets into a small number of distinct groups. Dendrograms were constructed for each year and sampling method to illustrate species shared between plots based on hierarchical clustering of Jaccard index values. The program MATLAB (MATLAB 2012) was used to construct dendrograms showing clustering patterns. The biodiversity and Jaccard indices were calculated using EstimateS (Colwell 2013).

Species accumulation curves were generated to assess Carabidae species richness. These curves show the rate at which added species are encountered as more individuals are collected. Species accumulation curves were constructed using EstimateS (Colwell 2013).

A repeated-measures analysis of variance (ANOVA) with cropping system as between-subjects factor was used to test whether natural enemy abundance differed between full season and double crop soybean plots across the growing season. A first-order autoregressive was used for covariance structure. Data were log(n+1)-transformed for statistical analysis in SAS (SAS Institute 2009). An ANOVA was used to analyze the effect of cropping system on the Shannon Index and the Simpson Index using PROC MIXED (SAS Institute 2009). For both analyses means were separated using F-protected least squares means test at $\alpha = 0.05$.

4.3 Results

4.3.1 Natural enemy assemblage

A total of 9,843 arthropods were captured and identified in this experiment. Overall, arthropods from 41 species, 28 genera, 18 families, seven orders and three classes were identified (Table 4.2).

Table 4.2. Genera and species of predatory arthropods identified from sweep net and pitfall trap samples taken in 20 soybean fields in southeastern Virginia in 2014-2015.

Insecta						Coccinellidae	
	Coleoptera						*Coleomegilla maculata* (Timberlake)
		Anthicidae					*Harmonia axyridis* (Pallas)
		Carabidae					*Hippodamia convergens* (Guerin)
			Agonum octopunctatum (Fabricius)			Lampryidae	
			Agonum pallipes (Fabricius)			Melyridae	
			Agonum punctiforme (Say)			Mordellidae	
			Amblygnathus mexicanus (Bates)			Staphylinidae	
			Anisodactylus merula (Germar)		Hemiptera		
			Anisodactylus sanctaecrucis (Fabricius)			Anthocoridae	
			Calosoma sayi (Dejean)				*Orius insidiosus* (Say)
			Calosoma externum (Say)			Geocoridae	
			Calathus opaculus (LeConte)				*Geocoris punctipes* (Say)
			Carabus vinctus (Weber)				*Geocoris uliginosus* (Say)
			Chlaenius laticollis (Say)			Nabidae	
			Chlaenius tomentosus (Say)			Pentatomidae	
			Chlaenius tricolor (Dejean)				*Podisus maculiventris* (Say)
			Cicindela punctulata (Olivier)			Reduviidae	
			Cyclotrachelus sigillatus (Say)		Diptera		
			Dicaelus ambiguus (Laferte-Senectere)			Dolichopodidae	
			Dicaelus elongatus (Bonelli)			Syrphidae	
			Diplocheila obtusa (Leconte)		Neuroptera		
			Galerita bicolor (Drury)			Hemerobiidae	
			Harpalus gravis (Leconte)			Chrysopidae	
			Harpalus katiae (Battoni)		Hymenoptera		
			Harpalus pensylvanicus (DeGeer)			Braconidae	
			Lebia grandis (Hentz)			Ichneumonidae	
			Lebia spp.	Arachnida			
			Paraclivina bipustulata (Fabricius)		Araneae		
			Poecilus chalcites (Say)		Opiliones		
			Pterostichus sculptus (LeConte)	Chilopoda			
			Scarites subterraneus (Fabricius)				
			Selenophorus opalinus (LeConte)				
			Selenophorus palliatus (Fabricius)				
			Stenocrepis mexicana (Chevrolat)				
			Stenolophus conjunctus (Say)				
			Stenolophus ochropezus (Say)				
			Tetracha carolina (Linnaeus)				
			Tetracha virginica (Linnaeus)				

In 2014, spiders (37%), *Orius insidiosus* (Say) (24%) , *Geocoris punctipes* (Say) (16%) and Dolichopodidae (7%) made up 84% of 3,218 specimens caught by sweep net, and spiders (61%), Staphylinidae (11%), *Cicindella punctulata* (Olivier) (5%) and *Pterostichus sculptus* (LeConte) (5%) made up 82% of 3,540 specimens caught by pitfall trap.

In 2015, spiders (32%), *O. insidiosus* (35%), *Hippodamia convergens* (Guerin) (7%) and Dolichopodidae (7%) made up 81% of the 2,140 specimens caught by sweep net, and spiders (72%), Staphylinidae (8%) and *P. sculptus* (8%) made up 88% of 945 specimens caught by pitfall trap.

4.3.2 Effect of cropping system on the natural enemy community

Seven species, eight genera, 16 families, 6 orders and two classes of natural enemy with 1418 individuals and eight species, eight genera, 18 families and 6 orders with 1800 individuals were identified from sweep net samples in full season and double-crop soybean, respectively in 2014. Seven species, seven genera, 16 families, six orders and two classes with 972 individuals and six species, six genera, 16 families, six orders and two classes of natural enemy with 1168 individuals were identified from sweep net samples in full season and double crop soybeans, respectively, in 2015.

Thirty-three species, 23 genera, 12 families, seven orders and three classes of natural enemies with 1273 individuals and 17 species, 14 genera, 10 families, six orders and three classes of natural enemies with 2,267 individuals were identified from pitfall trap samples in full season and double crop soybean, respectively in 2014. Ten species, nine genera, six families, four orders and three classes of natural enemies with 340 individuals and 16 species, 15 genera, six families, five orders and three classes of natural enemies with 605 individuals were identified from pitfall trap samples in full season and double crop soybean, respectively in 2015.

When examining species diversity of Carabidae caught per plot throughout the entire season, during 2014 pitfall trap samples in full season soybean had greater diversity, with a Shannon Index of 1.97 and

Simpson Index of 6.12, compared with 1.38 and 3.2 in double crop soybean (Shannon Index: $F = 6.44$; df = 1, 10; $P = 0.02$; Simpson Index: $F = 9.2$; df = 1,10; $P = 0.01$; Table 4.3). In 2015 pitfall traps, there was no significant difference in either index between the two cropping systems. (Shannon Index: $F = 0.05$; df = 1,6; $P = 0.84$; Simpson Index: $F = 0.03$; df = 1,6; $P = 0.87$).

Table 4.3. Diversity (mean+-SE) of Carabidae per plot in double crop and full season soybean caught in pitfall traps throughout the soybean growing season in southeastern Virginia in 2014-15. Each index was calculated using the total species counts over the growing season from each plot. There were six double crop plots and six full season plots in 2014 and four double crop plots and four full season plots in 2015.

Year	Diversity index	Full season	Double crop	F-ratio	DF	P-value
2014	Shannon Index	1.973 ± 0.157	1.388 ± 0.169	6.44	1,10	0.03
	Simpson Index	6.118 ± 0.861	3.203 ± 0.427	9.2	1,10	0.013
2015	Shannon Index	1.108 ± 0.184	1.218 ± 0.299	0.05	1,6	0.837
	Simpson Index	2.948 ± 0.504	2.963 ± 0.856	0.03	1,6	0.869

Individual species and families responded differently to cropping system in sweep net samples. Total natural enemies and spiders were more abundant in double crop soybean in 2014 (treatment × day interaction, total natural enemies: $F = 2.94$; df = 5,50; $P = 0.02$; treatment × day interaction, Araneae: $F = 19.18$; df = 5,50; $P < 0.0001$; Figs. 4.1-4.3, Table 4.4). *G. punctipes* was significantly more abundant in full season soybeans in 2014 and 2015 (treatment × day interaction, 2014: $F = 14.18$; df = 5,0; $P < 0.0001$; treatment, 2015: $F = 21.93$; df = 1,4; $P = 0.01$; Figs. 4.5 and 4.6). *O. insidiosus*, Braconidae, Dolichopodidae and *H. convergens* were significantly more abundant in double crop soybean in 2014 (treatment × day interactions: *O. insidiosus*: $F = 18.36$; df = 5,50; $P < 0.0001$; Fig. 4.7; Braconidae: $F = 9.7$; df = 5,50; $P < 0.0001$; Dolichopodidae: F = 8.84; df = 5, 50; $P < 0.0001$; *H. convergens*: $F = 6.12$; df = 5,50; $P < 0.0001$). Nabidae was more abundant in full season soybean in 2014 (treatment × day interaction, $F = 7.6$; df = 5,50; $P < 0.0001$; Fig. 4.8).

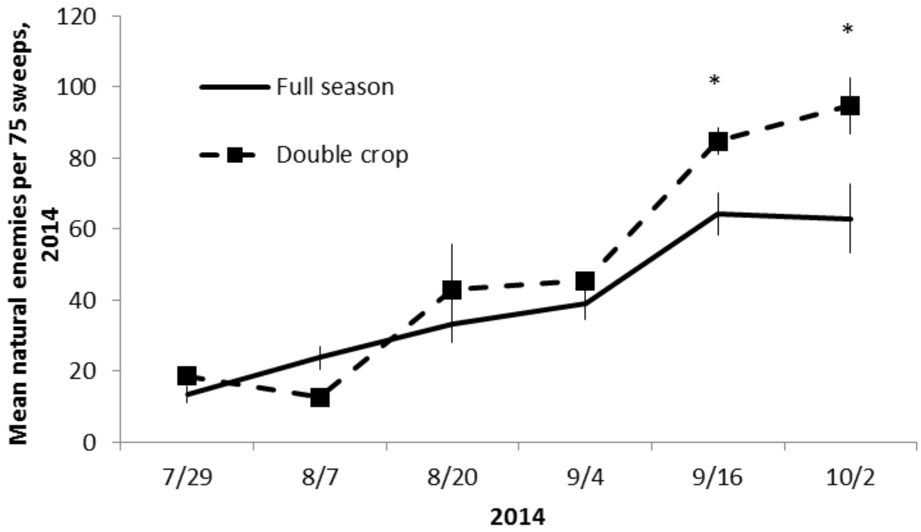


Figure 4.1. Mean natural enemies (± SE; *, P < 0.05) caught per 75 sweeps with a sweep net from six double crop and six full season fields in southeastern Virginia in 2014. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

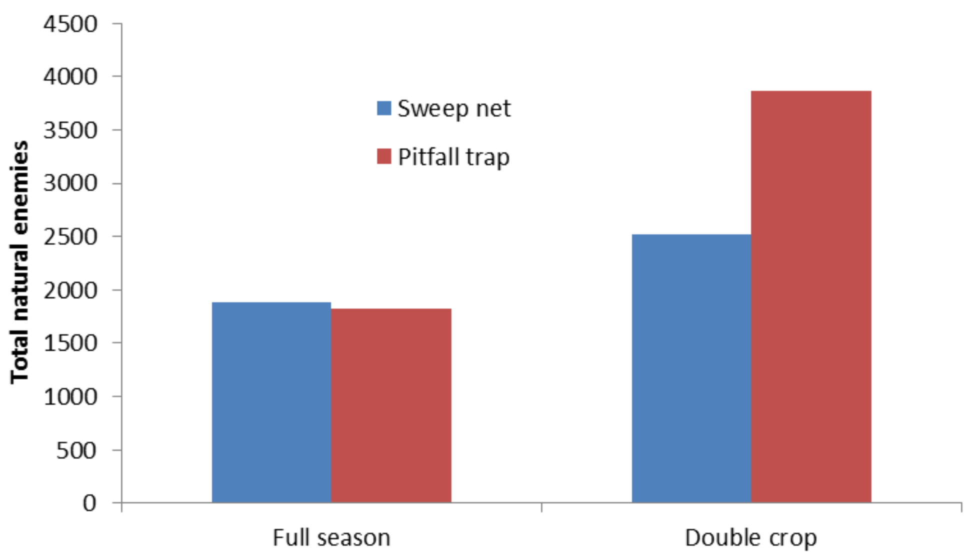


Figure 4.2. Total natural enemies caught with a sweep net and pitfall traps in six full season and six double crop soybean fields in southeastern Virginia on six sampling dates in 2014. Sweep net samples consisted of 75 sweeps. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

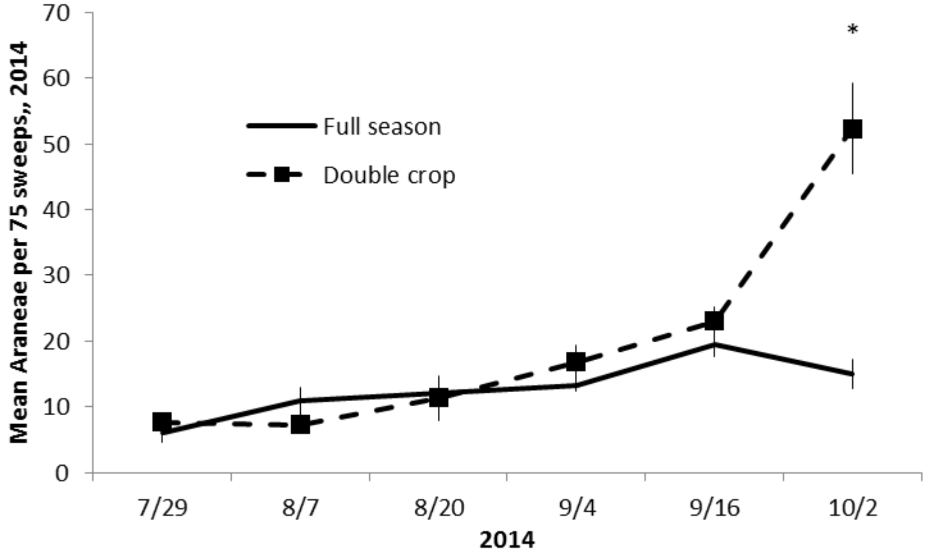


Figure 4.3. Mean Araneae (± SE; *, P < 0.05) caught per 75 sweeps with a sweep net from six double crop and six full season fields in southeastern Virginia in 2014. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

Table 4.4. Mean number of common natural enemies per plot caught with a sweep net in full season and double crop soybeans in southeastern Virginia in 2014 and 2015. Mean represents the mean natural enemies caught with 75 sweeps of a sweep net in six full season plots or six double crop plots. Counts were taken biweekly from 29 July 2014 to 2 October 2014, and 5 August 2015 to 30 September 2015.

		Treatment		RM ANOVA F-ratio (p-value)								
		Full season	Double crop	Treatment			Day			Interaction (T x D)		
Orius insidiosus				F-ratio	DF	p-value	F-ratio	DF	p-value	F-ratio	DF	p-value
	2014	1.64 ± 1.02	19.61 ± 7.53	43.39	1,10	<.0001	6.52	5,50	<.0001	18.36	5,50	<.0001
	2015	12.80 ± 6.32	24.35 ± 13.06	0.71	1,4	0.4475	6.84	4,16	0.0021	3.64	4,16	0.0271
Nabidae												
	2014	3.19 ± 1.89	0.36 ± 0.28	35.14	1,10	0.0001	17.97	5,50	<.0001	7.6	5,50	<.0001
	2015	1.10 ± 1.17	0.30 ± 0.46	0.6	1,4	0.4816	2.32	4,16	0.1016	0.78	4,16	0.5547
Geocoris punctipes												
	2014	13.81 ± 5.94	0.22 ± 0.20	211.99	1,10	<.0001	24.3	5,50	<.0001	14.18	5,50	<.0001
	2015	4.65 ± 2.36	0.70 ± 0.59	21.93	1,4	0.0094	5.23	4,16	0.0069	2.93	4,16	0.0538
Geocoris uliginosis												
	2014	0.72 ± 0.66	0.06 ± 0.09	3.97	1,10	0.0744	1.44	5,50	0.2253	2.24	5,50	0.0651
	2015	0.40 ± 0.50	0.80 ± 0.80	2.81	1,4	0.1688	0.84	4,16	0.5203	1.67	4,16	0.2058
Braconidae												
	2014	0.14 ± 0.14	1.03 ± 0.57	33.11	1,10	0.0002	9.55	5,50	<.0001	9.7	5,50	<.0001
	2015	2.15 ± 1.17	3.65 ± 1.86	7	1,4	0.0572	8.37	4,16	0.0008	1.21	4,16	0.3432
Hippodamia convergens												
	2014	0.56 ± 0.53	1.78 ± 1.00	17.76	1,10	0.0018	21.97	5,50	<.0001	6.12	5,50	0.0002
	2015	4.35 ± 3.55	2.65 ± 2.66	4.68	1,4	0.0964	9.22	4,16	0.0005	1.13	4,16	0.3757
Dolichopodidae												
	2014	2.31 ± 1.51	3.64 ± 1.45	3.48	1,10	0.0917	5.3	5,50	0.0006	8.84	5,50	<.0001
	2015	3.25 ± 1.49	3.90 ± 1.88	0.01	1,4	0.944	0.46	4,16	0.763	1.68	4,16	0.2041
Araneae												
	2014	12.83 ± 2.28	19.75 ± 7.20	7.13	1,10	0.0235	25.03	5,50	<.0001	19.18	5,50	<.0001
	2015	15.75 ± 4.99	18.55 ± 5.85	0.35	1,4	0.5878	1.07	4,16	0.4029	4.44	4,16	0.0132
Total natural enemies												
	2014	39.39 ± 9.42	49.75 ± 14.04	6.64	1,10	0.0276	38.79	5,50	<.0001	2.94	5,50	0.0209
	2015	48.60 ± 12.28	58.40 ± 16.84	0.07	1,4	0.8082	1.44	4,16	0.2658	2.15	4,16	0.1212

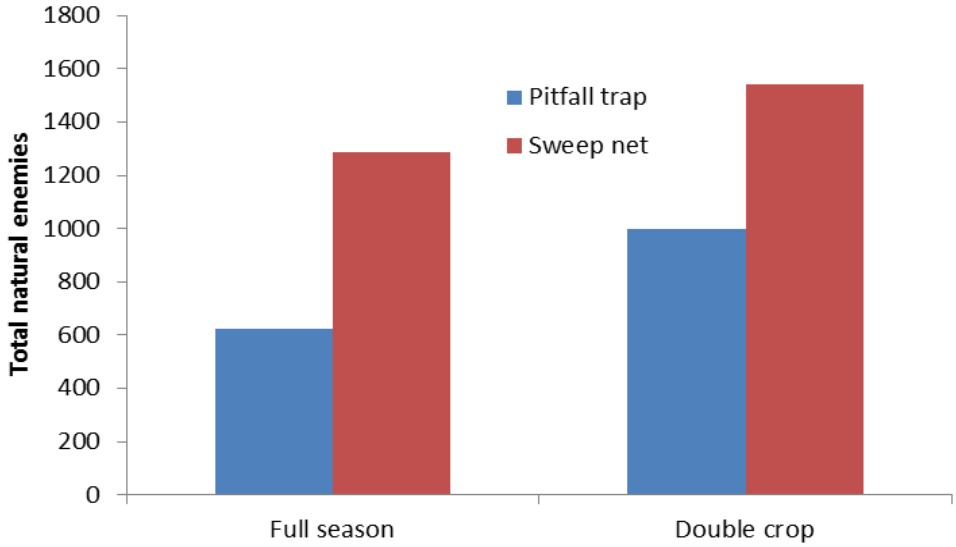


Figure 4.4. Total natural enemies caught with a sweep net and pitfall traps in four full season and four double crop soybean fields in southeastern Virginia on five sampling dates in 2015. Sweep net samples consisted of 75 sweeps. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 5 August 2015 to 30 September 2015.

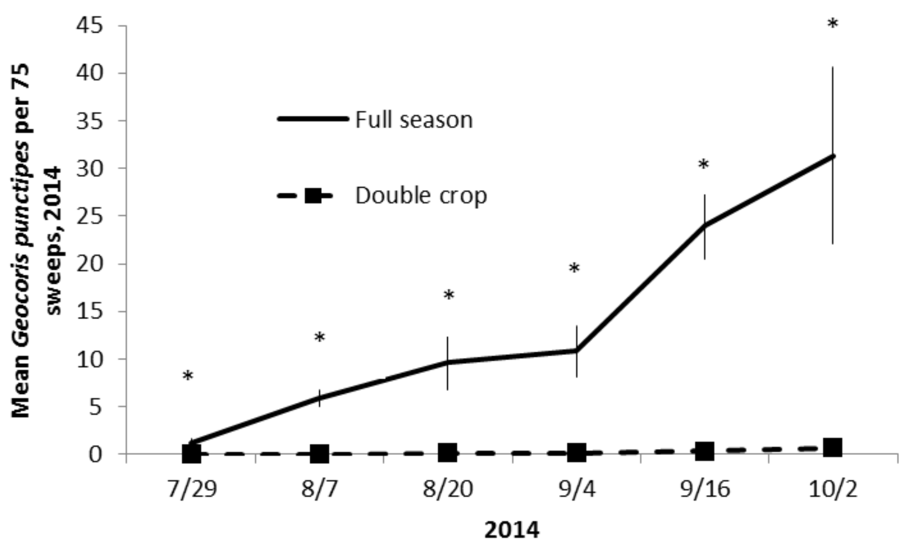


Figure 4.5. Mean *Geocoris punctipes* (± SE; *, P < 0.05) caught per 75 sweeps with a sweep net from six double crop and six full season fields in southeastern Virginia in 2014. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

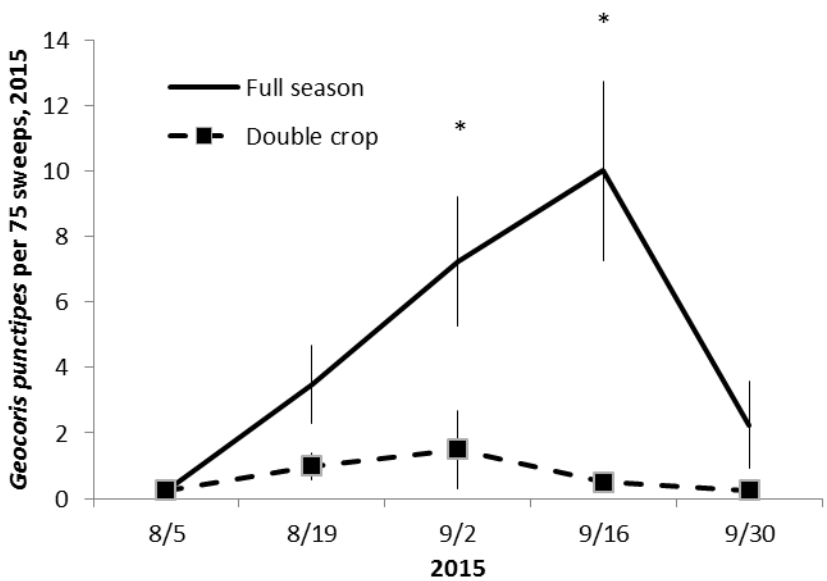


Figure 4.6. Mean *Geocoris punctipes* caught per 75 sweeps with a sweep net from four double crop and four full season fields in southeastern Virginia in 2015. Counts were taken biweekly from 5 August 2015 to 30 September 2015.

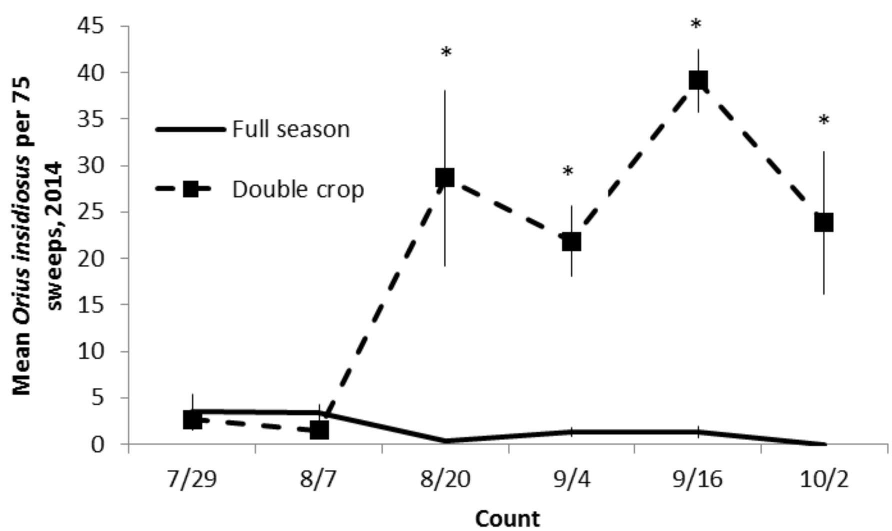


Figure 4.7. Mean *Orius insidiosus* (± SE; *, P < 0.05) caught per 75 sweeps with a sweep net from six double crop and six full season fields in southeastern Virginia in 2014. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

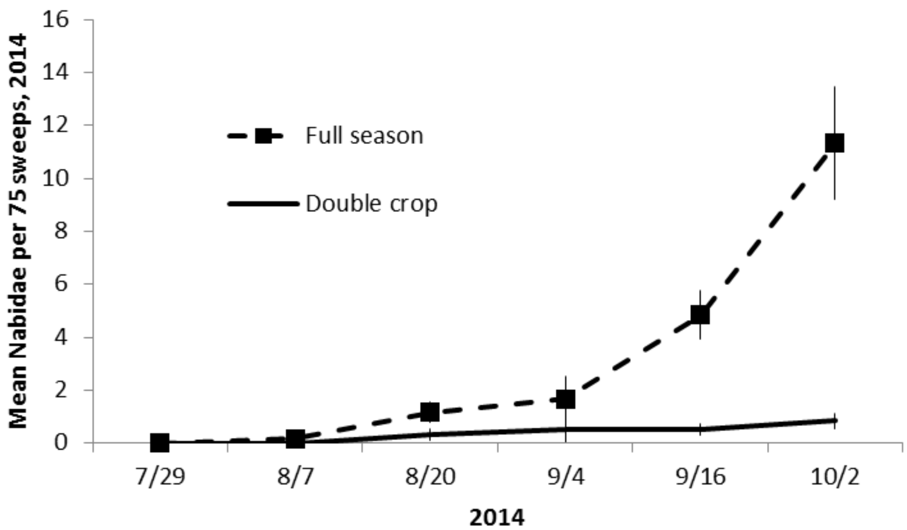


Figure 4.8. Mean Nabidae (± SE; *, P < 0.05) caught per 75 sweeps with a sweep net from six double crop and six full season fields in southeastern Virginia in 2014. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

Generally, the abundance of ground-dwelling natural enemies in total and of individual families and species differed depending on cropping system. In 2014 pitfall trap samples showed there were more natural enemies in double crop soybean than full season soybean (treatment × day interaction, $F = 2.93$; df = 5,50; $P = 0.02$; Figs. 4.2 and 4.9, Table 4.5). The population of the carabid *P. sculptus* was higher in double crop soybean in 2014 and 2015 (treatment × day interaction, 2014: $F = 10.81$; df = 5,50; $P < 0.0001$; 2015: $F = 21.78$; df = 4,16; $P < 0.0001$; Figs. 4.10 and 4.11). Carabid populations were more abundant in double crop soybean in 2015 ($F = 21.34$; df = 1,4; $P = 0.01$; Fig. 4.12) and centipedes were more abundant in double crop soybean in 2014 ($F = 8.95$; df = 1,10; $P = 0.01$). The carabid *C. punctulata* was more abundant in full season soybean in 2014 ($F = 6.75$; df = 1,10; $P = 0.03$; Fig. 4.13).

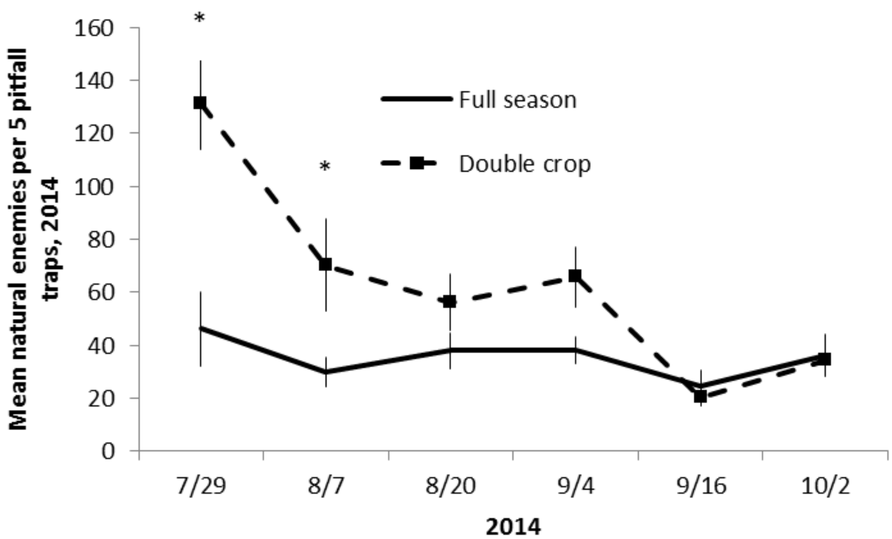


Figure 4.9. Mean natural enemies (± SE; *, P < 0.05) caught per five pitfall traps in six full season and six double crop soybean fields in southeastern Virginia in 2014. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

Table 4.5. Mean number of common natural enemies per plot caught with a pitfall traps in full season and double crop soybeans in southeastern Virginia in 2014 and 2015. Mean represents the mean arthropods in six full season plots or six double crop plots. In each plot there were five pitfall traps, which were combined after collection. Traps were open for seven days. Counts were taken biweekly from 29 July 2014 to 2 October 2014 and 5 August 2015 to 30 September 2015.

		Treatment		RM ANOVA F-ratio (p-value)								
		Full season	Double crop	Treatment			Day			Interaction (T x D)		
Cicindela punctulata				F-ratio	DF	p-value	F-ratio	DF	p-value	F-ratio	DF	p-value
	2014	4.11 ± 3.53	0.44 ± 0.60	6.75	1,10	0.0266	8.75	5,50	<.0001	2.09	5,50	0.0816
	2015	0.00	0.40 ± 1.10	2.67	1,4	0.1778	2.67	4,16	0.0705	2.67	4,16	0.0705
Tetracha carolina												
	2014	0.08 ± 0.11	0.25 ± 0.27	2.04	1,10	0.1839	3.27	5,50	0.0125	0.78	5,50	0.5667
	2015	0.00	0.00	0	0	0	0	0	0	0	0	0
Pterostichus sculptus												
	2014	0.44 ± 0.60	4.08 ± 3.45	15.09	1,10	0.003	31.82	5,50	<.0001	10.81	5,50	<.0001
	2015	0.05 ± 0.11	3.85 ± 8.96	21.78	1,4	0.0095	36	4,16	<.0001	21.78	4,16	<.0001
Harpalus pensylvanicus												
	2014	1.19 ± 0.98	1.67 ± 0.89	0.89	1,10	0.3686	5.92	5,50	0.0002	7.15	5,50	<.0001
	2015	0.65 ± 0.37	1.20 ± 2.44	1.41	1,4	0.3009	18.18	4,16	<.0001	3.29	4,16	0.0379
Calosoma sayi												
	2014	0.06 ± 0.09	0.00	2	1,10	0.1877	0.8	5,50	0.555	0.8	5,50	0.555
	2015	0.2 ± 0.21	0.5 ± 1.40	2.78	1,4	0.1709	4.19	4,16	0.0164	3.73	4,16	0.0251
Staphylinidae												
	2014	3.523 ± 2.07	7.53 ± 4.46	3.5	1,10	0.0908	3.19	5,50	0.014	2.22	5,50	0.0667
	2015	0.80 ± 0.53	2.85 ± 6.43	0.13	1,4	0.7378	3.34	4,16	0.036	9.33	4,16	0.0004
Centipede												
	2014	0.56 ± 0.56	1.97 ± 1.16	8.95	1,10	0.0135	1.87	5,50	0.1166	1.88	5,50	0.1152
	2015	0.10 ± 0.22	0.25 ± 0.64	1.18	1,4	0.339	0.85	4,16	0.5136	1.6	4,16	0.2223
Araneae												
	2014	15.47 ± 3.70	44.53 ± 16.56	2	1,10	0.1877	0.8	5,50	0.555	0.8	5,50	0.555
	2015	14.20 ± 6.95	19.70 ± 18.67	5.12	1,4	0.0864	4.26	4,16	0.0156	2.22	4,16	0.1131
Total natural enemies												
	2014	35.36 ± 8.13	62.97 ± 18.32	11.06	1,10	0.0077	9.02	5,50	<.0001	2.93	5,50	0.0214
	2015	17.00 ± 6.87	30.25 ± 24.05	6.27	1,4	0.0665	2.62	4,16	0.0743	1.78	4,16	0.1827
Total Carabidae												
	2014	12.83 ± 6.12	8.44 ± 3.36	2.33	1,10	0.1581	4.5	5,50	0.0018	2.47	5,50	0.0448
	2015	1.45 ± 0.47	6.95 ± 8.84	21.34	1,4	0.0099	3.78	4,16	0.024	1.18	4,16	0.3551

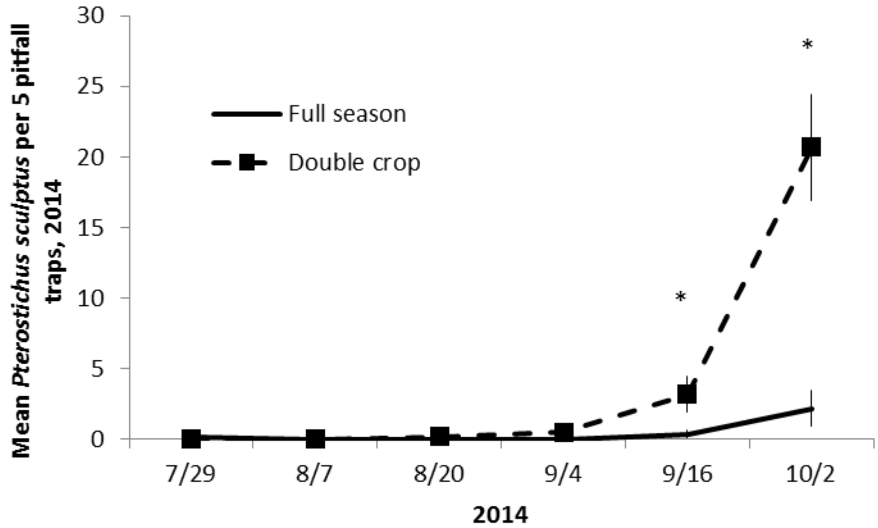


Figure 4.10. Mean *Pterostichus sculptus* (± SE; *, P < 0.05) caught per five pitfall traps from six double crop and six full season fields in southeastern Virginia in 2014. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

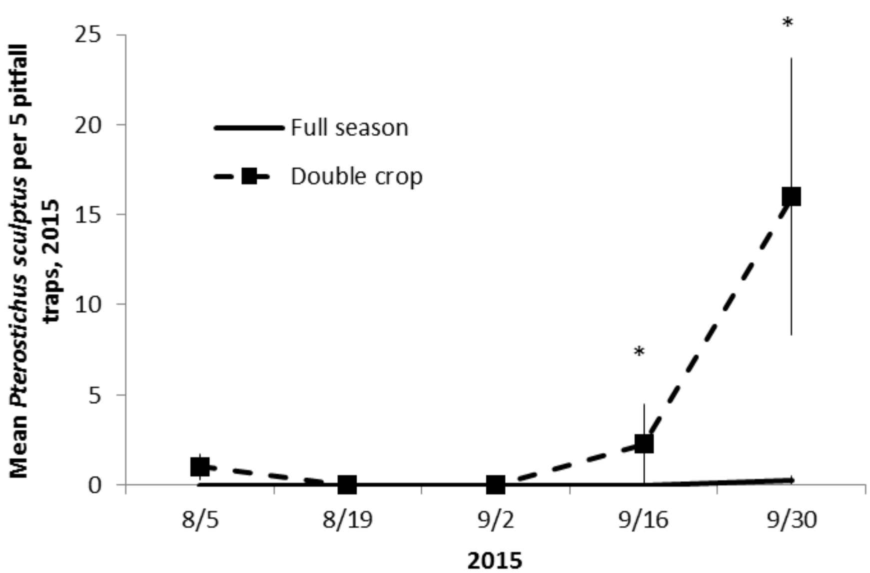


Figure 4.11. Mean *Pterosticus sculptus* (± SE; *, P < 0.05) caught per five pitfall traps from four double crop and four full season fields in southeastern Virginia in 2015. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 5 August 2015 to 30 September 2015.

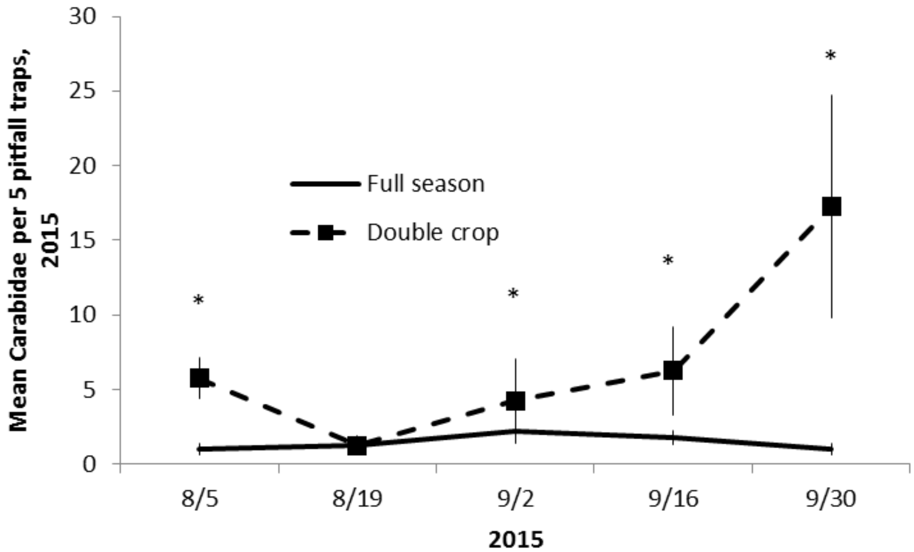


Figure 4.12. Mean Carabidae (± SE; *, P < 0.05) caught per five pitfall traps in four full season and four double crop soybean fields in southeastern Virginia in 2015. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 5 August 2015 to 30 September 2015.

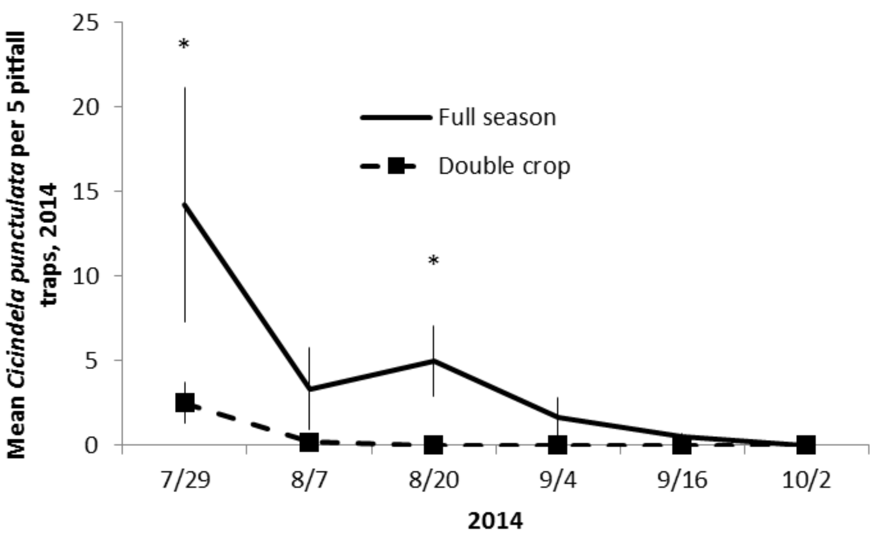


Figure 4.13. Mean *Cicindela punctulata* (± SE; *, P < 0.05) caught per five pitfall traps in six full season and six double crop soybean fields in southeastern Virginia in 2014. Each field held five pitfall traps and traps were open for seven days. Counts were taken biweekly from 29 July 2014 to 2 October 2014.

A total of 1,418 arthropods were identified from full season sweep net samples and 1,800 from double crop sweep net samples in 2014. *G. punctipes* was the dominant taxa in full season soybean (27%) and spiders and *O. insidiosus* were the dominant taxa in double crop soybean (39% and 40%; Figs. 4.14 and 4.15). A total of 972 arthropods were identified from full season sweep net samples and 1,168 from double crop sweep net samples in 2015. Spiders were the dominant taxa in full season soybean (32%) and *O. insidiosus* was the dominant taxa in double crop soybean (42%; Figs. 4.16 and 4.17). A total of 1,273 arthropods were identified from full season pitfall traps and 2,267 from double crop pitfall traps in 2014. Spiders were the dominant taxa collected in both systems (44% and 70% respectively; Figs. 4.18 and 4.19). A total of 340 arthropods were identified from full season pitfall traps and 605 in double crop pitfall traps in 2015. Spiders were the dominant taxa collected in both systems (84% and 65% respectively; Figs. 4.20 and 4.21).

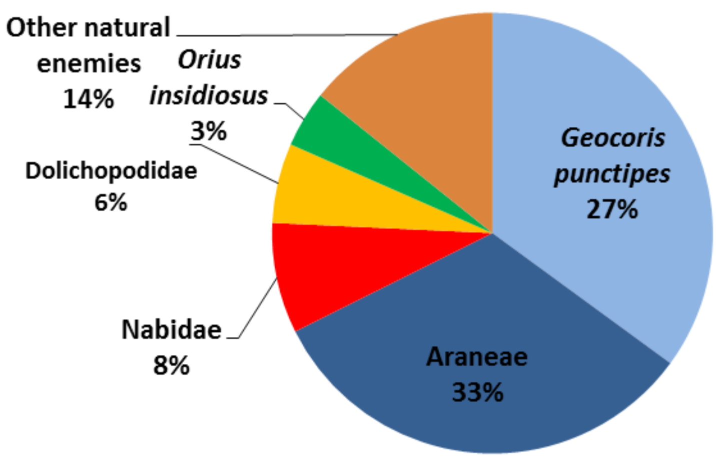


Figure 4.14. The natural enemy diversity in full season soybean caught with a sweep net throughout the growing season in southeastern Virginia in 2014.

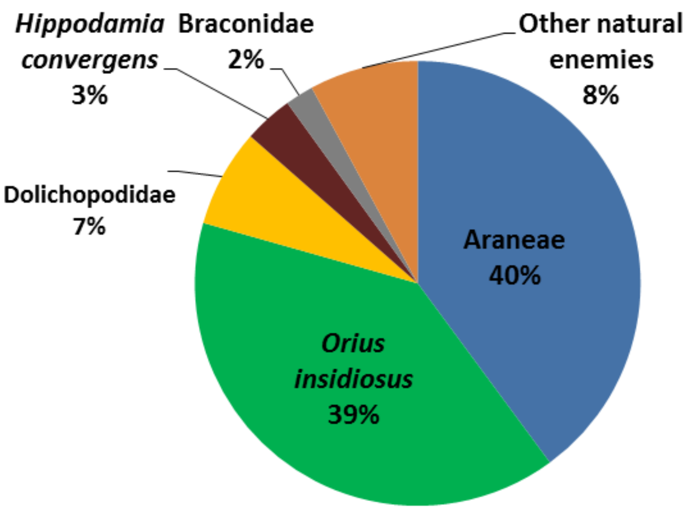


Figure 4.15. The natural enemy diversity in double crop soybean caught with sweep net throughout the growing season in southeastern Virginia in 2014.

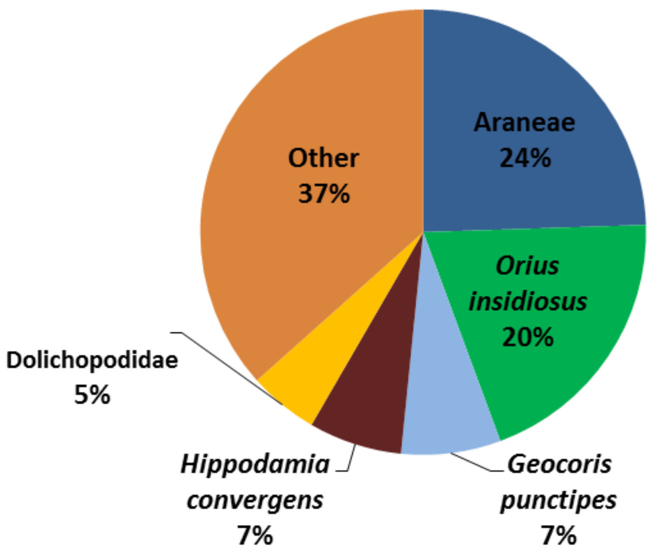


Figure 4.16. The natural enemy diversity in full season soybean caught with a sweep net throughout the growing season on southeastern Virginia in 2015.

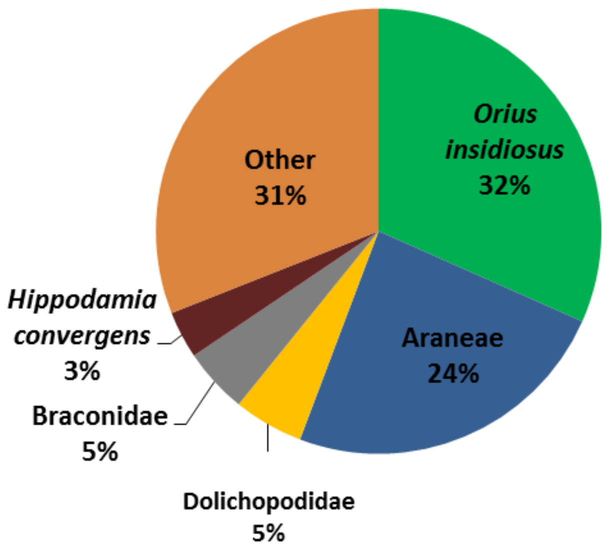


Figure 4.17. The natural enemy diversity in double crop soybean caught with a sweep net throughout the growing season in southeastern Virginia in 2015.

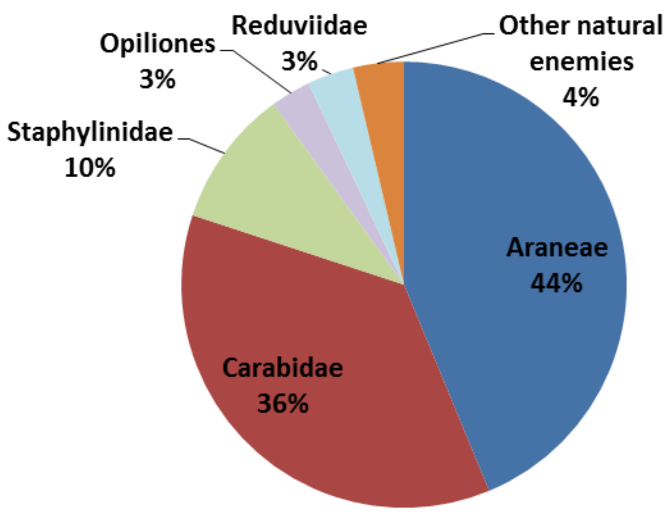


Figure 4.18. The natural enemy diversity in full season soybean caught with pitfall traps throughout the growing season in southeastern Virginia in 2014.

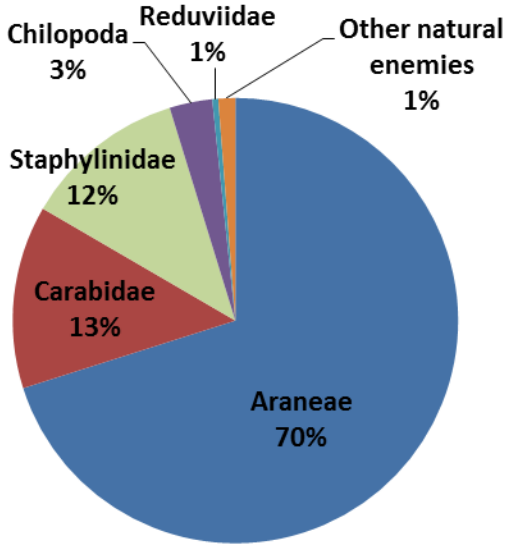


Figure 4.19. The natural enemy diversity in double crop soybean caught with pitfall traps throughout the growing season in southeastern Virginia in 2014.

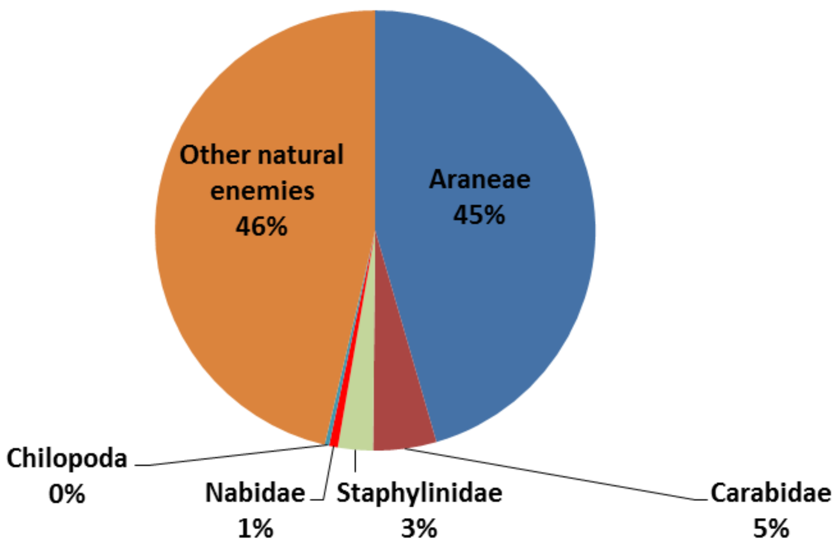


Figure 4.20. The natural enemy diversity in full season soybean caught with pitfall traps throughout the growing season in southeastern Virginia in 2015.

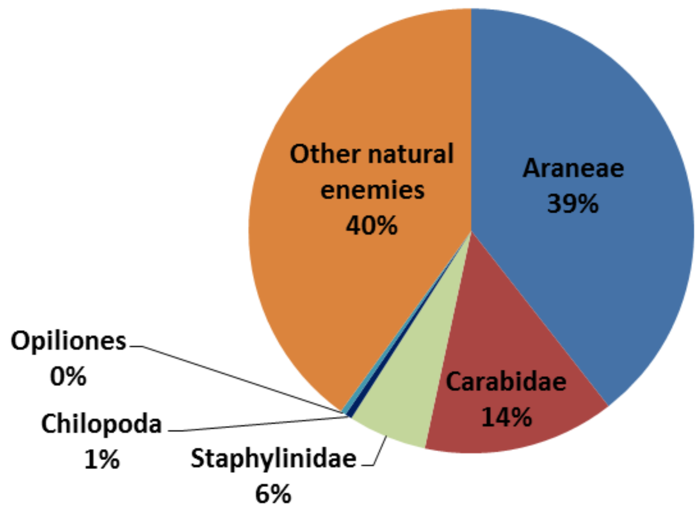


Figure 4.21. The natural enemy diversity in double crop soybean caught with pitfall traps throughout the growing season in southeastern Virginia in 2015.

In 2014 sweep net samples, spiders were the second most abundant taxa in full season soybean (33%), followed by Nabidae (8%), while in double crop, Dolichopodidae (7%) was second and *H. convergens* (4%) was third. In 2015 sweep net samples, *O. insidiosus* was second most abundant taxa in full season soybean (26%) followed by *H. convergens* (9%). In 2015 double crop sweep net samples, spiders were the second most dominant taxa (32%) followed by Dolichopodidae (7%). In 2014 and 2015 the second and third most abundant taxa were Carabidae and Staphylinidae in both full season and double crop soybean pitfall traps.

In 2014 the dominant carabid in full season pitfall traps was *C. punctulata* (32%) followed by *Tetracha. virginica* (Linnaeus) (10%), *Chlaenius tomentosus* (Say), *Harpalus pensylvanicus* (DeGeer) (9% each), and *Agonum punctiform* (Say) (6%). In double crop pitfall traps the dominant carabid was *P. sculptus* (48%) followed by *H. pensylvanicus* (20%), *Chlaenius latticolis* (Say) (12%), and *Tetracha carolina* (Linnaeus) (3%) in 2014. In 2015, the dominant carabid in full season pitfall traps was *H. pensylvanicus* (45%) followed by *Poelcites chalcites* (Say) (17%), *Calosoma sayi* (Dejean) (14%), and *Stenolophus* *mexicanus* (Chevrolat) (10%). In double crop pitfall traps the dominant carabid was *P. sculptus* (55%) followed by *H. pensylvanicus* (17%), *C. sayi* (7%), and *C. punctulata* (6%).

The dendrograms of the Jaccard similarity matrix for carabids in 2014 pitfall trap samples show the highest similarity between two double crop fields (DC9 and DC7 ; Fig. 4.22). The fields are split into two groups, one with four double crop fields and the other with six full season fields and two double crop fields. The double crop fields tend to have greater similarity with each other than with full season fields. In 2015, double crop fields DC17 and DC18 showed the highest amount of similarity, followed by double crop fields DC19 and DC20, then full season fields FS14 and FS15. While these pairings were between fields of the same cropping system, there was not a tendency of cropping system to cluster together as a whole, as a grouping of two double crop fields, DC17 and DC18 and one full season field, FS16, were in a completely separate cluster from the other fields.

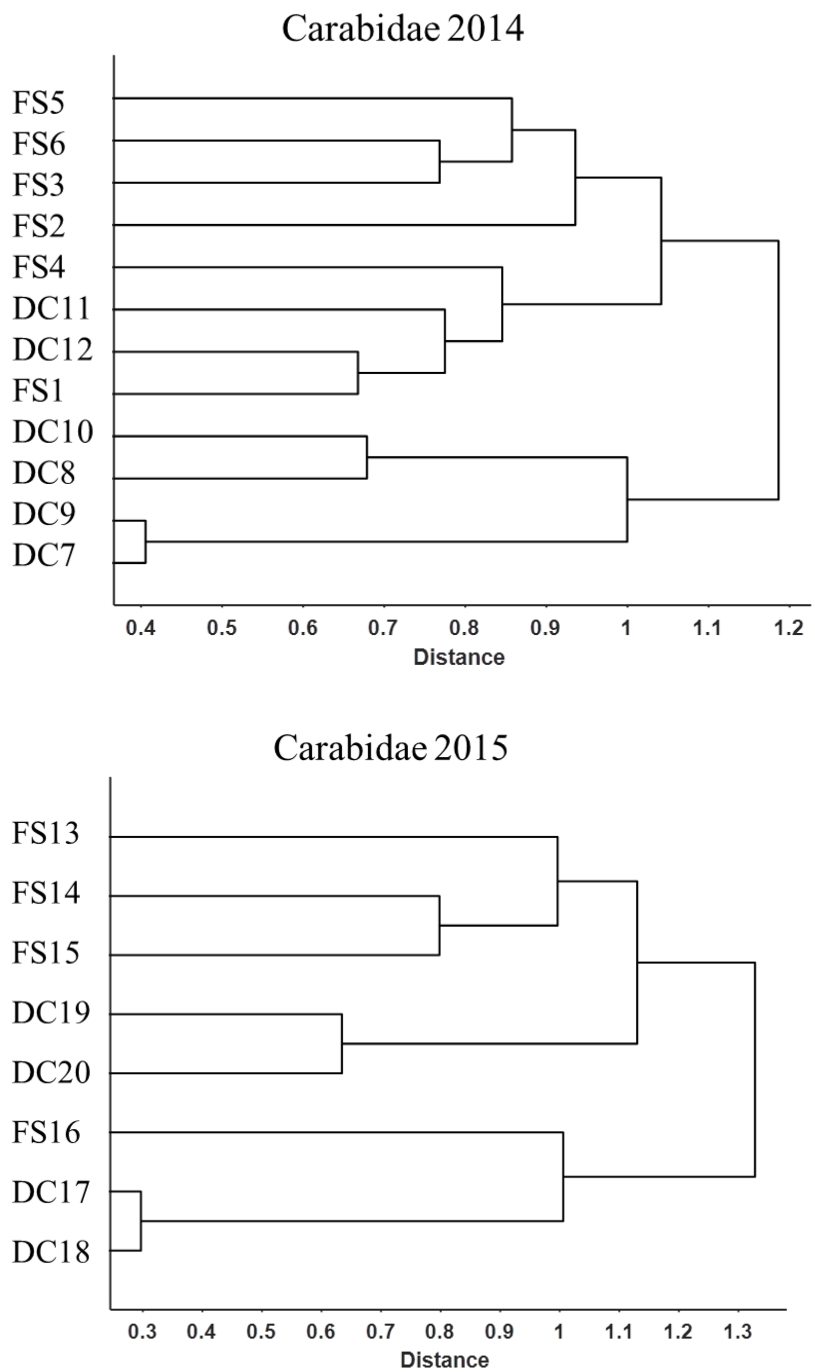


Figure 4.22. Cluster analysis dendrograms for Carabidae collected by pitfall trap in full season and double crop soybean fields in southeastern Virginia in 2014 and 2015. Distance represents Euclidean distance. (FS = Full season; DC = Double crop).

The species accumulation curve for pitfall trap samples in 2014 indicates that full season soybean plots contain more carabid species than double crop soybeans (Fig. 4.23). In 2015 many more carabids were caught in double crop than in full season fields. The rarefaction curves suggest similar expected species richness for 2015, but the steep rise in the full season curve indicates additional species beyond those captured are present in the system (Fig. 4.24). However, the lack of asymptote in the curves of both cropping systems suggests that there are more species present that have not been collected yet.

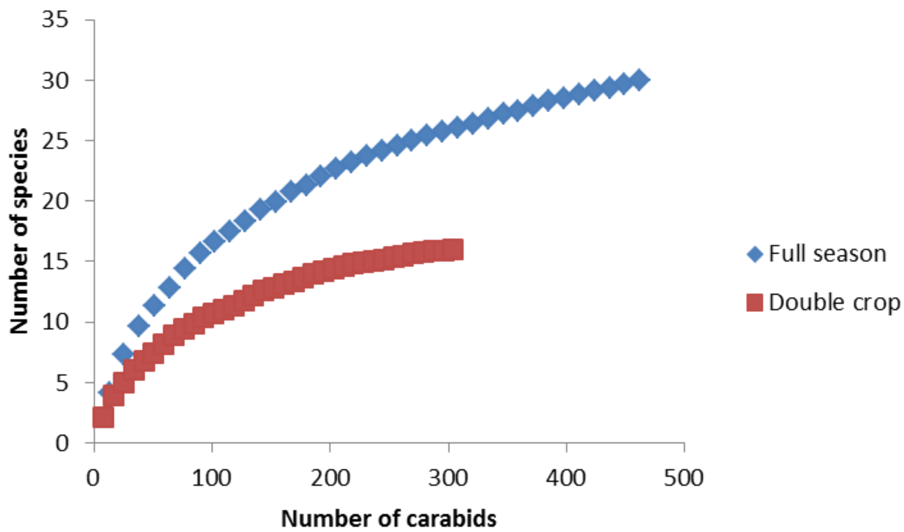


Figure 4.23. Species accumulation curves for Carabidae caught with pitfall traps in full season and double crop soybeans in southeastern Virginia in 2014.

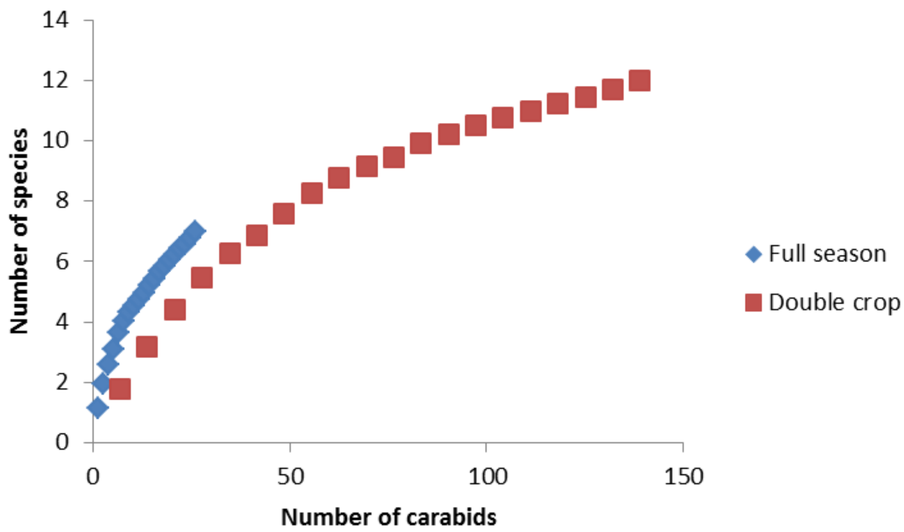


Figure 4.24. Species accumulation curves for Carabidae caught with pitfall traps in full season and double crop soybeans in southeastern Virginia in 2015.

4.4 Discussion

This research had two goals. First, we sought to identify and determine abundance of the arthropod natural enemy community in Virginia soybean. Second, we tested the hypothesis that full season soybean would have greater natural enemy diversity than double crop soybean.

Overall, there tended to be a higher abundance of ground and foliar dwelling natural enemies in double crop fields, while diversity tended to be higher in full season soybean. Spiders accounted for approximately 25-40% of natural enemies in the foliage in both systems. *G. punctipes* was a dominant predator in full season soybean but not double crop soybean.

Although total natural enemies were not significantly different between the two systems in 2015, the mean number of natural enemies per plot was almost twice as high in double crop soybean. Significant rainfall in June 2015 could be responsible for the relatively low numbers of ground-dwelling natural enemies in full season soybean. Full season soybean were planted in mid-May, and double crop beans were planted in mid- to late-June. Natural enemies that were moving into growing full season soybean could have been driven out of fields, or the early season colonization could have beenslowed, by the 19 cm of rain that fell in June.

Surrounding habitat provided a stable environment for natural enemies during times of low food resources in crops and during harvest and winter. Habitats including forest and meadows typically shelter a larger proportion of beneficial insects than pests (Denys and Tscharntke 2002, Rusch et al. 2010). Forests protect natural enemies against extreme temperature variation (Landis et al. 2000, Rusch et al. 2010). In 2014, several full season and double crop plots were situated next to dense forest. In 2015 plots were more likely to be near patchy trees or no trees at all. This could have affected the overall abundance of natural enemies caught.

The stability of the taxa identity of the ground-dwelling community was evident in 2014 and 2015. In both years and both cropping systems, spiders, carabids and staphylinid beetles were the first, second and third most abundant taxa captured in pitfall traps. This is interesting since the species identity of carabids changes depending on the cropping system, as it does with spiders (see Chapter 3). These arthropods are well adapted to the transient environment of a soybean field, and persist over the two years by either occupying wheat over the winter in double crop fields or re-invading from adjacent habitats in full season soybean. Different species fill in to create a stable proportion of dominant arthropods that occupy different niches in the natural enemy community.

Detritivores are considered early season food for generalist predators (Settle et al. 1996). Detritus from decaying wheat stubble that is common in double crop soybean provides food for detritivores such as Collembola, which could be an important dietary source for ground predators early in the season (Gurr et al. 2012). Organisms in detritus provide year-round food for carabids and these are higher in fields with crop residue (Stinner and House 1990, Hatten et al. 2007). Consumption of detritivores can help build up the predator community before potential pests arrive. Generalist predators can arrive early and, as their populations increase, they are in place to feed on potential pests as soon as they start arriving (Gurr et al. 2012).

High natural enemy diversity could be more important than abundance, since diversity increases stability of ecological function after disturbances such as harvesting (Rusch 2010). Research on the effects of diversity shows a strong relationship between high natural enemy diversity and herbivore suppression in agricultural systems (Letourneau et al. 2009, Rusch et al. 2010). A highly diverse and abundant natural enemy community could be more important in double crop than full season soybean in Virginia. *H. zea*, for example, arrives in early to mid-August, and at high densities can cause significant damage to soybean. Full season soybean planted in April or May is usually able to progress beyond the flowering and podding stages when *H. zea* can cause much damage to soybean pods, but double crop soybean may still be in the vulnerable stages (Herbert et al. 2003).

Most carabids are predators or omnivores (Kromp 1999). By examining the carabid community we were able to calculate two indexes in an attempt to compare diversity in the two cropping systems. In 2014 full season soybean had higher diversity by both indexes. This is due to more species in full season soybean - 29 compared to 16 in double crop soybean. The carabid community was also more even in full season soybean, while in double crop *P. sculptus* and *H. pensylvanicus* made up almost 70% of total carabids caught. Carabids are selective about microclimate and habitat (Wallin 1986, Hatten et al. 2007). The full season soybean fields, even with comparatively little detritus, were more conducive to a greater number of species than double crop soybean. Certain species might be prone to moving into crops early in the season, and by the time double crop soybean has a full canopy many of these species have migrated into other crops. For instance, *C. punctulata*, the dominant carabid in full season soybean in 2014, was at peak abundance at the first counting date in late July. Double crop soybean was still in an early growth stage and this could have deterred *C. punctulata* from migrating into the fields.

Future research can determine what can cause large shifts in natural enemy populations from year to year. If precipitation is a major factor, steps can be taken to supplement the natural enemy community after heavy rainfall. Similarly, if the surrounding landscape is influential to the abundance of natural enemies steps can be taken to enhance the natural enemy community, such as adding hedgerows attractive to carabids and spiders.

References

Anderson, A. C. and K. V. Yeargan. 1998. Influence of soybean canopy closure on predator abundances and predation on *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs. Environmental Entomology 27: 1488-1495.

Ciegler, J. 2000. Ground Beetles and Wrinkled Bark Beetles of South Carolina (Coleoptera: Geadephaga: Carabidae and Rhysodidae). Biota of South Carolina. Vol. 1. Clemson, S.C., Clemson University.

Colwell, R. K. 2013. "EstimateS: Statistical estimation of species richness and shared species from samples. Version 9." Persistent URL <purl.oclc.org/estimates>.

Denys, C. and T. Tscharntke. 2002. Plant-insect communities and predator-prey ratios in field margin strips, adjacent crop fields, and fallows. Oecologia 130: 315-324.

Ferguson, H. J., R. M. McPherson and W. A. Allen. 1984. Effect of 4 soybean cropping systems on the abundance of foliage-inhabiting insect predators. Environmental Entomology 13: 1105-1112.

Hatten, T. D., N. A. Bosque-Perez, J. R. Labonte, S. O. Guy and S. D. Eigenbrode. 2007. Effects of tillage on the activity density and biological diversity of carabid beetles in spring and winter crops. Environmental Entomology 36: 356-368.

Herbert, A., C. Hull and E. Day. 2003. Corn earworm biology and management in soybeans. Virginia Cooperative Extension publication 444-770.

Kajak, A. 1978. Analysis of consumption by spiders under laboratory and field conditions. Ekologia Polska-Polish Journal of Ecology 26: 411-428.

Kogan, M. and S. G. Turnipseed. 1987. Ecology and management of soybean arthropods. Annual Review of Entomology 32: 507-538.

Kromp, B. 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agriculture Ecosystems & Environment 74: 187-228.

Landis, D. A., S. D. Wratten and G. M. Gurr. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. Annual Review of Entomology 45: 175-201.

Letourneau, D. K., J. A. Jedlicka, Bothwell, S. G. and C. R. Moreno. 2009. Effects of natural enemy biodiversity on the suppression of arthropod herbivores in terrestrial ecosystems. Annual Review of Ecology Evolution and Systematics. Palo Alto, Annual Reviews. 40: 573-592.

Post, W. M. and C. C. Travis. 1979. Quantitative stability in models of ecological communities. Journal of Theoretical Biology 79: 547-553.

Rusch, A., M. Valantin-Morison, J. P. Sarthough and J. Roger-Estrade. 2010. Biological control of insect pests in agroecosystems: Effects of crop management, farming systems, and seminatural habitats at the landscape scale: A review. Advances in Agronomy, Vol 109. D. L. Sparks. San Diego, Elsevier Academic Press Inc. 109: 219-259.

SAS Institute. 2009. SAS 9.2 reference, 2nd ed. SAS Institute, Cary, NC.

Settle, W. H., H. Ariawan, E. T. Astuti, W. Cahyana, A. L. Hakim, D. Hindayana, A. S. Lestari and Pajamingsih. 1996. Managing tropical rice pests through conservation of generalist natural enemies and alternative prey. Ecology 77: 1975-1988.

Shannon, C. E. and W. Weaver. 1949. The mathematical theory of communication, University of Illinois Press, Urbana.

Simpson, E. H. 1949. Measurement of diversity. Nature 163: 688.

Stinner, B. R. and G. J. House. 1990. Arthropods and other invertebrates in conservation-tillage agriculture. Annual Review of Entomology 35: 299-318.

Swenson, S. J., D. A. Prischmann-Voldseth and F. R. Musser. 2013. Corn earworms (Lepidoptera: Noctuidae) as pests of soybean. Journal of Integrated Pest Management 4.

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

Turnipseed, S. G. and M. Kogan. 1976. Soybean entomology. Annual Review of Entomology 21: 247-282.

Wallin, H. 1986. Habitat choice of some field-inhabiting carabid beetles (Coleoptera, Carabidae) studied by recapture of marked individuals. Ecological Entomology 11: 457-466.

Whalen, R. 2016. Abundance and diversity of foliar and ground-dwelling spiders in two soybean cropping systems. Dissertation chapter, Department of Entomology, Virginia Tech.

Witmer, J. E., J. A. Hough-Goldstein and J.D. Pesek. 2003. Ground-dwelling and foliar arthropods in four cropping systems. Environmental Entomology 32: 366-376.

Conclusions

Arthropod natural enemies are imperative for controlling herbivore populations in soybean with minimal insecticide inputs. Broad-spectrum insecticide application can decrease natural enemy densities and allow for secondary pest outbreaks. Selective insecticides that have less impact on natural enemy populations might allow for pest control while preserving important predators. By counting common natural enemies in soybean for three weeks after insecticide application, we found two selective diamide insecticides did not disrupt the natural enemy community. Natural enemies exposed to broad-spectrum insecticide decreased in counts done with a beat sheet. The two selective insecticides, flubendiamide and chlorantraniliprole, which target Lepidopteran pets, were highly effective at decreasing caterpillar abundance. These insecticides would be good alternatives to broad-spectrum insecticides for producers that need Lepidoptera control in their fields while at the same time keeping the natural enemy community intact.

We identified 7,371 specimens and found 76 spider species in soybean fields over the course of two years. This predator community was much more diverse in 2014 than in 2015, which could be due to many factors including precipitation, row spacing and field borders. In both years of the study we found diversity of foliar-dwelling spiders in full season and double crop soybean to be similar, but in 2014 the ground-dwelling spider community in full season soybean had higher diversity. Double crop soybeans had higher abundance on the ground and in the foliage compared with full season soybean. This was unexpected, since double crop soybeans are planted later than full season and spider populations would have less time to colonize and grow. However, since double crop soybean fields are continuously covered with an early crop such as wheat, followed by wheat stubble, then soybean, some spider species might live year-round in the fields. Future research could investigate the spider community in double-crop soybean fields from soybean harvest through wheat harvest and compare this community with that in a bare full season field. Information from this research can guide possible conservation efforts with dominant species. For instance, the dominant spider in both systems was *Pardosa milvina.* This wolf spider is known to occupy fields regardless of availability of prey; they are motivated more by habitat complexity (Schmidt and Rypstra 2010). This spider was abundant in both years of the study in both systems and its stability even in times of low prey availability makes it a good candidate for conservation, perhaps by increasing habitat complexity along field borders.

When we examined the natural enemy community in double crop and full season soybeans we found more natural enemies in double crop fields, both on the ground and in the foliage. This is similar to our findings on spider abundance in the two systems. There were some differences in dominant predators. *Geocoris punctipes* (Say) was a dominant predator in full season soybean foliage and was almost non-existent in double crop soybean. This is an important egg predator in both its immature and adult stages. Its absence in double crop soybean leaves a niche open which egg predator *Orius insidiosus* (Say) seemed to fill. When we compared diversity of a family of predatory beetles we found higher diversity in full season soybean, which was again similar to our spider data. The similarity in spider and insect natural enemy diversity and abundance trends suggests that a greater number of species can co-exist in full season soybean, while in double crop soybeans a few dominant natural enemies thrive. Future research can determine what factors of double crop soybean contribute to this higher abundance. This is difficult to discern in growers fields, since two possible contributors to natural enemy density, habitat complexity from wheat stubble, and planting date, occur simultaneously.

Virginia soybean, both full season and double crop, has plant architectural complexity that is highly attractive to a variety of natural enemies. Our research on this natural source of pest control can be conveyed to producers and perhaps encourage them to survey their fields before application of broad-spectrum insecticides.

References

Schmidt, J. M. and A. L. Rypstra. 2010. Opportunistic predator prefers habitat complexity that exposes prey while reducing cannibalism and intraguild encounters. Oecologia 164: 899-910.