

ABSTRACT

GRINNAN, ROSEMARY. The Effects of Climate Change on Plant-Herbivore Interactions in Soybean. (Under the direction of Dr. Marc T. J. Johnson.)

Future climate change is predicted to include increases in global temperature and shifts in global precipitation patterns that will result in increased frequency and severity of drought. These abiotic environmental changes are predicted to benefit herbivorous insects resulting in increased population outbreaks. It has been hypothesized that plants experiencing increased abiotic stress may be more susceptible to insect herbivory. If these predictions are all correct, then it will be important to gain an understanding of how plants will respond to these changes, especially in agriculture. If our agricultural plants are in danger of compounded decreases in performance due to synergistic negative effects of stress, then we must begin preparations now to ensure future food security. This study presents results from four ecological experiments on an important model crop system, soybean (*Glycine max* (L.) Merr., Fabaceae), conducted in controlled growth experiments and in the field. In these experiments, I asked the following questions (i) How will combinations of drought, increased temperature, and insect herbivory impact soybean performance?, (ii) How will drought and temperature impact soybean resistance to insect herbivory?, and (iii) How do soybean genotypes vary in their phenotypic response to stress? I conducted one growth chamber experiment, two greenhouse experiments and one field experiment during 2009-2010 in North Carolina in which I tested combinations of drought, temperature, and insect

herbivory on up to 51 soybean genotypes, and up to three insect herbivores indoors and natural field herbivory outdoors. I measured soybean performance as plant biomass, seed yield, and phenology; I measured insect resistance through no-choice feeding assays and herbivore damage to the plants. I also measured a number of phenotypic traits and evaluated them for strength in genotypically predicting plant performance and herbivore resistance. Overall, I found that drought will be the dominant stressor of soybean and that drought, temperature and herbivory did not interact to impact soybean performance. Also, drought and temperature were not as impactful on herbivore resistance as soybean genotype, although it does appear that drought may increase resistance. Plant genotype had over-arching impacts in most phenotypic measurements and stress responses. The traits that most strongly predicted soybean yield were plant growth rate and herbivore damage (both negative correlations). Plant growth rate most strongly predicted herbivore damage (positive correlation). I conclude that the discovery of phenotypic traits that can predict soybean response to stress coupled with the understanding of genetic mechanisms underlying soybean response to stress will be very important in ensuring the viability of soybean in light of predicted climate change.

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The Effects of Climate Change on Plant-Herbivore Interactions in Soybean

by
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DEDICATION

This work is dedicated to my mom and dad, Cynthia and Stephen Smith, who worked tirelessly to ensure that I received the best education possible. Their support and encouragement in my academic endeavors played a fundamental role in my success. Through their enduring efforts I was able to attend a well respected private high school, which was well outside of their financial abilities. Not only did they make my (and my brothers') attendance at this school financially possible somehow, but they participated in every aspect of my academics, assisting with homework, attending functions and reminding me to expect more of myself every day. Without their support, I would not have accomplished so much. Thanks mom and dad!

BIOGRAPHY

I was born in 1974 in Camden, NJ and grew up in Merchantville, NJ, a small suburb just outside Philadelphia, PA. I received my high school diploma in 1992 from Camden Catholic High School, Cherry Hill, NJ and went on to attend LaSalle University, Philadelphia, PA for a brief time. I then took some time off of school, in which I traveled around the U.S. and lived in numerous states. In my 30th year, I returned to school with fresh ideas and goals. I earned an Associate of Science in Biology in 2006 at Camden County College, Blackwood, NJ. I then attended North Carolina State University, Raleigh, NC where I earned my Bachelor of Science in Plant Biology in 2009 (and anticipate earning my Master of Science in Plant Biology in 2011).

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CHAPTER 1

Ecological Implications of Climate Change on Agricultural Systems

This ever-changing world presents great challenges to its inhabitants to understand and adapt to the environments in which we exist. There is no end to the surprises nature can bring us. Humans have endured the gamut of environmental stress from pestilence and plagues to catastrophic natural disasters; and we have always prevailed through determination, intellect, and ingenuity. But currently, the planet is experiencing changes in climate at rates never before witnessed by humans. Global temperatures are steadily rising, atmospheric gas composition is changing, precipitation and weather events are redistributing and becoming more variable and biological activity seems driven more and more towards invasion and extinction. So how will we prepare for what is to come? The answer lies in investigation and discovery. We must ask: What will these changes mean and what factors will be most important? This research attempts to investigate which factors associated with climate change will be most important to one biological system, soybean.

Global implications of predicted climate change

Global warming is a fairly new term, but has a great deal of supportive data. We have been recording global temperatures for ~150 years and have seen the average temperature rise approximately 0.5°C in this time (Hansen et al. 1999).

While this temperature change is a global average, and not consistent from region to region, it represents a large overall change for our planet (Easterling et al. 2000). Biological organisms may respond in a variety of ways and there are already observations recording temperature-related shifts in species' range distributions (Easterling et al. 2000, Walther et al. 2002, Parmesan and Yohe 2003, Kelly and Goulden 2008, Willis et al. 2008), phenologies (Walther et al. 2002, Willis et al. 2008), and abundances and interactions (Levine and Paige 2004, Roy et al. 2004, Hamilton et al. 2005, Dermody et al. 2008, Adams et al. 2009, O'Connor 2009, van Mantgem et al. 2009). Much of these changes can also be related to abiotic repercussions of global temperature increases, such as changes in precipitation.

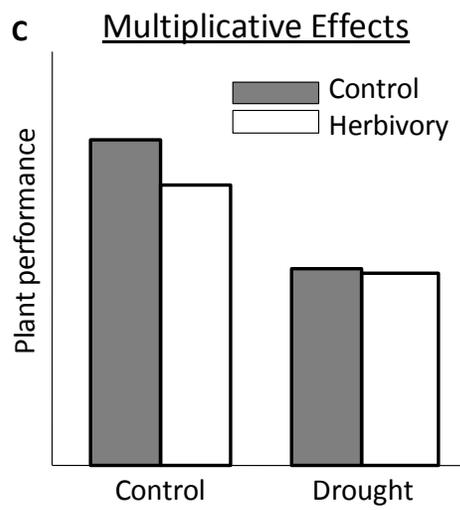
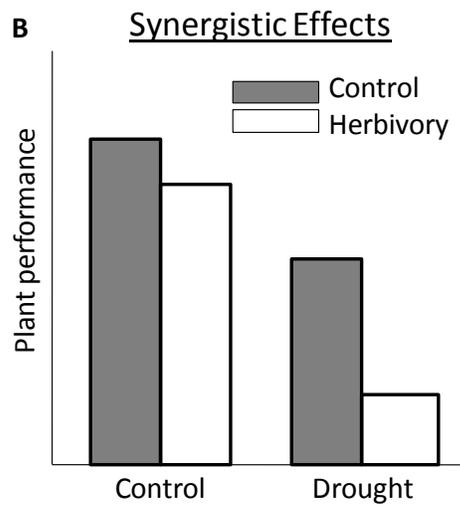
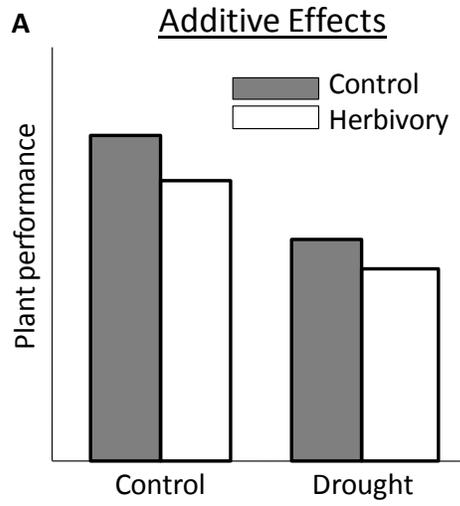
Temperature fluctuations on a global scale alter convection currents, resulting in changes in global precipitation patterns. This means that areas of high and low precipitation levels will shift, resulting in more frequent and extreme weather phenomena, such as drought and floods, for many areas (Easterling et al. 2000, IPCC 2007, Portmann et al. 2009, Seager et al. 2009). This is also resulting in altered species' occurrences and interactions (Williams and Jackson 2007, Kelly and Goulden 2008, Adams et al. 2009, Seager et al. 2009). This is troubling for many scientists, as we do not yet have a reasonable understanding of how these changes will impact humans, or if and how we might mitigate some of the negative effects of climate change.

Ecological and agricultural implications related to climate change

One unsettling prediction that has emerged from climate change studies suggests that plants may be in danger of herbivorous insect population outbreaks (Levine and Paige 2004, Hamilton et al. 2005, Dermody et al. 2008, Zhang et al. 2009). The Plant Stress Hypothesis posits that plants under abiotic stress, such as increased temperature and drought, may be more susceptible to damage by insect herbivores due to biochemical changes in their leaves that result in increased palatability to the insects, namely decreased defense compounds and increased nitrogen: carbon ratios (White 1984). If this is true, then plant systems can be in danger of decreased abundance, fecundity and fitness. It is important that scientists gain more understanding of the likelihood of these predictions, as we rely on plants for our survival.

Our agricultural plants should be of primary concern in light of these predictions. Without sustainable agricultural systems, feeding the world will be very challenging. The World Bank (2010) predicted significant decreases in global crop yields due to climate change by the year 2050, if we make no changes to our current agricultural methods and crop varieties. It will be important to understand if abiotic changes in environment coupled with biotic stress (insect herbivory) will result in additive or synergistic negative effects (Fig. 1.1). For instance, if droughted plants are more likely to experience dramatically increased herbivore damage as compared to non-droughted plants, then we must begin to understand what genes and traits may help mitigate this scenario. This will help scientists, crop breeders and

Figure 1.1 Potential outcomes of stress interactions for a hypothetical scenario, where effects of drought and herbivory are evaluated for their impact on plant performance. **A** Additive effects suggest that drought and herbivory will not interact to greatly reduce plant performance, but instead that the reductions can be added together to predict total effects. **B** Synergistic effects suggest that drought and herbivory will interact to greatly reduce plant performance more than their additive effects predict. **C** Multiplicative effects suggest that drought and herbivory interact to produce a smaller reduction in plant performance than predicted by their additive effects.



farmers prepare for future climate change. Knowledge will be our first step in mitigating possible negative impacts of climate change.

Protecting our agricultural systems

Studying impacts of multiple stresses in our agricultural systems has emerged as a new trend in ecological understanding of managed biological systems. New studies are focusing more on dynamic understanding of complex interactions that occur in nature, exploring effects of multiple stress interactions in both natural and managed systems (Brown et al. 2001, Boling et al. 2004, Relyea 2004, Hull-Sanders and Eubanks 2005, Deegan et al. 2007, Daleo and Iribarne 2009, Mody et al. 2009, Silva et al. 2010). However, understanding responses of one system will not necessarily equate to understanding the responses of all systems, and the road of exploration that lies ahead is long. Studies that focus specifically on crop systems in this regard will be invaluable (Smelser and Pedigo 1992, Hamilton et al. 2005, Dermody et al. 2008, Li et al. 2009, Piao et al. 2010, Yesudas et al. 2010, Olesen et al. 2011). These will be the studies that guide and inspire our management practices and crop selection.

For millennia, humans have been selecting crops that can endure regional climatic stress and provide us with higher yields. In recent decades, the advancement of molecular technologies has allowed scientists to better understand how genetic selection and manipulation can provide more accurate breeding practices. Currently, there are many crop varieties, or genotypes, that have been

bred to withstand environmental stresses such as drought and insect herbivory. Some of these may already be able to remain viable in light of predicted climate change, or they may be useful for breeding of such crops. It will be important to test these genotypes to determine both their viability against stress and their traits and genes responsible for inferring this viability. This level of understanding will serve to ensure future food security in light of predicted climate changes to come.

Research objectives and questions

This study seeks to understand the effects of predicted climate change on plant-herbivore interactions in a model crop system, soybean (*Glycine max* (L.) Merr., Fabaceae). While this is, essentially, an ecological study in an agricultural system, the results may help scientists, breeders and farmers gauge what factors related to climate change will be most important to focus on in future studies and breeding. In my research I tried to address the following questions: (i) How will factors such as increased temperature, drought and insect herbivory interact to impact soybean performance? (ii) How will abiotic stress, such as temperature and drought, impact the resistance of soybean to insect herbivores? And, (iii) How do soybean genotypes vary phenotypically in their responses to stress? It is my goal to provide the results of this study to the science community to improve our understanding of these complicated ecological problems and move forward in our preparations for changes ahead.

Experimental system

Soybean, an herbaceous legume, provides an ideal system to test our research questions. Soybean is among the most important crops globally, with a yield of 258 million metric tons and \$39 billion in sales in 2010 alone (American Soybean Association 2011). The US has emerged as the world's leading soybean producer with 31 million hectares planted in 2010 (American Soybean Association 2011).

Soybeans have been bred for a variety of phenotypic traits including resistance to drought and herbivory (Carter et al. 2004, Charlson et al. 2009), which makes them strong candidates for understanding how plant genotypes will respond to multiple environmental stresses. For this study, I chose 51 genotypes (Appendix A) to answer my research questions. These genotypes represent the wide range of maturity groups (2-8; a categorical descriptor of the time it takes a variety to flower and produce mature fruits) grown across North America. Many genotypes were chosen because they were bred as slow-wilting or offspring of slow-wilting parents, some notable genotypes being PI 416937 (Sloane et al. 1990), and 'Jackson'. Others were chosen for resistance to insects. Some notable herbivore resistant lines include 5 genotypes from Dr. H. Roger Boerma at the University of Georgia and 8 genotypes from Dr. M.A. Rouf Mian at the USDA in Wooster, OH. Other notable genotypes include 'Stressland' (Cooper et al. 1999), 'Boggs' (Boerma et al. 2000), 'NC-Roy' (Burton and Carter 2005), and 'Benning' (Boerma et al. 1997). All 51 lines

were chosen because they have a known genetic lineage and have been proven through experimental testing to possess relevant phenotypic traits.

The soybean ecosystem is also host to a diverse variety of organisms, including insect herbivores. Many of its generalist insect herbivores are available commercially, increasing the consistency and ease with which to study this system. For this study, I chose three generalist agricultural pests common to the soybean system as well as the southeast US, where this study was conducted. These were: *Heliothis virescens* Fabricius (Noctuidae), the tobacco earworm; *Helicoverpa zea* Boddie (Noctuidae), the corn earworm; and *Spodoptera exigua* Hübner (Noctuidae), the beet armyworm. These insects are relevant pests, easy to obtain commercially, and ideal for experimental research, i.e. easily transferred, incubated, collected, etc.

Experimental sites and conditions are explained in Chapters 2 and 3.

REFERENCES

- Adams, H. D., M. Guardiola-Claramonte, G. A. Barron-Gafford, J. C. Villegas, D. D. Breshears, C. B. Zou, P. A. Troch, and T. E. Huxman. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* **106**:7063-7066.
- American Soybean Association. 2011. Soy Stats.
<http://www.soystats.com/2010/Default-frames.htm>
- Boerma, H. R., R. S. Hussey, D. V. Phillips, E. D. Wood, G. B. Rowan, and S. L. Finnerty. 1997. Registration of 'Benning' soybean. *Crop Science* **37**:1982-1982.
- Boerma, H. R., R. S. Hussey, D. V. Phillips, E. D. Wood, G. B. Rowan, and S. L. Finnerty. 2000. Registration of 'Boggs' soybean. *Crop Science* **40**:294-295.

- Boling, A., T. P. Tuong, S. Y. Jatmiko, and M. A. Burac. 2004. Yield constraints of rainfed lowland rice in Central Java, Indonesia. *Field Crops Research* **90**:351-360.
- Brown, J. H., T. G. Whitham, S. K. M. Ernest, and C. A. Gehring. 2001. Complex species interactions and the dynamics of ecological systems: Long-term experiments. *Science* **293**:643-650.
- Burton, J. W. and T. E. Carter. 2005. Registration of 'NC-Roy' soybean. *Crop Science* **45**:2654-2654.
- Carter, T.E., R. L. Nelson, C. Sneller, and Z. Cui, 2004. Genetic Diversity in Soybean. *Soybeans: Improvement, Production, and Uses, Third Edition (Agronomy)*. Boerma, H. R. and J. E. Specht (Ed.). American Society of Agronomy, Madison, WI. 303-416.
- Charlson, D. V., S. Bhatnagar, C. A. King, J. D. Ray, C. H. Sneller, T. E. Carter, and L. C. Purcell. 2009. Polygenic inheritance of canopy wilting in soybean *Glycine max* (L.) Merr. *Theoretical and Applied Genetics* **119**:587-594.
- Cooper, R. L., R. J. Martin, S. K. St Martin, A. Calip-DuBois, R. J. Fioritto, and A. F. Schmitthenner. 1999. Registration of 'Stressland' soybean. *Crop Science* **39**:590-591.
- Daleo, P. and O. Iribarne. 2009. Beyond competition: The stress-gradient hypothesis tested in plant-herbivore interactions. *Ecology* **90**:2368-2374.
- Deegan, L. A., J. L. Bowen, D. Drake, J. W. Fleeger, C. T. Friedrichs, K. A. Galvan, J. E. Hobbie, C. Hopkinson, D. S. Johnson, J. M. Johnson, L. E. Lemay, E. Miller, B. J. Peterson, C. Picard, S. Sheldon, M. Sutherland, J. Vallino, and R. S. Warren. 2007. Susceptibility of salt marshes to nutrient enrichment and predator removal. *Ecological Applications* **17**:S42-S63.
- Dermody, O., B. F. O'Neill, A. R. Zangerl, M. R. Berenbaum, and E. H. DeLucia. 2008. Effects of elevated CO₂ and O₃ on leaf damage and insect abundance in a soybean agroecosystem. *Arthropod-Plant Interactions* **2**:125-135.
- Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl, and L. O. Mearns. 2000. Climate extremes: Observations, modeling, and impacts. *Science* **289**:2068-2074.
- Hamilton, J. G., O. Dermody, M. Aldea, A. R. Zangerl, A. Rogers, M. R. Berenbaum, and E. H. DeLucia. 2005. Anthropogenic changes in tropospheric composition

- increase susceptibility of soybean to insect herbivory. *Environmental Entomology* **34**:479-485.
- Hansen, J., R. Ruedy, J. Glascoe, and M. Sato. 1999. GISS analysis of surface temperature change. *Journal of Geophysical Research-Atmospheres* **104**:30997-31022.
- Hull-Sanders, H. M. and M. D. Eubanks. 2005. Plant defense theory provides insight into interactions involving inbred plants and insect herbivores. *Ecology* **86**:897-904.
- Intergovernmental Panel on Climate Change Fourth Assessment Report. 2007. *Climate Change 2007: Synthesis Report*. Allali, A., R. Bojariu, S. Diaz, I. Elgizouli, D. Griggs, D. Hawkins, O. Hohmeyer, B. P. Jallow, L. Kajfež-Bogataj, N. Leary, H. Lee, and D. Wratt (Ed.). Cambridge University Press, Cambridge.
- Kelly, A. E. and M. L. Goulden. 2008. Rapid shifts in plant distribution with recent climate change. *Proceedings of the National Academy of Sciences of the United States of America* **105**:11823-11826.
- Levine, M. T. and K. N. Paige. 2004. Direct and indirect effects of drought on compensation following herbivory in scarlet gilia. *Ecology* **85**:3185-3191.
- Li, Y. P., W. Ye, M. Wang, and X. D. Yan. 2009. Climate change and drought: A risk assessment of crop-yield impacts. *Climate Research* **39**:31-46.
- Mody, K., D. Eichenberger, and S. Dorn. 2009. Stress magnitude matters: Different intensities of pulsed water stress produce non-monotonic resistance responses of host plants to insect herbivores. *Ecological Entomology* **34**:133-143.
- O'Connor, M. I. 2009. Warming strengthens an herbivore-plant interaction. *Ecology* **90**:388-398.
- Olesen, J. E., M. Trnka, K. C. Kersebaum, A. O. Skjelvag, B. Seguin, P. Peltonen-Sainio, F. Rossi, J. Kozyra, and F. Micale. 2011. Impacts and adaptation of European crop production systems to climate change. *European Journal of Agronomy* **34**:96-112.
- Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**:37-42.

- Piao, S. L., P. Ciais, Y. Huang, Z. H. Shen, S. S. Peng, J. S. Li, L. P. Zhou, H. Y. Liu, Y. C. Ma, Y. H. Ding, P. Friedlingstein, C. Z. Liu, K. Tan, Y. Q. Yu, T. Y. Zhang, and J. Y. Fang. 2010. The impacts of climate change on water resources and agriculture in China. *Nature* **467**:43-51.
- Portmann, R. W., S. Solomon, and G. C. Hegerl. 2009. Spatial and seasonal patterns in climate change, temperatures, and precipitation across the United States. *Proceedings of the National Academy of Sciences of the United States of America* **106**:7324-7329.
- Relyea, R. A. 2004. Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. *Environmental Toxicology and Chemistry* **23**:1080-1084.
- Roy, B. A., S. Gusewell, and J. Harte. 2004. Response of plant pathogens and herbivores to a warming experiment. *Ecology* **85**:2570-2581.
- Seager, R., A. Tzanova, and J. Nakamura. 2009. Drought in the southeastern United States: causes, variability over the last millennium, and the potential for future hydroclimate change. *Journal of Climate* **22**:5021-5045.
- Silva, E. N., S. L. Ferreira-Silva, A. D. Fontenele, R. V. Ribeiro, R. A. Viegas, and J. A. G. Silveira. 2010. Photosynthetic changes and protective mechanisms against oxidative damage subjected to isolated and combined drought and heat stresses in *Jatropha curcas* plants. *Journal of Plant Physiology* **167**:1157-1164.
- Sloane, R. J., R. P. Patterson, and T. E. Carter. 1990. Field drought tolerance of a soybean plant introduction. *Crop Science* **30**:118-123.
- Smelser, R. B. and L. P. Pedigo. 1992. Bean leaf beetle (Coleoptera, Chrysomelidae) herbivory on leaf, stem, and pod components of soybean. *Journal of Economic Entomology* **85**:2408-2412.
- van Mantgem, P. J., N. L. Stephenson, J. C. Byrne, L. D. Daniels, J. F. Franklin, P. Z. Fule, M. E. Harmon, A. J. Larson, J. M. Smith, A. H. Taylor, and T. T. Veblen. 2009. Widespread increase of tree mortality rates in the western United States. *Science* **323**:521-524.
- Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. *Nature* **416**:389-395.

- White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* **63**:90-105.
- Williams, J. W. and S. T. Jackson. 2007. Novel climates, no-analog communities, and ecological surprises. *Frontiers in Ecology and the Environment* **5**:475-482.
- Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, and C. C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences of the United States of America* **105**:17029-17033.
- World Bank, The. 2010. *World Development Report 2010: Development and Climate Change*. The International Bank for Reconstruction and Development / The World Bank. Washington D.C.
- Yesudas, C. R., H. Sharma, and D. A. Lightfoot. 2010. Identification of QTL in soybean underlying resistance to herbivory by Japanese beetles (*Popillia japonica*, Newman). *Theoretical and Applied Genetics* **121**:353-362.
- Zhang, Z. B., B. Cazelles, H. D. Tian, L. C. Stige, A. Brauning, and N. C. Stenseth. 2009. Periodic temperature-associated drought/flood drives locust plagues in China. *Proceedings of the Royal Society B-Biological Sciences* **276**:823-831.

CHAPTER 2

The Effects of Temperature and Drought on Plant-Herbivore Interactions in Soybeans in Controlled Growth Experiments

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ABSTRACT

Global climate change is predicted to result in increased global temperatures and increased precipitation variability, resulting in drought for many regions. Some studies also predict that these environmental changes will result in more frequent outbreaks of herbivorous insect populations. Here we sought to understand whether abiotic stress in combination with biotic stress leads to synergistic negative effects on soybean performance; if abiotic stress affects soybean resistance to insect herbivores; and how genetic variation impacts soybean responses to these stresses. We performed three experiments in controlled growth environments using up to 51 genotypes of soybean and 3 different herbivore species. Drought and herbivory had the largest and most consistent negative effects on plant performance, reducing above and belowground biomass by 10-45%, whereas increased temperature (2.2°C) had little to no effect on plants. Drought, temperature and herbivory rarely

interacted to influence soybean performance, although the combined effects of drought and temperature suggest a weak relationship in one large experiment. We also found that drought increased susceptibility to multiple generalist herbivore species on average, but these results varied dramatically in magnitude and direction among plant genotypes. Results suggest that some genotypes may already exist that can withstand predicted climate change.

INTRODUCTION

The earth is experiencing climatic changes and models predict continued increases in temperature and variability in precipitation (Easterling et al. 2000, IPCC 2007, Portmann et al. 2009). The effects of these changes on communities and ecosystems are less certain, although it is widely believed that ongoing environmental change will alter the ecology, behavior and evolution of species and ecosystems in diverse ways (Williams and Jackson 2007). For example, increased temperatures over the last several decades have been associated with shifts in species' phenologies (Walther et al. 2002, Willis et al. 2008), altered range distributions (Easterling et al. 2000, Walther et al. 2002, Parmesan and Yohe 2003, Kelly and Goulde 2008, Willis et al. 2008), and changes in species' abundances and interactions (Levine and Paige 2004, Roy et al. 2004, Hamilton et al. 2005, Dermody et al. 2008, Adams et al. 2009, O'Connor 2009, van Mantgem et al. 2009). These predictions and initial results indicate the imperative need for ecological studies to investigate how the combined effects of multiple environmental stresses

affect species interactions (Deegan et al. 2007, Memmott et al. 2007, McNulty and Boggs 2010). This will be complex, as there are countless systems and interactions to study and numerous combinations of predicted stresses to test. Here we seek to disentangle some of this complexity by investigating how simultaneous changes in temperature and drought could influence plant-herbivore interactions in a model crop system.

Increases in drought and temperature associated with climate change may facilitate corresponding increases in the frequency and severity of insect population outbreaks (Hammond et al. 2001, Levine and Paige 2004, Hamilton et al. 2005, Dermody et al. 2008). The Plant Stress Hypothesis predicts that environmental stress increases a plant's susceptibility to insect herbivory by altering leaf chemistry and whole plant physiology (White 1984). Specifically, abiotic stress can cause a reduction in plant defense compounds and an increase in available nitrogen and digestible proteins relative to carbon availability (White 1984), leading to more palatable food for insect herbivores. Thus, the compounded effects of increased population outbreaks and environmental stress could lead to larger reductions in growth and production in natural and managed systems. It is therefore important to begin to understand how climate change will influence plant-herbivore interactions, especially in agriculture.

The predicted changes in the biotic and abiotic environment associated with climate change could have large negative effects in agriculture. Many studies have investigated how crops will respond to predicted change in drought, temperature and

other factors, like atmospheric gas concentrations (Liu et al. 2003, Hamilton et al. 2005, Charlson et al. 2009b, Farooq et al. 2009, Alam et al. 2010). However, few studies have examined the combined effects of multiple abiotic and biotic factors on plant performance in agriculture (Smelser and Pedigo 1992, Rogers et al. 1994). For example, understanding whether changes in drought and herbivory will lead to synergistic negative effects on plants, as opposed to independent additive effects, is essential to understanding the potential impacts of climate change on species interactions (Fig. 2.1). This is especially important to secure the future productivity of agricultural plants, as The World Bank (2010) predicts that many regions of the world will experience loss of crop yield due to changes in climate by 2050 if we make no changes to our current methods or crop varieties.

Most crops contain many varieties that vary in a wide diversity of ecologically important traits. These traits can include tolerance to drought, resistance to pests and pathogens, and increased yield and nutritional content. As a result, there is a wide array of crop varieties available now, some of which may already possess the ability to withstand environmental change as well as herbivorous insect outbreaks. It will be important over the next decade to explore our crop varieties, or genotypes, to understand what traits are important in predicting a plant's endurance of stress, and eventually what physiological mechanisms are involved in this endurance. Understanding the ecological importance of genetic variation in crops under a wide array of environmental conditions will hopefully enable agriculture to mitigate any negative effects of climate change.

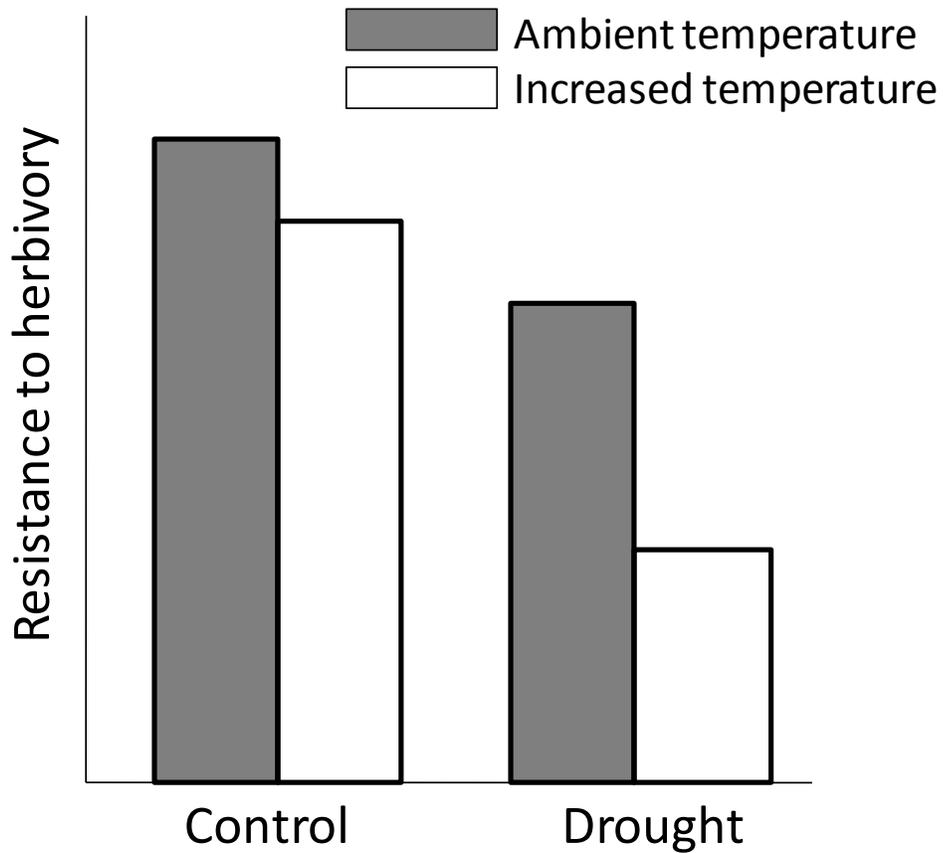


Figure 2.1 Hypothetical interaction between temperature and drought affecting the resistance of a plant to insect herbivory. Here, an increase in temperature in ambient watered (control) plants may decrease the plant's resistance to herbivory slightly, but in droughted plants, resistance may be much lower due to effects being synergistic rather than additive.

Here we seek to understand how predicted climate change might influence plant-herbivore interactions in a model crop system, soybean (*Glycine max* (L.) Merr.). To address this goal we conducted three experiments to answer the following questions: (i) How do the combined effects of drought, temperature, and herbivory affect plant performance? (ii) Do drought and temperature affect a plant's resistance to herbivory? The answer to these first two questions will provide insight into whether plant responses to climate change can be predicted by the independent effects of abiotic and biotic stressors, or whether multiple stressors might interact in complex ways to influence plant performance and their resistance to natural enemies. Finally, we asked: (iii) Do plant genotypes vary in their response to abiotic stress and resistance to insect herbivores? If soybean genotypes exist that are both tolerant to abiotic stresses associated with climate change, and are resistant to herbivores, then it might be possible to use these genotypes to develop new crops that are able to maintain high yields in the face of negative effects associated with future climate change.

METHODS

Study system

Soybean, a leguminous herbaceous plant, is an ideal system to test the effects of climate change on plants and plant-herbivore interactions. Soybean varieties have been bred for a diverse array of phenotypes, including increased resistance to drought and herbivory (Carter et al. 2004, Charlson et al. 2009b).

There are many genotypes available commercially and there are many natural and commercial insect herbivores available that are economically important pests of soybean.

Here, we chose 51 genotypes (Appendix A) to answer our research questions. These genotypes represented the wide range of maturity groups (2-8; a categorical descriptor of the time it takes a variety to flower and produce mature fruits) grown across North America. Our experiments utilized three agricultural pests common to the southeast US: *Heliothis virescens* Fabricius (Noctuidae), the tobacco earworm; *Helicoverpa zea* Boddie (Noctuidae), the corn earworm; and *Spodoptera exigua* Hübner (Noctuidae), the beet armyworm. These were chosen because they are both readily available commercially and relevant regional soybean pests.

Controlled growth experiments

To understand the combined effects of drought, temperature, herbivory and plant genotype on plant and insect performance, we conducted a series of three greenhouse and growth chamber experiments under controlled growth conditions.

Experiment 1: Effects of drought and herbivory on plant performance

We conducted a fully factorial experiment to test the combined effects of drought and herbivory on the performance of soybeans to investigate question 1. The experiment was performed at the Method Road Greenhouse facility at North Carolina State University (NCSU) during the summer of 2009 (for greenhouse

layout, see Appendix B) using the conventional soybean variety 'NC-Roy' (Burton and Carter 2005). 80 plants were grown in terra cotta pots (1650 ml, standard 15 cm round pot), with a 1:1 mix of sterile sand and sterile soil. Plants were fertilized 6 days after seed was sown with 4.2g of Osmocote slow release fertilizer pellets (14-14-14, N-P-K; The Scotts Co., Marysville, OH, USA) dispersed on the soil surface and 11-12mL of micronutrient fertilizer (8mg/L water; Scotts Peters Professional STEM Fertilizer, The Scotts Co., Marysville, OH, USA).

On day 18 after planting, we imposed drought to a random half of the plants through cessation of watering. Plants were qualitatively measured for wilting stress daily according to the following scale: 0 - no wilt; 1 - apical stem erect, a mixture of turgid and wilted leaves; 2 - apical stem $\leq 45^\circ$ angle from upright, most leaves wilting; 3 - apical stem $> 45^\circ$ angle from upright, most turgor lost; 4 - plant completely wilting with many dry leaves. Plants were 'rescued' by watering once they reached level 3, and they were given water daily thereafter. Overall, plants were subjected to drought for an average of 4.2 days.

On day 26 after planting, herbivory was imposed on a random half of the drought and control plants by placing 3rd instar *Heliothis virescens* caterpillars (supplied by the Fred Gould lab, NCSU, Dept. of Entomology) directly onto plants. This was one week after all plants had been rescued from drought. All plants were contained within bags constructed of white polyester tulle. Herbivores were left on plants to feed until ~30% of the plant was damaged (average damage = 30%) before being removed.

Entire plants were harvested 2.5 weeks after the removal of insects (on days 48 and 49) and dried at 38°C for 3 days. We then weighed above- and belowground biomass to evaluate plant performance.

Statistical analyses - The effects of drought and herbivory on soybean performance were analyzed as a two-factor analysis of variance (ANOVA). Specifically we used the model: Soybean performance = Mean_{overall} + Drought + Herbivory + Drought X Herbivory + Error. We examined the residuals of the untransformed data for each variable separately to determine whether assumptions of homogeneity of variance or normality were met. When these assumptions were violated we examined whether log- or square-root transformations of the data improved the distribution of residuals. All factors were treated as fixed effects and analyses were performed in Proc GLM of SAS (SAS Institute, Cary, NC, USA).

Experiment 2: Effects of drought, temperature and herbivory on plant performance

To further understand the combined effects of drought, temperature and herbivory on the performance of soybean to address question 1, we conducted a fully factorial experiment at the NCSU Phytotron, a growth chamber facility that allowed us to control temperature. We used 8 growth chambers, each containing 12 pots with one 'NC-Roy' plant per pot. The experiment was staggered over three trials as we could not use all chambers simultaneously, and each trial included ambient and increased temperature chambers. Chambers were programmed as follows: 13.5h light and 9.5h dark periods with 30-minute transition periods at the beginning

and end of each light period; temperature in 4 chambers were ambient, at 28.9°C and 16.1°C during day and night hours respectively, and the other 4 chambers reflected a 2.2°C increase, at 31.1°C and 18.3°C, day and night hours respectively. The mean relative humidity in the chambers was measured at 28% and 26% in ambient and increased temperature chambers respectively. The day length, ambient temperature and water conditions were chosen to mimic conditions in Raleigh, NC in mid-June, the time of year that experienced the greatest correlations between temperature and drought during the last 80 years (Portmann et al. 2009). The temperature increase was chosen to slightly exceed the 2°C target threshold set by the United Nations (UN) and the European Union (EU) panels on climate change to understand the consequences of surpassing this target (EU Climate Change Expert Group 2008). This target is the maximum amount of warming desired by the year 2050.

Soybeans were planted in plastic pots (1650 ml, standard 15 cm pot) with 1:1 mixture of sand and Perlite. Plants were fertilized 7-14 days after planting with 2.5g of Osmocote (14-14-14 N-P-K) slow release fertilizer pellets added to the soil surface and micronutrient fertilizer as described in Experiment 1.

Drought was induced on day 20 via cessation of watering. Plants were qualitatively assessed for wilt daily according to the following scale: 0 - no wilt; 1 - leaves curling, turning, or very slight loss of turgor; 2 - apical meristem erect, some leaves with wilt; 3 - apical meristem at $\leq 45^\circ$ angle from erect, most leaves with wilt; 4 - apical meristem pointed downward ($>45^\circ$ angle from erect). If the plant reached

level 4 then the plant was 'rescued' with water, and maintained a daily watering schedule thereafter. Plants were droughted for an average of 8.8 days.

Herbivory was imposed on days 36-41, depending on the length of drought within the trial, by placing 3rd instar *Heliothis virescens* (supplied by the Fred Gould lab, NCSU, Dept. of Entomology) onto one-half of soybean plants and allowing them to remove ~25% of leaf tissue (average damage = 25%). All plants were isolated in bags constructed of polyester Casa Collection White Organza fabric which were removed at the end of the herbivory period (ca. 5 days). Plants were allowed to resume normal growth for one week before harvest, which occurred between days 46-56, depending on the length of drought and herbivory within each trial. Above and belowground biomass was harvested, dried, and weighed as in Experiment 1.

Statistical analyses - Here, we examined the effects of drought, temperature and herbivory on plant performance, and the experimental design necessitated a more complex statistical model. The unit of replication varied among the three main treatment factors, whereby temperature had the fewest number of experimental units because this treatment was randomly assigned to entire chambers, whereas drought and herbivory were randomly assigned to the individual plants within chambers. As we wanted to maximize replication but had limited chamber space, we conducted multiple experimental trials, repeating the same experimental design each time. We started all analyses with the following "full model": Soybean performance = $\text{Mean}_{\text{overall}} + \textit{Trial} + \textit{Temperature} + \textit{Trial} \times \textit{Temperature} + \textit{Chamber}(\textit{Temperature}) + \textit{Drought} + \textit{Herbivory} + \textit{Trial} \times \textit{Drought} + \textit{Trial} \times \textit{Herbivory} + \textit{Temperature} \times \textit{Drought}$

+ Temperature X Herbivory + Temperature X Drought X Herbivory + Error. All effects in italics (i.e. trial, chamber and interactions involving these factors) were treated as random effects and appropriate denominator terms and degrees of freedom were determined using the 'test' statement in Proc GLM of SAS. To decrease the parameterization of the model and to increase statistical power, we removed non-significant interactions (cutoff $p > 0.15$) and blocking effects (trial and chamber) using a backwards sequential stepwise procedure. This procedure was applied to each performance variable separately and applied by starting at the highest-order interaction term. We continued the backwards removal of parameters until we reached the "reduced model"; simpler interactions and main effects were retained when higher order interactions containing the simpler terms were found to be significant, according to our criterion. Residuals were examined and transformations were performed as described in Experiment 1.

Experiment 3: Effects of drought, temperature and plant genotype on plant performance and resistance to herbivores

To determine the impact of drought on resistance to herbivores (question 2), as well as genetic variation in response to drought (question 3), we conducted a drought x genotype factorial experiment. The motivation for this experiment was to understand whether our results from Experiments 1 and 2 were consistent among a diverse array of soybean varieties, and to understand whether there existed plant genotypes that were both resistant to drought and to common herbivorous insects of

soybean. The experiment was conducted in the NCSU Method Road Greenhouse facility in the summer of 2009 (for greenhouse layout, see Appendix C).

The experiment used 51 soybean genotypes (Appendix A) with 10 replicate plants per genotype. Soybeans were planted in terra cotta pots (1650 ml, standard 15 cm pot) with 1:1 mixture of sterile sand and germinating mix soil, fertilized as described in Experiment 1, and randomized spatially. We measured the height of every plant 2 times per week until the beginning of the drought treatment from the soil surface to the primary shoot apical meristem and calculated the change in plant height per day through time, which I refer to as "plant growth rate" hereafter.

To study the effects of temperature on plants we used an existing temperature gradient in the greenhouse, which we characterized using five Extech® Instruments TH10 Temperature Dataloggers (Extech Instruments Corp., Waltham, MA, USA) evenly spaced along the length of the bench. The average day and night temperature in the greenhouse were 30.10°C and 25.77°C, respectively.

We imposed drought to half of the replicates from each genotype 24 days after planting and scored wilt daily as described in Experiment 2. Plants were droughted for an average of 11 days prior to resuming daily watering. Wilt avoidance was calculated as the number of days it took a droughted plant to reach level 3 wilt.

On day 41, we performed a no-choice resistance assay of all plants to three common agricultural insect pests of soybean (*H. virescens*, *H. Zea*, *S. exigua*). Three 3.8 cm round holes (11.34 cm²) were punched from the 2nd fully expanded trifoliate leaf below the primary apical meristem of each plant with a Marvy Uchida

LVEJCP craft punch (Uchida of America, Corp., Torrance, CA, USA). These leaf punches were placed individually into 60 x 15mm polystyrene petri dishes lined with moistened filter paper. Newly hatched caterpillars of *H. virescens*, *H. zea*, and *S. exigua* ordered from Benzon Research, Inc. (Carlisle, PA, USA) were placed individually on punched leaves, such that we assayed resistance of each plant to all three insect species. The insects were allowed to feed on a leaf punch in the petri dish for one week, at which point the insects were removed and placed in a 1.5 mL centrifuge tube. They were allowed to void their gut contents for approximately 24 hours and then were frozen. The wet weight of the insects was measured on a Mettler AT20 FACT microbalance digital scale (Mettler-Toledo, Inc., Columbus, OH, USA) and this biomass was used as a proxy for insect performance.

On day 59, aboveground biomass was harvested from all soybeans by cutting plants at the soil surface and placing them in a paper bag. The bags were dried at 38°C for approximately two days. The dried plant material was then weighed as in Experiment 1.

Statistical analyses - Here, we examined the effects of drought, temperature, soybean maturity group and plant genotype nested within maturity group, on both plant and insect performance. We analyzed these data using restricted maximum likelihood (REML) in Proc Mixed of SAS. These analyses employed the following full model: Performance = Mean_{overall} + Drought + Temperature + Maturity Group + *Genotype(Mat. Grp.)* + Drought X Temperature + Drought X Maturity Group + *Drought X Genotype(Mat. Grp.)* + Temperature X Maturity Group + *Temperature X*

Genotype(Mat. Grp.) + Drought X Temperature X Maturity Group + Drought X Temperature X Genotype(Mat. Grp.) + Error. Temperature varied continuously among plants and was treated as a covariate in analyses. Factors in italics were all treated as random effects and their significance was tested using log-likelihood ratio tests based on the comparison of -2 x restricted log-likelihood of nested models, fit to a χ^2 distribution with 1 degree of freedom. All other effects were treated as fixed and their significance was determined using *F*-tests and the appropriate degrees of freedom. As with Experiment 2, we reduced the complexity of models using a backwards stepwise selection procedure and assessed whether our residuals met assumptions of homogenous variance and normality.

To identify genetically variable plant traits correlated with plant and insect responses, we estimated the genetic variance for phenotypic traits and calculated their breeding values for each genotype. We then calculated Pearson Product Moment correlation coefficients and their corresponding p-values.

RESULTS

Experiment 1: Effects of drought and herbivory on plant performance

Drought and herbivory had independent negative effects on all measures of plant biomass, including aboveground, belowground, and total biomass (Table 2.1), reducing aboveground biomass by 24% and 20%, and belowground biomass by 10% and 19%, respectively (Fig. 2.2A,B). There was no significant interaction between drought and herbivory on either aboveground (*D x H*, *F* = 5.79, *p* = 0.07) or belowground (*D x H*, *F* = 0.01, *p* = 0.92) biomass. Drought and herbivory

Table 2.1 Effects of drought and herbivory on multiple aspects of plant performance (Experiment 1). For each response variable we include results from analysis of variance (ANOVA). We show transformations applied to the data in parentheses, which were only used when required to meet assumptions of ANOVA.

	df	SS	F	P
Total biomass				
Model	3	193.42	17.50	<.001
Drought	1	84.52	22.94	<.001
Herbivory	1	104.67	28.40	<.001
D x H	1	7.98	2.16	0.15
Error	75	276.39		
Belowground biomass (square root transformed)				
Model	3	2.62	10.01	<.001
Drought	1	0.51	5.89	0.018
Herbivory	1	2.13	24.45	<.001
D x H	1	0.001	0.01	0.92
Error	75	6.55		
Aboveground biomass				
Model	3	85.85	16.78	<.001
Drought	1	48.19	28.26	<.001
Herbivory	1	33.66	19.75	<.001
D x H	1	5.79	5.79	0.0694
Error	75	127.86		
Above: belowground biomass ratio				
Model	3	6.85	4.18	0.009
Drought	1	0.26	0.47	0.49
Herbivory	1	5.60	10.26	0.002
D x H	1	0.91	1.67	0.20
Error	75	40.95		

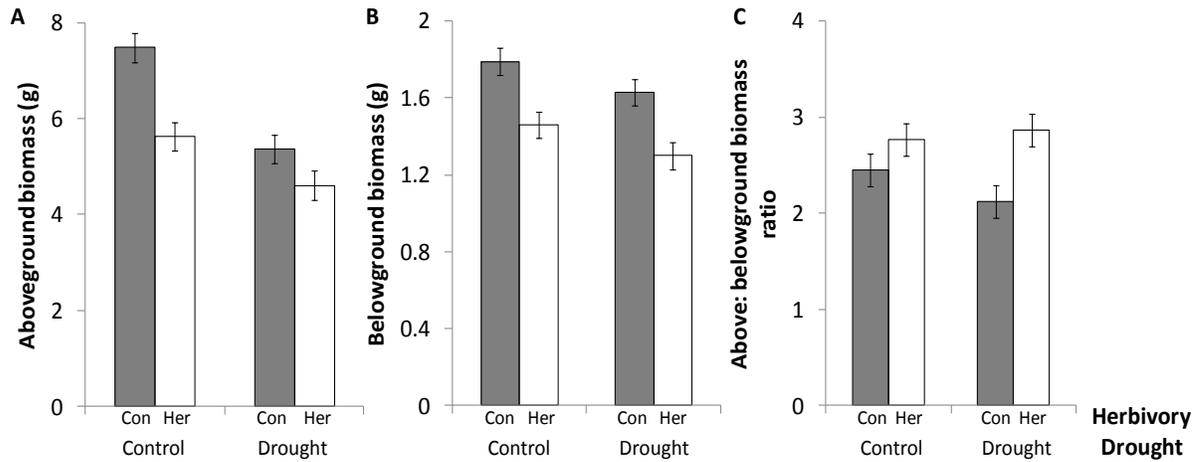


Figure 2.2 Soybean biomass across drought and herbivory treatments (Experiment 1), for **A** aboveground biomass, **B** belowground biomass, and **C** above: belowground biomass ratio.

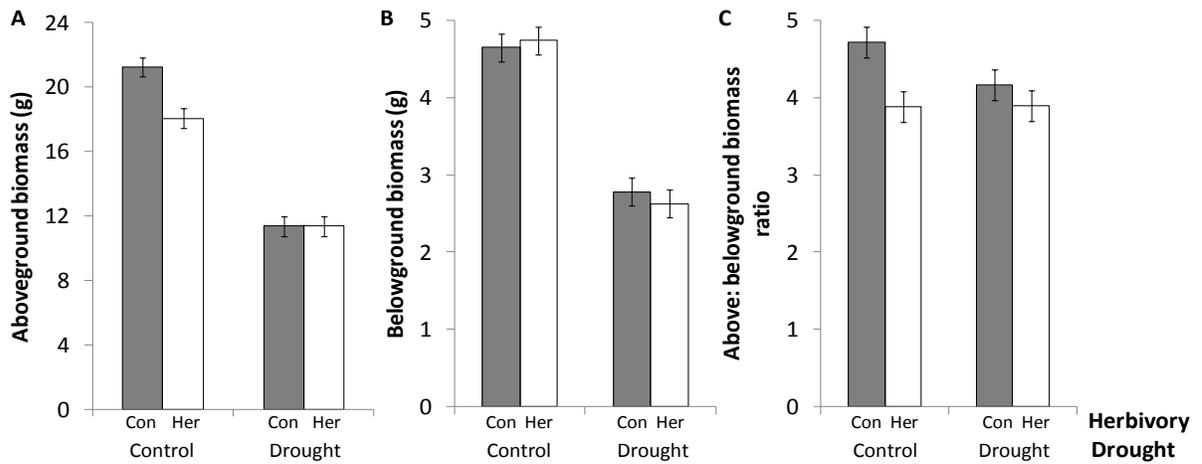


Figure 2.3 Soybean biomass across drought and herbivory treatments (Experiment 2), for **A** aboveground biomass, **B** belowground biomass, and **C** above: belowground biomass ratio.

differentially affected the relative allocation to aboveground and belowground biomass, such that drought had no significant effect on the ratio of above: belowground biomass (Table 2.1), while herbivory caused a 23% increase in the ratio. This effect was primarily caused by herbivore-damaged plants compensating by increasing/decreasing allocation to aboveground/belowground tissue (Fig. 2.2C).

Experiment 2: Effects of drought, temperature and herbivory on plant performance

Consistent with Experiment 1, drought and herbivory reduced most components of plant biomass (Table 2.2, Fig. 2.3). Drought reduced total, aboveground and belowground biomass by 43-45% (Fig. 2.3A,B). Herbivory had a weaker effect than drought, reducing total and aboveground biomass by 12-13%, while negative effects of herbivory on belowground biomass depended on the experimental trial (Table 2.2), with the only significant negative effect detected in trial 2 ($F = 3.28$, $p = 0.04$). Increasing temperature by 2.2°C had no discernible effect on any component of plant biomass (Table 2.2). As in Experiment 1, the combined stresses did not interact to influence plant performance, indicating that the negative effects of these stressors on plant performance were independent of one another.

The effects of drought and herbivory on above: belowground biomass ratios were inconsistent with Experiment 1. In Experiment 2, drought and herbivory treatments reduced the ratio by 3.5% ($F = 0.74$, $p = 0.48$) and 9.5% ($F = 24.56$, $p < 0.001$), respectively (Fig. 2.3C); recall that herbivory increased the ratio in Experiment 1 (Fig. 2.2C). In Experiment 2, there was also a weak 3-way interaction

Table 2.2 Effects of drought, temperature and herbivory on multiple aspects of plant performance (Experiment 2). For each response variable we include results from analysis of variance (ANOVA). We show transformations applied to the data in parentheses, which were only used when required to meet assumptions of ANOVA. (Table continued on following page.)

	df	SS	F	P
Total biomass				
Drought	1	2463.26	23.65	0.039
Error (drought x trial)	2.02	210.29		
Trial	2	1593.96	7.39	0.119
Error (MS[drought x trial])	2	215.59		
Temperature	1	0.44	0.03	0.853
Herbivory	1	127.82	10.03	0.002
Drought x trial	2	215.59	8.45	0.0004
Error MS (MS error)	85	1083.77		
Aboveground biomass				
Drought	1	1639.09	20.11	0.046
Error (drought x trial)	2.015	164.21		
Trial	2	1329.24	7.87	0.113
Error (MS[drought x trial])	2	168.81		
Temperature	1	1.23	0.15	0.696
Herbivory	1	107.33	13.36	0.0004
Drought x trial	2	168.81	10.51	<.001
Error MS(MS Error)	85	682.74		
Belowground biomass				
Drought	1	74.56	43.69	0.021
Error (drought x trial)	2.05	3.49		
Trial	1	11.07	4.37	0.125
Error (MS[drought x trial])	3.11	7.88		
Temperature	1	1.31	0.6	0.494
Herbivory	1	0.38	0.22	0.687
Chamber (temperature)	3	6.39	3.88	0.012
Error (herbivory x trial)	2.05	3.59		
Drought x trial	2	3.5	3.19	0.047
Herbivory x trial	2	3.6	3.28	0.043
Error MS(MS error)	79	43.33		

Table 2.2 (cont'd.)

	df	SS	F	P
Aboveground: belowground biomass ratio (log transformed)				
Drought	1	0.05	0.74	0.48
Error .966 x MS(drought x trial)	2.04	0.14		
Trial	1	2.56	42.45	0.017
Error 0.8115 x (MS[drought x trial])	2.25	0.14		
Temperature	1	0.004	0.03	0.867
Error (chamber (temperature))	2.98	0.39		
Herbivory	1	0.45	24.56	<.001
Chamber (temperature)	3	0.39	7.05	0.0003
Error (herbivory x trial)				
Drought x trial	2	0.14	3.84	0.026
Drought x temperature	1	0.04	2.37	0.128
Drought x temperature x herbivory	3	0.12	2.26	0.088
Error MS(MS error)	77	1.4		

between drought, temperature and herbivory in above: belowground biomass ratio, indicating that these three stresses in combination may influence the plant's relative allocation of biomass.

Experiment 3: Effects of drought, temperature and plant genotype on plant performance and resistance to herbivores

Plant performance - As in the previous two experiments, drought significantly reduced aboveground biomass, here by 37%, while the main effect of temperature had no effect on plant performance (Table 2.3). There was, however, a significant interaction between drought and temperature, which was caused by a negative effect of temperature on biomass for plants that received ambient water, but no effect of temperature when plants were subjected to drought (Fig. 2.4).

Soybean genotypes varied in aboveground biomass by 90% from the smallest to largest genotype (for experiment means see Appendix A), and this effect was not dependent on either drought ($G \times D$, $F = 0.1$, $p = 0.38$) or temperature ($G \times T$, $F = 0.0$, $p = 0.5$). The lack of an interaction here suggests that the 51 soybean genotypes did not express any genetic variation for tolerance to water or temperature stress (tolerance being defined as proportionally non-significant reductions in biomass due to stress). As expected, maturity groups varied in aboveground biomass (Table 2.3), and this effect of maturity group did not depend on either drought or temperature ($p > 0.5$ for all 2-way and 3-way interactions).

Genotypes varied in the ability to resist wilting (i.e. wilting avoidance, Table

Table 2.3 Effects of drought and temperature on soybean aboveground biomass (Experiment 3). We include results from analysis of variance (ANOVA) for aboveground biomass. This experiment tested 51 soybean genotypes (510 plants total) from 6 maturity groups. Abbreviations ndf and ddf refer to numerator and denominator degrees of freedom respectively.

Aboveground biomass (reduced model)					
	ndf	ddf	SS/Var	X ² /F	P
Drought	1	499		11.5	0.0008
Temperature	1	499		0.72	0.396
Maturity group	5	499		3.89	0.005
Temperature x drought	1	499		9.02	0.003
Genotype (maturity group) ¹	1	3048.8	5.3604	50.2	6.94E-13
Error			19.5737		

¹This effect was tested with a Chi-squared statistic while all other effects were tested with F-tests.

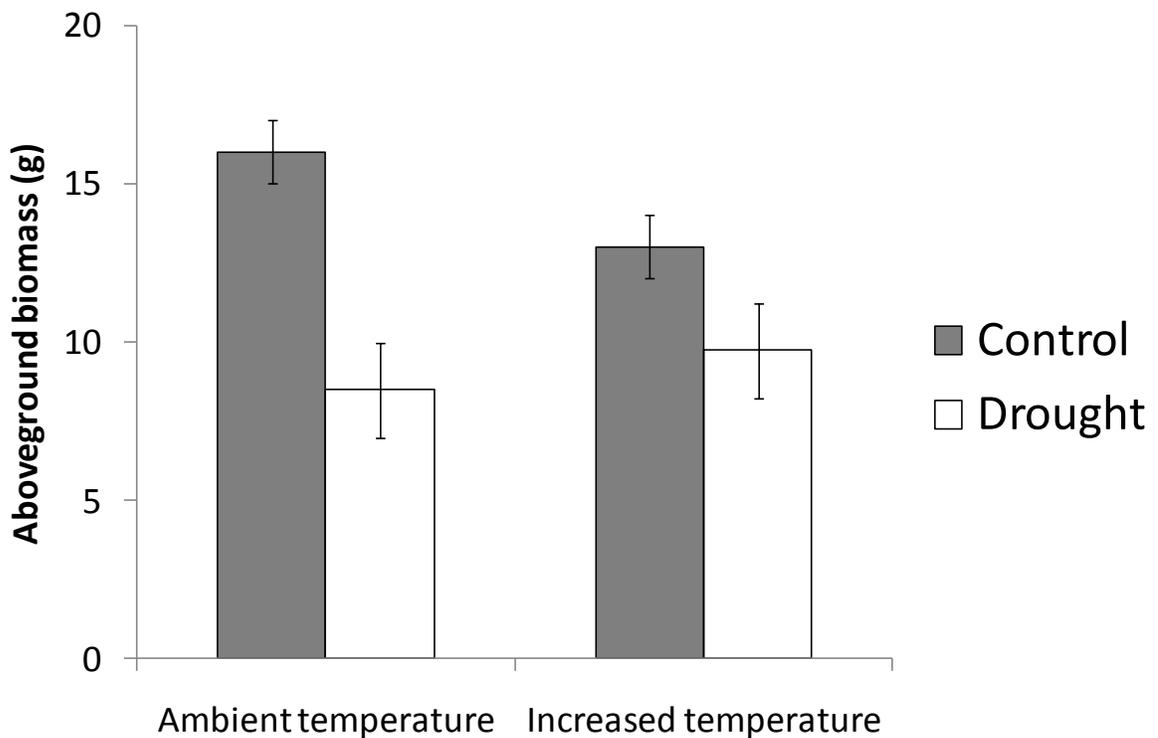


Figure 2.4 Soybean aboveground biomass for drought x temperature interaction (Experiment 3).

2.4), as genotypes began to exhibit severe wilting after 3 days, while others still had not wilted after 15 days (wilt avoidance was measured as the number of days until level 3 wilt was reached, i.e. apical meristem at $\leq 45^\circ$ angle from erect, most leaves with wilt). This is important, because it indicates that some existing soybean varieties are already able to endure moderate levels of water stress.

Plant resistance to herbivores – Abiotic stress had conflicting impacts on the susceptibility of plants to herbivores. Overall, drought increased susceptibility of plants to herbivores when all genotypes are observed together, where the three caterpillar species gained 16-24% more biomass on leaves excised from droughted plants compared to controls. This increase in susceptibility was statistically significant for *H. virescens* and *H. zea*, but not *S. exigua* ($F = 2.95$, $p = 0.09$; Table 2.5). Increasing temperature resulted in an overall decrease in insect performance (Table 2.5, Fig. 2.5), where insect biomass decreased 8-17%. However, variation in biomass fluctuated throughout the temperature gradient, such that the highest temperatures did not result in the smallest insect mass, and, it is important to note that the plants were exposed to temperature variation, not the insects. The temperature results were, again, significant for *H. virescens* and *H. zea*, but not *S. exigua* ($F = 0.06$, $p = 0.8$; Table 2.5).

Plant genotypes varied in their resistance to herbivores, but these effects depended greatly on whether or not plants experienced drought and on the focal insect species. Despite the overall trend for plants to exhibit increased susceptibility

Table 2.4 Effects of temperature and plant genotype on wilt avoidance in droughted soybeans (Experiment 3). We include results from analysis of variance (ANOVA) for wilt avoidance, which was measured as the number of days until level 3 wilt (apical meristem at $\leq 45^\circ$ angle from erect, most leaves with wilt) was reached.

Wilt avoidance (reduced model)				
	df	Error	X ² /F	P
Temperature	1	199	0.06	0.8
Maturity group	5	45	1.38	0.25
Genotype (maturity group) ¹	1		6.2	0.006

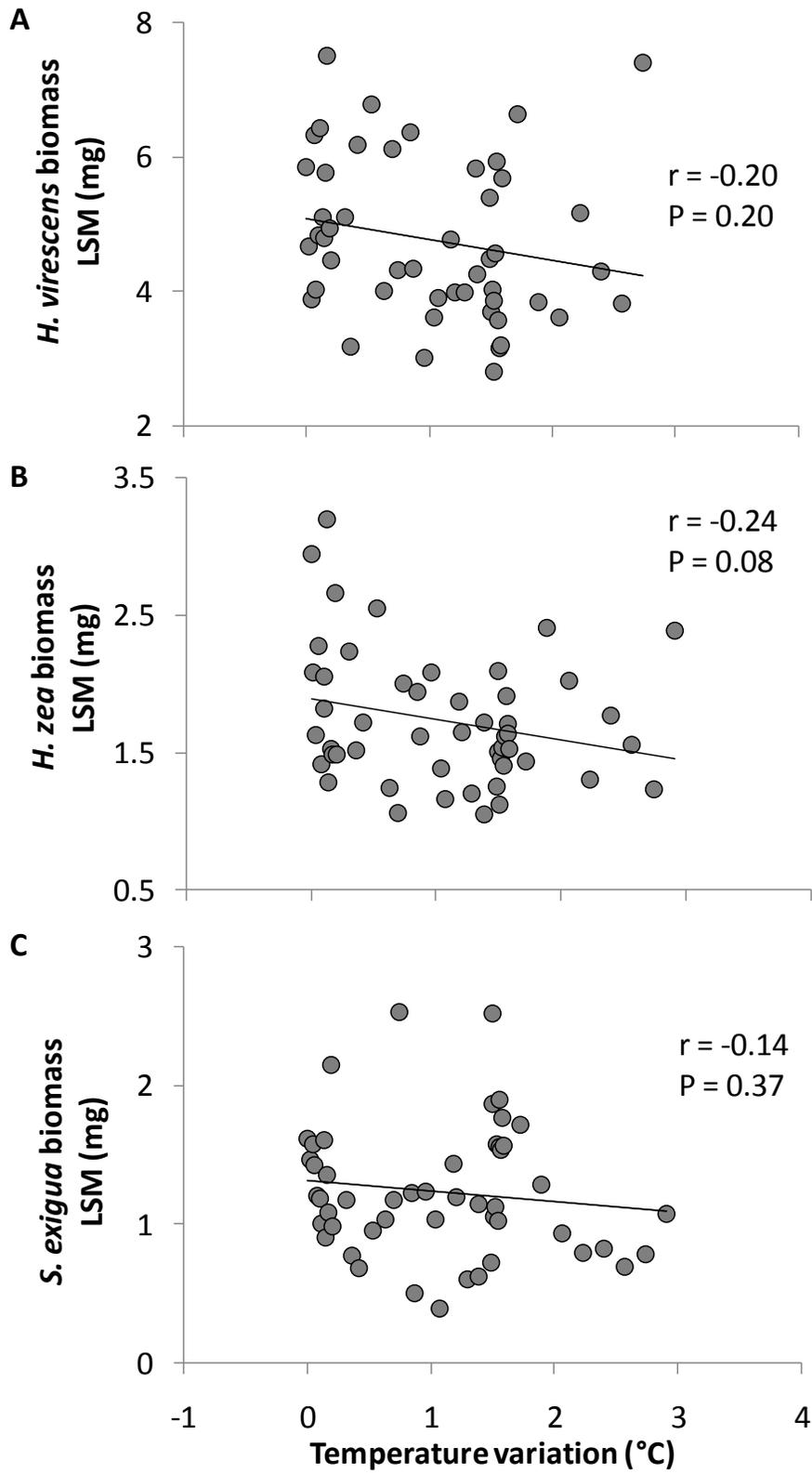
¹This effect was tested with a Chi-squared statistic while all other effects were tested with F-tests.

Table 2.5 Effects of drought, temperature and plant genotype on insect biomass for 3 common regional soybean herbivores (Experiment 3). We include results from analysis of variance (ANOVA) for biomasses, which were measured after a 7 day no-choice feeding assay of each insect on 51 soybean genotypes.

Insect biomass (reduced models)				
	df	X ² /F	P	
<i>Heliothis virescens</i>				
Drought	1,95	9.42	0.0028	
Temperature	1,366	7.66	0.0059	
Maturity group	5,95	0.55	0.74	
Genotype ¹	1	0.1	0.38	
Genotype x drought	1	2.5	0.057	
<i>Helicoverpa zea</i>				
Drought	1,50	12.9	0.0007	
Temperature	1,373	10.4	0.001	
Maturity group	5,45	2.61	0.024	
Genotype (maturity group) ¹		0.1	0.376	
Genotype x drought		5.9	0.0076	
<i>Spodoptera exigua</i>				
Drought	1,50	2.95	0.0919	
Temperature	1,353	0.06	0.8	
Maturity group	5,45	1.38	0.25	
Genotype (maturity group) ¹	1	0	0.5	
Genotype x drought	0.016	5.2	0.0113	

¹This effect was tested with a Chi-squared statistic while all other effects were tested with F-tests.

Figure 2.5 Effects of temperature on the performance of herbivorous insects (Experiment 3), for **A** *Heliothis virescens*, **B** *Helicoverpa zea*, and **C** *Spodoptera exigua* from a no-choice bioassay, where the x-axis represents temperature variation in the experiment from the lowest value measured (shown by 0°C), and each point represents the mean biomass for all insects feeding on the plants (across all genotypes) grown at that temperature. Fit lines have been added.



to herbivores following drought, plant genotype and drought interacted to influence all herbivore species studied (Table 2.5). Reaction norm plots (Fig. 2.6) show that drought increased the susceptibility of many plant genotypes to herbivores (e.g. bold solid lines in Fig. 2.6A), but for each herbivore species, reaction norms also show that drought increased the resistance of some plant genotypes to herbivores (e.g. bold dashed lines in Fig. 2.6A). Soybean genotypes exhibited positive genotypic correlations in susceptibility among all three herbivore species (Table 2.6, Fig. 2.7), indicating that if a soybean genotype was susceptible to one herbivore species, it was likely to be susceptible to the others.

We were particularly interested in whether a plant's ability to tolerate drought or avoid wilting might be genotypically correlated with the response of plant susceptibility to herbivores. When drought tolerance was measured as the dry weight of the droughted soybean biomass minus the dry weight of the control soybean biomass, there were no correlations between drought tolerance and insect susceptibility following drought. All correlations of drought tolerance to insect susceptibility were negative, however the correlation was only mildly significant for one insect (*H. zea*: $p = 0.03$, $r = -0.31$), and there was a great deal of scatter in all three correlations. When wilt avoidance was measured as a number of days until a critical level of drought was observed (qualitative drought scoring level 3), there were correlations between genotypic wilt avoidance and susceptibility to insect herbivory (Fig. 2.8). These were significant for *H. zea* and *S. exigua*, indicating that there may be some genotypes of soybean that are resistant to both drought and certain

Figure 2.6 Effects of drought treatment x plant genotype on insect biomass (Experiment 3), for **A** *Heliiothis virescens*, **B** *Helicoverpa zea*, and **C** *Spodoptera exigua*. For each graph, black lines represent one soybean genotype and reflect its corresponding directional influence on insect performance following drought. Note in **A**: the two heavy solid lines represent two arbitrary soybean genotypes whose droughted plant means resulted in higher insect performance than their control means; the two heavy dotted lines represent two arbitrary genotypes whose droughted plant means resulted in lower insect performance than their control means. Heavy black dots in each chart represent overall means for control and drought. These charts illustrate vast genotypic differences in soybean resistance to herbivory following drought.

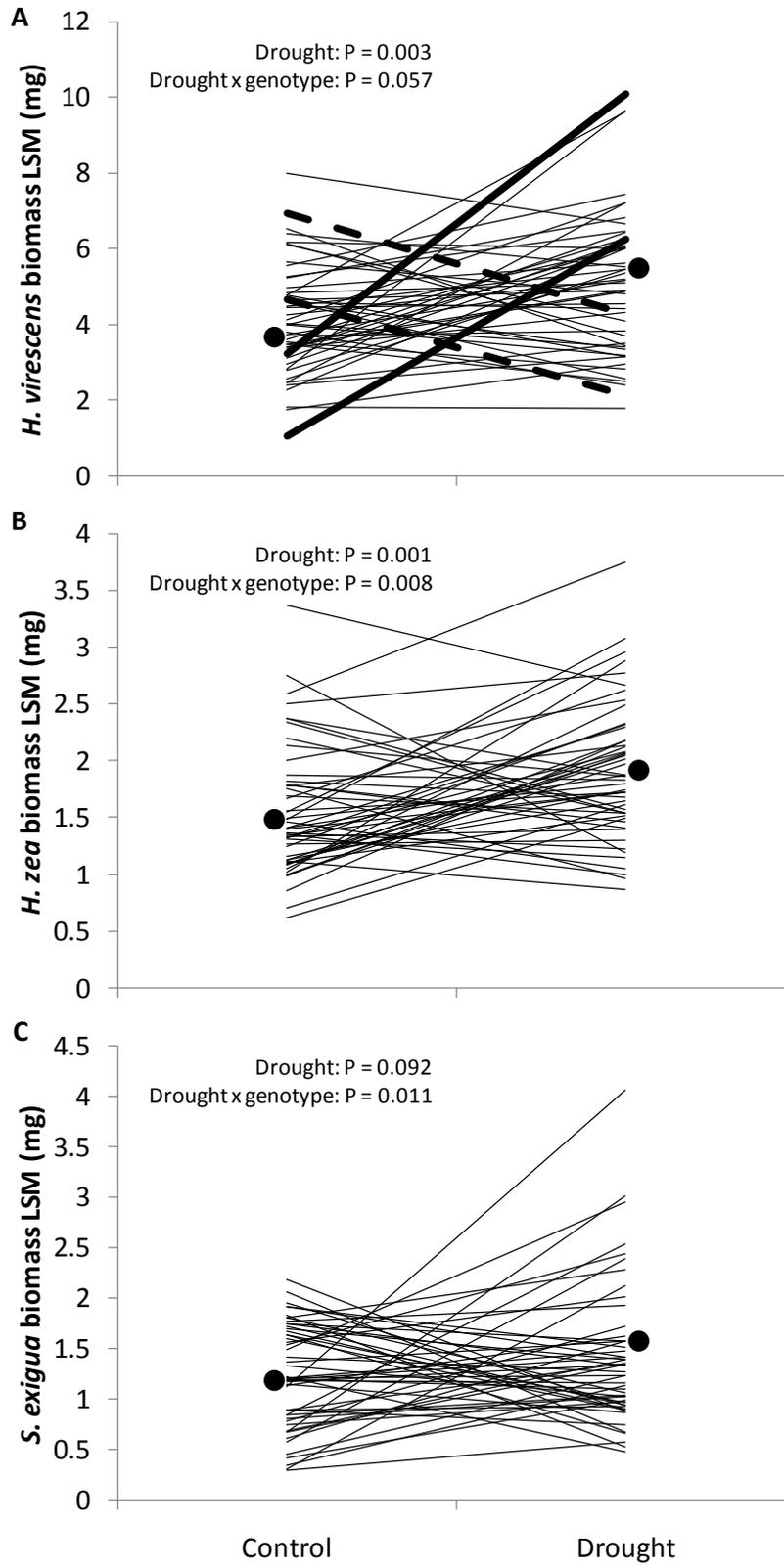


Table 2.6 Genotypic correlations between herbivorous insect biomasses (Experiment 3). We include results from analysis of variance (ANOVA) for 3 insect species that each fed on 51 soybean genotypes in a no-choice feeding assay following a drought treatment.

Insect biomass correlations					
	Treatment	df	F	P	Error
<i>H. virescens x H. zea</i>					
	Control	1,50	9.0077	0.0042	1.7581
	Drought	1,50	21.9541	<0.0001	2.4989
<i>H. virescens x S. exigua</i>					
	Control	1,50	15.9475	0.0002	1.5702
	Drought	1,50	8.3805	0.0056	3.0900
<i>S. exigua x H. zea</i>					
	Control	1,50	12.0609	0.0011	0.25801
	Drought	1,50	12.7611	0.0008	0.28523

Figure 2.7 Soybean genotypic variation in insect performance correlations (Experiment 3), for **A** *H. virescens* x *H. zea*, **B** *H. virescens* x *S. exigua*, and **C** (below) *S. exigua* x *H. zea*, where axes represent insect biomass (mg) and each point represents the least squares means (LSM) for insects feeding on all controls (ambient water) of each soybean genotype. Trend lines have been added. For each graph, there is a positive correlation in insect performance suggesting that if a soybean genotype is susceptible to herbivory by one insect, it is likely to be susceptible to herbivory by the other insects.

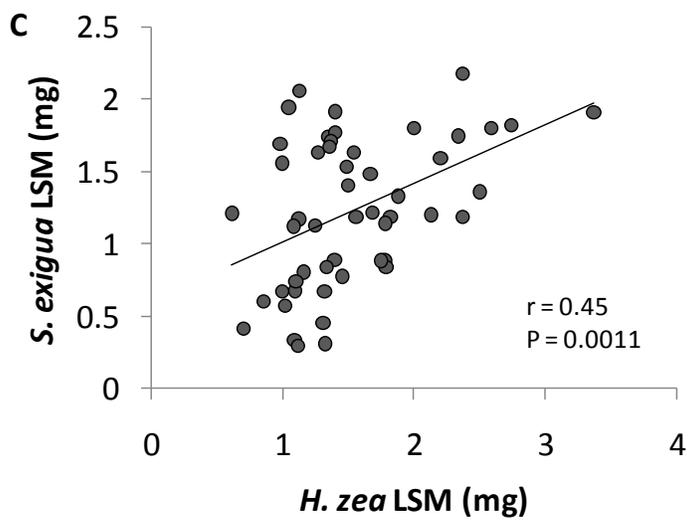
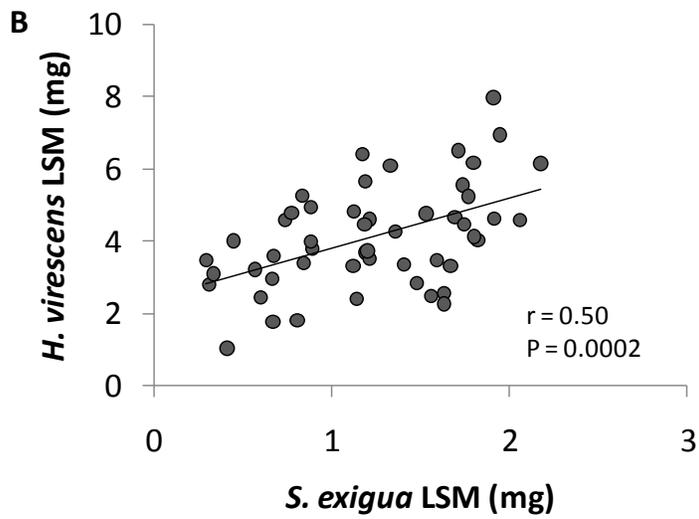
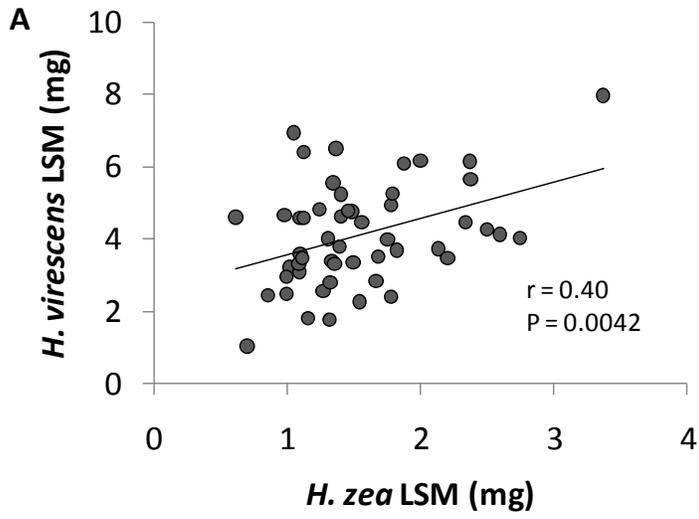
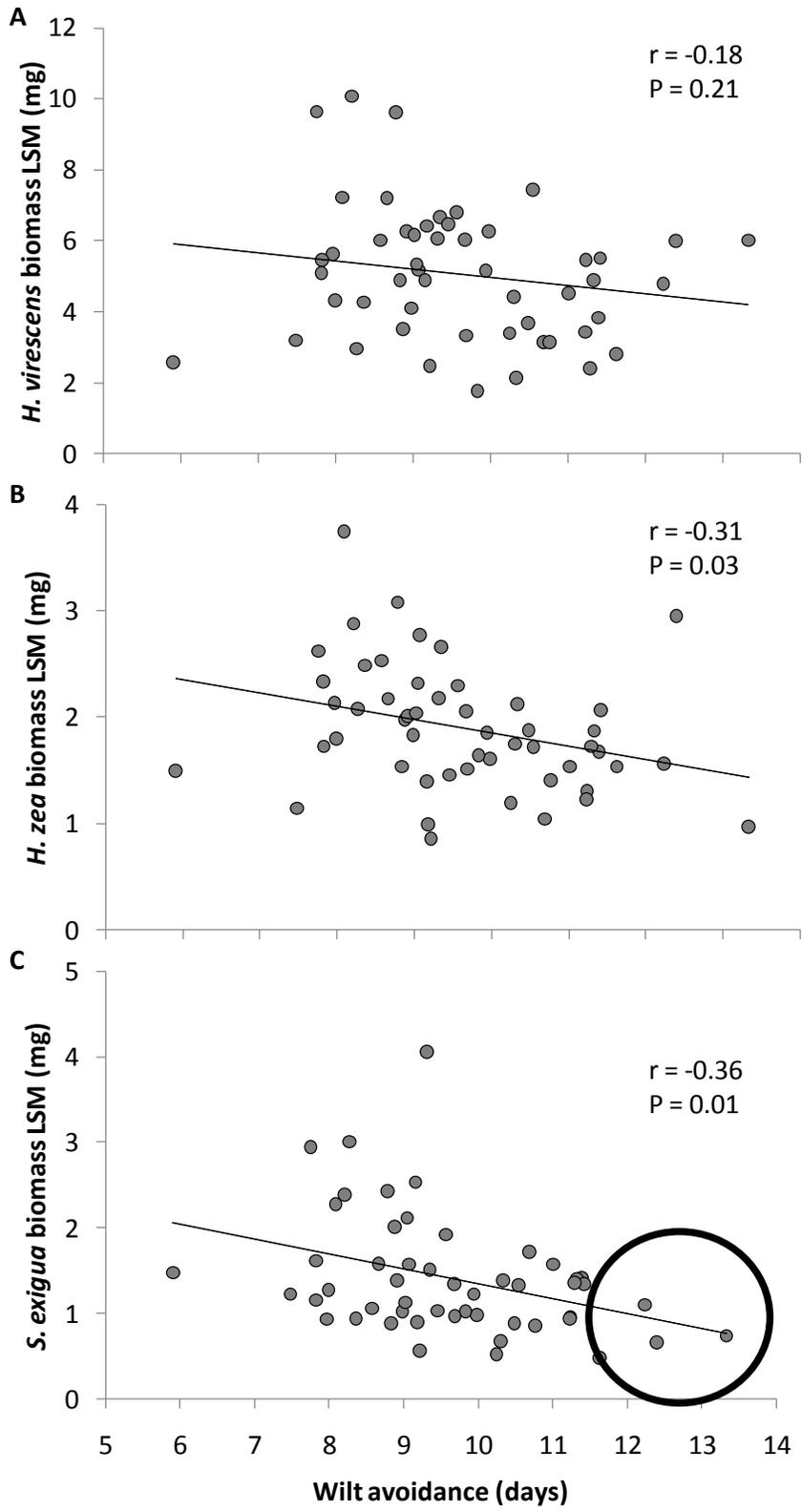


Figure 2.8 Soybean genotypic variation in wilt avoidance x insect biomass (Experiment 3), for **A** *Heliothis virescens*, **B** *Helicoverpa zea*, and **C** *Spodoptera exigua*. Wilt avoidance was measured as a number of days until level 3 wilt (apical meristem at $\leq 45^\circ$ angle from erect, most leaves with wilt) was reached. Each point represents the least squares means of insect biomass of insects feeding on one soybean genotype. Trend lines have been added. Note in **C**: the large circle illustrates soybean genotypes that were able to avoid wilt the longest and also resulted in the poorest performance of *S. exigua*, indicating that these genotypes are potential candidates for being tolerant of drought and resistant to herbivory.



herbivorous insects. All three insects negatively correlated with wilt avoidance, however they only explained less than 10% of the variation. Wilt avoidance did not correlate with drought tolerance ($p > 0.3$). We also looked at the correlation between plant growth rate (calculated rate of increase in plant height) and insect performance among drought treatments and found that there were no significant correlations, as each correlation (6 total) explained less than 6% of the variation (all $p > 0.09$).

Overall, there were no consistent effects of maturity group on susceptibility to herbivores. Maturity groups varied in susceptibility to *H. zea* (Fig. 2.9), with the earliest maturity groups (2 and 4) being most susceptible. Maturity group had no clear effect on the other two herbivore species and maturity group never interacted with drought or temperature to affect herbivores.

DISCUSSION

Our experiments provide insight into how abiotic and biotic stressors associated with climate change will influence plant performance and plant-herbivore interactions in a model crop system. Four specific results are most important in this regard. First, drought and herbivory had strong and consistent negative effects on plant performance, but these factors did not interact with one another. Second, modest increases in temperature had, at best, weak effects on soybean performance and the resistance of soybean to herbivores. Third, drought tended to increase the susceptibility of plants to herbivores (Experiment 3), but this effect varied dramatically among existing soybean genotypes. And fourth, we show that

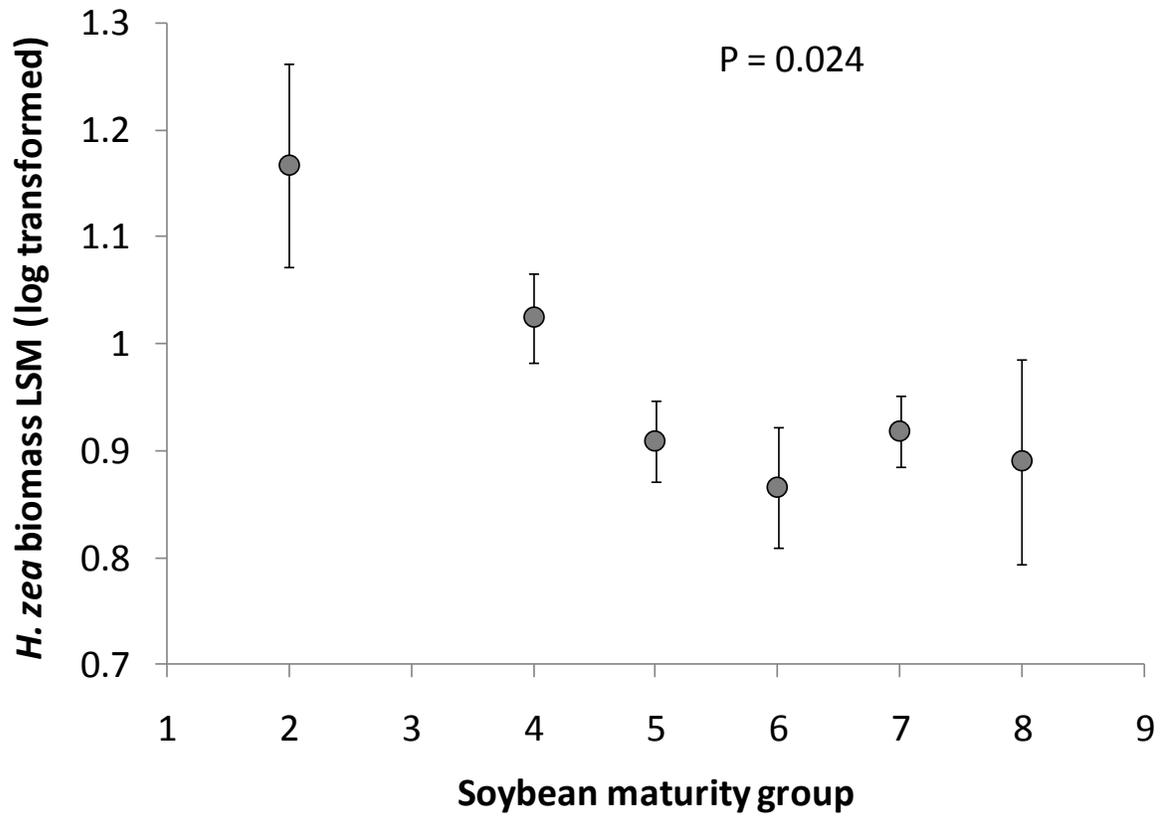


Figure 2.9 *Helicoverpa zea* biomass as a function of soybean maturity group (Experiment 3). Each point represents the mean biomass of insects feeding on a maturity group. The larger the maturity group, the longer the time until fruit maturation.

the ability of soybean to avoid wilting can be genetically correlated with resistance to two of our herbivores tested.

Effects of drought and herbivory on plant performance

Drought and herbivory negatively impacted plant performance consistently and independently. Drought consistently reduced all measurements of soybean biomass in all three experiments. Herbivory generally reduced performance, but not for every measurement – belowground biomass in Experiment 2 was not affected; the reason for this difference is not clear.

These results suggest that the effects of changes in drought and herbivory related to climate change on plant performance can be predicted by studying these factors in isolation. This is important because it suggests that the effects of these multiple stressors do not lead to synergistic negative effects, as is sometimes seen in other biological systems (Relyea 2003, 2004, 2005, Silva et al. 2010). Many studies report negative impacts of drought (Hoogenboom et al. 1987, Ray and Sinclair 1998, Liu et al. 2003, James et al. 2008) and herbivory (Kamalay et al. 1997, Rypstra and Marshall 2005, Costamagna et al. 2007) on soybean biomass, however there are few studies to our knowledge that investigate biotic stresses in combination with drought in soybean (Smelser and Pedigo 1992).

Our results have important implications for theories that predict effects of stress on plant defenses against herbivores. While the Plant Stress Hypothesis states that abiotic stress will increase the nitrogen: carbon ratio in leaves, increasing

susceptibility to insect herbivores, our results imply that this is not the case in soybean. If the nitrogen: carbon ratios were altered in our soybean due to drought, it appears as though it will not be enticing to the three insects we studied. It is questionable whether a droughted plant would be able to continue nitrogen uptake in light of reduced water uptake from drought and reduced carbon substrate synthesis due to insect pressure (Johnson 1985). Another theory, the Optimal Partitioning Theory, which states that a plant under physiological stress will apportion its new biomass to the plant organ responsible for acquiring the limiting resource (Brouwer 1963, Johnson 1985, McCarthy and Enquist 2007), presents a logical biomass partitioning model of plants under stress. This theory may be able to explain some of our above: belowground ratio results, however it is also not logical that this theory can apply to a plant under both drought and herbivory stress, as both water and carbohydrate needed for building tissue would be limited.

Effects of temperature on soybean performance and plant resistance to herbivores

Our experiments suggest that modest increases in temperature (2.2°C) have little effect on soybean performance. Thus, if the UN and EU's temperature increase target of 2°C (EU Climate Change Expert Group 2008) is met, then the increased temperature may have no discernible impact on soybean yields and their interactions with herbivores, at least in areas with ambient temperatures comparable to 28.9°C. The only interaction that impacted any performance measurement was the drought x temperature interaction in Experiment 3, potentially because the

variation in temperature for this experiment at times was much greater than 2°C (overall temperature variation range was 5°C).

Abiotic stress can impact a plant's ability to defend itself against herbivorous attacks by affecting the plant's biochemistry. Temperature independently impacted insect performance in *H. virescens* and *H. zea* but not in *S. exigua*, however it never explained more than 6% of the total variation in herbivore performance. This suggests that temperature was not a primary influence and that only some of our insects, and none of our soybean types, responded to temperature increases, contrary to the Plant Stress Hypothesis. But this also suggests that not all species of insect will respond to the physiological changes that soybean exhibit under abiotic stress in the manner presented in the Plant Stress Hypothesis, supporting Deutsch et al. (2008), who suggests that insect species will respond differently to global warming based on physiological tolerance. Specifically, insects at greater latitudes have broader thermal tolerances and will not be impacted by predicted temperature increases or may even benefit from them, whereas tropical insect species that are currently existing at their thermal optima will be strongly impacted by temperature increases due to lack of physiological tolerance. In our experiment, the plants were exposed to temperature variation and not the insects. However, we speculate that it may be possible that a similar argument related to thermal tolerance due to life history traits and genetics can be made for plants. We argue below that some of the inconsistency in these results may be explained by genetic variation in the response of different plant genotypes to abiotic stress.

Effects of drought on plant resistance to herbivores and impacts of genetic variation

Drought increased susceptibility of soybean to insect herbivores across all soybean genotypes tested, but this result varied substantially when observing genotypes individually. As illustrated in Fig. 2.6, there was broad variability in both direction and magnitude of the soybean genotypes' response to herbivores following drought. This means that while drought can be thought of as an overall stressor that can weaken soybean defenses, supporting the Plant Stress Hypothesis, it might be possible to select soybean genotypes that can increase their resistance following drought. These genotypes will make good candidates for future breeding of stress-resistant soybeans.

The effect of genetic variation in this study was important and overarching. It is intuitive that genotype would affect aboveground biomass, as this study focused on vegetative phases only, preventing later maturity groups (6 represented here) from "catching up" in their development. Genotype impacted wilt avoidance in droughted plants (Table 2.4) indicating that there will be genetic variation in response to drought stress. This also seems intuitive, as it has been studied thoroughly (James et al. 2008, Charlson et al. 2009b, Alam et al. 2010, Stolf-Moreira et al. 2010b). The variation in the insects' responses to the genotype x drought interaction was broad, and while genotype did not independently influence those responses, it is clear that there are physiological differences that occur in each genotype after stress that directly influences the palatability of the plant to the insects, resulting in some genotypes showing marked resilience in the face of stress.

It has been shown that plant genotype x environment interactions can strongly impact insect herbivory (Johnson and Agrawal 2005, Cotter and Edwards 2006, Tack et al. 2010) and this study seems to support this.

Genotypic correlations in plant traits and insect resistance

Soybean variation in phenotypic traits has been of interest to humans since the domestication of soybean. With the advent of modern technological advances in genetics, scientists have been able to advance plant breeding such that genes responsible for selected traits are now being identified and utilized. Currently, the US Dept. of Agriculture (USDA) maintains over 18,000 accessions in their germplasm collection and globally there are over 156,000 accessions available (Carter et al. 2004). The USDA also maintains a collection of over 550 near isogenic lines of soybean using 11 recurrent parents (Carter et al. 2004). This allows breeders to study the agronomic impact and molecular basis of selected traits such as leaf morphology and pubescence, plant growth rate, and disease resistance, among others. The current sequencing of the soybean genome will also allow breeders to gain better insight into the genes that correspond to desired traits and the expression of those genes (Kim et al. 2010, Lam et al. 2010, Wu et al. 2010). Here we discuss the phenotypic traits that correlated to herbivory resistance in this study.

Some of the most interesting results of this study related to the correlation between phenotypic traits of soybean genotypes and herbivory resistance. We found that there was a positive genotypic correlation among all three insects studied for

drought and control plants (Table 2.6, Fig. 2.7). This implies that, in general, susceptibility to one herbivore can predict susceptibility to other herbivores (Maddox and Root 1990, Leimu and Koricheva 2006, Johnson et al. 2009). We also mentioned previously that wilt avoidance correlated with herbivory resistance (Table 2.4, Fig. 2.8), where, on average, slower wilting plants tended to be more resistant to insects. Identifying these types of correlations and genotypes that maintain high performance under multiple stresses could provide breeders, and eventually farmers, with new varieties that might be better suited to future climates.

Predicting insect responses to stressed plants can be very difficult. It has been shown that the intensity level of stress as well as the age of the leaves will affect the insect response, and that traits such as size, biomass, water, nitrogen content and leaf mass per area were not good predictors of responses of a *Spodoptera* species to stressed plants (Mody et al. 2009). This reinforces the notion that different insect species will not always exhibit the same responses in their interactions with stressed plants. This seems to agree with Schoonhoven et al. (2005) who suggests that there is no simple prediction to describe the interaction between insects and water stressed plants, and Gutbrodt et al. (2011) who found that variations in intensity of drought and insect specificity will produce varying insect responses. This may help clarify why Zhang et al. (2009) found that global warming and flood/drought events will not have a significant impact on pest outbreaks. And, there is yet another theory, the Plant Vigor Hypothesis, which suggests that insects will preferentially feed on healthy, vigorous plants (Price 1991). This contradicts the

view by Rhoades (1979) that under drought stress, a plant's synthesis of defensive compounds would be hindered. This leaves the subject of abiotic stress' impact on herbivory of plants without strong generalizations. But our work presented here will hopefully illustrate that it is possible to investigate these challenging ecological questions within specific systems in order to disseminate some of the complexity.

Implications and future directions for research

Our finding that some soybean genotypes can withstand multiple stresses has important implications for farmers and soybean breeders alike. It appears that current varieties of soybean can be used to mitigate negative effects of climate change. These varieties may be commercially viable themselves, or they may be used to breed even stronger future varieties. The key missing component to this is the physiological and molecular understanding of plant responses to stress. While morphological and phenological traits may guide our investigations (Sadok and Sinclair 2010, Sinclair et al. 2010), they do not tell the complete story. It will be important for future stress studies on soybean to investigate the molecular basis for abiotic stress tolerance, and these studies are beginning to surface (Charlson et al. 2009a, Du et al. 2009a, Du et al. 2009b, Manavalan et al. 2009, Nguyen et al. 2009, Alam et al. 2010, Stolf-Moreira et al. 2010a, Stolf-Moreira et al. 2010b). Once scientists can synthesize this molecular data we can gain a clearer picture of soybean stress tolerance.

Experiments like the ones presented here are essential to understanding how natural and managed ecosystems will be affected by climate change. While no study can investigate every potential factor at once, we can proceed by doing our best to test as many factors as possible. This can only serve to broaden our understanding of the potential impacts of climate change. This is especially true when discussing crop systems, as securing our food supply throughout all phases of climate change is important. Here, we have investigated three stresses on one crop system in controlled, common environment experiments. It will be very important to take these questions to the field where we can examine whether our main results are observed in a more natural agricultural setting. And, as discussed above, understanding plant physiology and molecular mechanisms associated with plant responses would provide further insight into the mechanisms underlying these responses.

This study attempts to address some of our lack of dynamic understanding of the interactions between our rapidly changing environment and the biological responses that will result. Some believe that ultimately, species have the potential to adapt to climate change, and that it is human influence that will prove most detrimental to our ecological systems (Grime et al. 2008). But finding complacency in this view can be very dangerous, as we should never stifle our curiosity about the impact of nature or the unpredictability of our planet's ever-changing climate. Understanding climate change and its effects will likely prove an eternal process.

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REFERENCES

- Adams, H. D., M. Guardiola-Claramonte, G. A. Barron-Gafford, J. C. Villegas, D. D. Breshears, C. B. Zou, P. A. Troch, and T. E. Huxman. 2009. Temperature sensitivity of drought-induced tree mortality portends increased regional die-off under global-change-type drought. *Proceedings of the National Academy of Sciences of the United States of America* **106**:7063-7066.
- Alam, I., S. A. Sharmin, K. H. Kim, J. K. Yang, M. S. Choi, and B. H. Lee. 2010. Proteome analysis of soybean roots subjected to short-term drought stress. *Plant and Soil* **333**:491-505.
- Brouwer, R. 1963. Some aspects of the equilibrium between overground and underground plant parts. *Jaarboek van het Instituut voor Biologisch en Scheikundig onderzoek aan Landbouwgewassen* **213**:31-39.
- Burton, J. W. and T. E. Carter. 2005. Registration of 'NC-Roy' soybean. *Crop Science* **45**:2654-2654.

- Carter, T.E., R. L. Nelson, C. Sneller, and Z. Cui, 2004. Genetic Diversity in Soybean. Soybeans: Improvement, Production, and Uses, Third Edition (Agronomy). Boerma, H. R. and J. E. Specht (Ed.). American Society of Agronomy, Madison, WI. 303-416.
- Charlson, D., K. Korth, and L. Purcell. 2009a. Allantoate amidohydrolase expression is independent of drought tolerance in soybean. *Plant Biology (Rockville)* **2009**:135.
- Charlson, D. V., S. Bhatnagar, C. A. King, J. D. Ray, C. H. Sneller, T. E. Carter, and L. C. Purcell. 2009b. Polygenic inheritance of canopy wilting in soybean *Glycine max* (L.) Merr. *Theoretical and Applied Genetics* **119**:587-594.
- Costamagna, A. C., D. A. Landis, and C. D. Difonzo. 2007. Suppression of soybean aphid by generalist predators results in a trophic cascade in soybeans. *Ecological Applications* **17**:441-451.
- Cotter, S. C. and O. R. Edwards. 2006. Quantitative genetics of preference and performance on chickpeas in the noctuid moth, *Helicoverpa armigera*. *Heredity* **96**:396-402.
- Deegan, L. A., J. L. Bowen, D. Drake, J. W. Fleeger, C. T. Friedrichs, K. A. Galvan, J. E. Hobbie, C. Hopkinson, D. S. Johnson, J. M. Johnson, L. E. Lemay, E. Miller, B. J. Peterson, C. Picard, S. Sheldon, M. Sutherland, J. Vallino, and R. S. Warren. 2007. Susceptibility of salt marshes to nutrient enrichment and predator removal. *Ecological Applications* **17**:S42-S63.
- Dermody, O., B. F. O'Neill, A. R. Zangerl, M. R. Berenbaum, and E. H. DeLucia. 2008. Effects of elevated CO₂ and O₃ on leaf damage and insect abundance in a soybean agroecosystem. *Arthropod-Plant Interactions* **2**:125-135.
- Deutsch, C. A., J. J. Tewksbury, R. B. Huey, K. S. Sheldon, C. K. Ghalambor, D. C. Haak, and P. R. Martin. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings of the National Academy of Sciences of the United States of America* **105**:6668-6672.

- Du, W. J., M. Wang, S. X. Fu, and D. Y. Yu. 2009a. Mapping QTLs for seed yield and drought susceptibility index in soybean (*Glycine max* L.) across different environments. *Journal of Genetics and Genomics* **36**:721-731.
- Du, W. J., D. Y. Yu, and S. X. Fu. 2009b. Detection of quantitative trait loci for yield and drought tolerance traits in soybean using a recombinant inbred line population. *Journal of Integrative Plant Biology* **51**:868-878.
- Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl, and L. O. Mearns. 2000. Climate extremes: Observations, modeling, and impacts. *Science* **289**:2068-2074.
- EU Climate Change Expert Group 'EG Science'. 2008. The 2°C Target: Information Reference Document. Meinshausen, M. (Ed.).
- Farooq, M., A. Wahid, N. Kobayashi, D. Fujita, and S. M. A. Basra. 2009. Plant drought stress: Effects, mechanisms and management. *Agronomy for Sustainable Development* **29**:185-212.
- Grime, J. P., J. D. Fridley, A. P. Askew, K. Thompson, J. G. Hodgson, and C. R. Bennett. 2008. Long-term resistance to simulated climate change in an infertile grassland. *Proceedings of the National Academy of Sciences of the United States of America* **105**:10028-10032.
- Gutbrodt, B., K. Mody, and S. Dorn. 2011. Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos* **000**:1-9.
- Hamilton, J. G., O. Dermody, M. Aldea, A. R. Zangerl, A. Rogers, M. R. Berenbaum, and E. H. DeLucia. 2005. Anthropogenic changes in tropospheric composition increase susceptibility of soybean to insect herbivory. *Environmental Entomology* **34**:479-485.
- Hammond, R. B., P. Bierman, E. Levine, and R. L. Cooper. 2001. Field resistance of two soybean germplasm lines, HC95-15MB and HC95-24MB, against bean leaf beetle (Coleoptera : Chrysomelidae), western corn rootworm (Coleoptera : Chrysomelidae), and Japanese beetles (Coleoptera : Scarabidae). *Journal of Economic Entomology* **94**:1594-1601.

- Hoogenboom, G., M. G. Huck, and C. M. Peterson. 1987. Root-growth rate of soybean as affected by drought stress. *Agronomy Journal* **79**:607-614.
- Intergovernmental Panel on Climate Change Fourth Assessment Report. 2007. *Climate Change 2007: Synthesis Report*. Allali, A., R. Bojariu, S. Diaz, I. Elgizouli, D. Griggs, D. Hawkins, O. Hohmeyer, B. P. Jallow, L. Kajfež-Bogataj, N. Leary, H. Lee, and D. Wratt (Ed.). Cambridge University Press, Cambridge.
- James, A. T., R. J. Lawn, and M. Cooper. 2008. Genotypic variation for drought stress response traits in soybean. I. Variation in soybean and wild *Glycine* spp. for epidermal conductance, osmotic potential, and relative water content. *Australian Journal of Agricultural Research* **59**:656-669.
- Johnson, I. R. 1985. A model of the partitioning of growth between the shoots and roots of vegetative plants. *Annals of Botany* **55**:421-431.
- Johnson, M. T. J. and A. A. Agrawal. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology* **86**:874-885.
- Johnson, M. T. J., A. A. Agrawal, J. L. Maron, and J. P. Salminen. 2009. Heritability, covariation and natural selection on 24 traits of common evening primrose (*Oenothera biennis*) from a field experiment. *Journal of Evolutionary Biology* **22**:1295-1307.
- Kamalay, J. C., J. H. Barger, and P. E. Pierson. 1997. Survival and development of gypsy moths (Lepidoptera: Lymantriidae) on corn and soybean foliage. *Journal of Economic Entomology* **90**:147-153.
- Kelly, A. E. and M. L. Goulden. 2008. Rapid shifts in plant distribution with recent climate change. *Proceedings of the National Academy of Sciences of the United States of America* **105**:11823-11826.
- Kim, M. Y., S. Lee, K. Van, T. H. Kim, S. C. Jeong, I. Y. Choi, D. S. Kim, Y. S. Lee, D. Park, J. Ma, W. Y. Kim, B. C. Kim, S. Park, K. A. Lee, D. H. Kim, K. H. Kim, J. H. Shin, Y. E. Jang, K. Do Kim, W. X. Liu, T. Chaisan, Y. J. Kang, Y. H. Lee, J. K. Moon, J. Schmutz, S. A. Jackson, J. Bhak, and S. H. Lee. 2010.

- Whole-genome sequencing and intensive analysis of the undomesticated soybean (*Glycine soja* Sieb. and Zucc.) genome. Proceedings of the National Academy of Sciences of the United States of America **107**:22032-22037.
- Lam, H. M., X. Xu, X. Liu, W. B. Chen, G. H. Yang, F. L. Wong, M. W. Li, W. M. He, N. Qin, B. Wang, J. Li, M. Jian, J. A. Wang, G. H. Shao, J. Wang, S. S. M. Sun, and G. Y. Zhang. 2010. Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. Nature Genetics **42**:1053-U1041.
- Levine, M. T. and K. N. Paige. 2004. Direct and indirect effects of drought on compensation following herbivory in scarlet gilia. Ecology **85**:3185-3191.
- Leimu, R. and J. Koricheva. 2006. A meta-analysis of genetic correlations between plant resistances to multiple enemies. American Naturalist **168**:E15-E37.
- Liu, F. L., M. N. Andersen, and C. R. Jensen. 2003. Loss of pod set caused by drought stress is associated with water status and ABA content of reproductive structures in soybean. Functional Plant Biology **30**:271-280.
- Maddox, G. D. and R. B. Root. 1990. Structure of the encounter between goldenrod (*Solidago altissima*) and its diverse insect fauna. Ecology **71**:2115-2124.
- Manavalan, L. P., S. K. Guttikonda, L. S. P. Tran, and H. T. Nguyen. 2009. Physiological and molecular approaches to improve drought resistance in soybean. Plant and Cell Physiology **50**:1260-1276.
- McCarthy, M. C. and B. J. Enquist. 2007. Consistency between an allometric approach and optimal partitioning theory in global patterns of plant biomass allocation. Functional Ecology **21**:713-720.
- McNulty, S. G. and J. L. Boggs. 2010. A conceptual framework: Redefining forest soil's critical acid loads under a changing climate. Environmental Pollution **158**:2053-2058.
- Memmott, J., P. G. Craze, N. M. Waser, and M. V. Price. 2007. Global warming and the disruption of plant-pollinator interactions. Ecology Letters **10**:710-717.

- Mody, K., D. Eichenberger, and S. Dorn. 2009. Stress magnitude matters: Different intensities of pulsed water stress produce non-monotonic resistance responses of host plants to insect herbivores. *Ecological Entomology* **34**:133-143.
- Nguyen, H. T., B. Valliyodan, S. Tran, M. Hanumappa, S. Guttikonda, T. N. Quach, and H. N. T. Tran. 2009. Discovery and characterization of regulatory factors associated with drought tolerance in soybean. *Plant Biology (Rockville)* **2009**:153-154.
- O'Connor, M. I. 2009. Warming strengthens an herbivore-plant interaction. *Ecology* **90**:388-398.
- Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**:37-42.
- Portmann, R. W., S. Solomon, and G. C. Hegerl. 2009. Spatial and seasonal patterns in climate change, temperatures, and precipitation across the United States. *Proceedings of the National Academy of Sciences of the United States of America* **106**:7324-7329.
- Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. *Oikos* **62**:244-251.
- Ray, J. D. and T. R. Sinclair. 1998. The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. *Journal of Experimental Botany* **49**:1381-1386.
- Relyea, R. A. 2003. Predator cues and pesticides: A double dose of danger for amphibians. *Ecological Applications* **13**:1515-1521.
- Relyea, R. A. 2004. Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. *Environmental Toxicology and Chemistry* **23**:1080-1084.
- Relyea, R. A. 2005. The lethal impacts of roundup and predatory stress on six species of North American tadpoles. *Archives of Environmental Contamination and Toxicology* **48**:351-357.

- Rhoades, D. F. 1979. Evolution of plant chemical defense against herbivores. Rosenthal, G. A. and D. H. Janzen (Ed.). *Herbivores: Their Interaction with Secondary Plant Metabolites*. Academic Press, Inc., New York, London.
- Rogers, H. H., G. B. Runion, and S. V. Krupa. 1994. Plant-responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83**:155-189.
- Roy, B. A., S. Gusewell, and J. Harte. 2004. Response of plant pathogens and herbivores to a warming experiment. *Ecology* **85**:2570-2581.
- Rypstra, A. L. and S. D. Marshall. 2005. Augmentation of soil detritus affects the spider community and herbivory in a soybean agroecosystem. *Entomologia Experimentalis Et Applicata* **116**:149-157.
- Sadok, W. and T. R. Sinclair. 2010. Transpiration response of 'slow-wilting' and commercial soybean (*Glycine max* (L.) Merr.) genotypes to three aquaporin inhibitors. *Journal of Experimental Botany* **61**:821-829.
- Schoonhoven, L. M., J. J. A. van Loon, and M. Dicke. 2005. *Insect-plant biology*. Second edition. Oxford University Press, Oxford, New York.
- Silva, E. N., S. L. Ferreira-Silva, A. D. Fontenele, R. V. Ribeiro, R. A. Viegas, and J. A. G. Silveira. 2010. Photosynthetic changes and protective mechanisms against oxidative damage subjected to isolated and combined drought and heat stresses in *Jatropha curcas* plants. *Journal of Plant Physiology* **167**:1157-1164.
- Sinclair, T. R., C. D. Messina, A. Beatty, and M. Samples. 2010. Assessment across the United States of the benefits of altered soybean drought traits. *Agronomy Journal* **102**:475-482.
- Smelser, R. B. and L. P. Pedigo. 1992. Bean leaf beetle (Coleoptera, Chrysomelidae) herbivory on leaf, stem, and pod components of soybean. *Journal of Economic Entomology* **85**:2408-2412.

- Stolf-Moreira, R., M. E. Medri, N. Neumaier, N. G. Lemos, R. L. Brogin, F. C. Marcelino, M. C. N. de Oliveira, J. R. B. Farias, R. V. Abdelnoor, and A. L. Nepomuceno. 2010a. Cloning and quantitative expression analysis of drought-induced genes in soybean. *Genetics and Molecular Research* **9**:858-867.
- Stolf-Moreira, R., M. E. Medri, N. Neumaier, N. G. Lemos, J. A. Pimenta, S. Tobita, R. L. Brogin, F. C. Marcelino-Guimaraes, M. C. N. Oliveira, J. R. B. Farias, R. V. Abdelnoor, and A. L. Nepomuceno. 2010b. Soybean physiology and gene expression during drought. *Genetics and Molecular Research* **9**:1946-1956.
- Tack, A. J. M., O. Ovaskainen, P. Pulkkinen, and T. Roslin. 2010. Spatial location dominates over host plant genotype in structuring an herbivore community. *Ecology* **91**:2660-2672.
- van Mantgem, P. J., N. L. Stephenson, J. C. Byrne, L. D. Daniels, J. F. Franklin, P. Z. Fule, M. E. Harmon, A. J. Larson, J. M. Smith, A. H. Taylor, and T. T. Veblen. 2009. Widespread increase of tree mortality rates in the western United States. *Science* **323**:521-524.
- Walther, G. R., E. Post, P. Convey, A. Menzel, C. Parmesan, T. J. C. Beebee, J. M. Fromentin, O. Hoegh-Guldberg, and F. Bairlein. 2002. Ecological responses to recent climate change. *Nature* **416**:389-395.
- White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* **63**:90-105.
- Williams, J. W. and S. T. Jackson. 2007. Novel climates, no-analog communities, and ecological surprises. *Frontiers in Ecology and the Environment* **5**:475-482.
- Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, and C. C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences of the United States of America* **105**:17029-17033.
- World Bank, The. 2010. World Development Report 2010: Development and Climate Change. The International Bank for Reconstruction and

Development / The World Bank. Washington D.C.

Wu, X. L., C. W. Ren, T. Joshi, T. Vuong, D. Xu, and H. T. Nguyen. 2010. SNP discovery by high-throughput sequencing in soybean. *Bmc Genomics* **11**.

Zhang, Z. B., B. Cazelles, H. D. Tian, L. C. Stige, A. Brauning, and N. C. Stenseth. 2009. Periodic temperature-associated drought/flood drives locust plagues in China. *Proceedings of the Royal Society B-Biological Sciences* **276**:823-831.

CHAPTER 3

The Influence of Drought on Plant-Herbivore Interactions across 16 Soybean Genotypes in a Field Experiment

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ABSTRACT

Drought related to climate change is predicted to increase in many areas of the world and it is important to understand how this will impact our agricultural systems. The Plant Stress Hypothesis predicts that plants experiencing drought will be more susceptible to insect herbivory due to biochemical changes in the plant, while the Plant Vigor Hypothesis suggests that insects will preferentially feed on healthy plants. We would like to know which of these hypotheses more strongly predicts the response of a model crop system to herbivory under drought. We conducted a field experiment using 16 soybean genotypes to determine if combined effects of drought and herbivory resulted in synergistic decreases in plant performance, if drought impacted soybean resistance to herbivory, and if there are phenotypic traits that predict these responses. We found that drought had the largest negative effect on soybean performance and that drought and herbivory did not

interact to impact performance. Droughted plants resulted in lower levels of insect herbivory, suggesting that the Plant Vigor Hypothesis more accurately predicted soybean resistance to herbivory under drought. Genotypic correlations showed that higher plant growth rate and lower seed yield correlated with higher levels of herbivory, and higher growth rates correlated with lower seed yield. This suggests that plants investing large amounts of resources in vegetative growth may be more desirable to generalist insects and may result in less available resources for fruit and seed production. Our results also indicate that mitigating negative impacts of drought should be a primary focus in preparing soybean for future climate change.

INTRODUCTION

Most climate change predictions agree that many areas of the globe will experience extreme fluctuations in precipitation (Easterling et al. 2000, Portmann et al. 2009, Seager et al. 2009). Some factors expected to contribute to this include changes in atmospheric, surface, and ocean water temperatures (Easterling et al. 2000, Cook et al. 2007, Portmann et al. 2009, Seager et al. 2009). While it is yet unclear which specific regions will receive increases or decreases in precipitation, it is generally accepted that drought will become more common for many areas (IPCC 2007, Li et al. 2009, Seager et al. 2009). Predictions regarding the impacts of drought on communities and ecosystem processes are more complex, and therefore less resolved (Williams and Jackson 2007). This is due to the fact that the various species in ecosystems can respond very differently to changes in their environment

(Andrewartha and Birch 1954, Parmesan and Yohe 2003, Willis et al. 2008). As the climate changes, it will be necessary to observe individual systems independently in order to understand the varied ecological responses to these changes.

Understanding the effects of abiotic stress on species' interactions within plant-insect systems is important if we are to predict and prepare for environmental changes. It has been hypothesized that increases in drought associated with climate change could increase the frequency and severity of insect population outbreaks (Hammond et al. 2001, Levine and Paige 2004, Hamilton et al. 2005, Dermody et al. 2008). This is important because if the negative impacts of drought on plants are compounded by increases in herbivorous insect populations, then plants may be at risk of increased damage during periods of drought. It is therefore necessary to understand the combined effects of drought and insect herbivory on the performance of plants. For instance, the effects of drought and herbivory on plant performance may be independent of each other and therefore additive. Alternatively, drought and herbivory could lead to synergistic negative effects resulting in a larger decrease in performance than predicted by their additive effects. Another possible outcome is that drought and herbivory could have multiplicative effects, resulting in a smaller decrease in performance than predicted by their additive effects. The exact nature of the combined effects of abiotic and biotic stressors will have implications for how breeders and farmers develop strategies to mitigate any harmful effects of climate change.

There are two conflicting hypotheses that offer predictions about how abiotic stress can affect a plant's susceptibility to insect herbivores - the Plant Stress Hypothesis and the Plant Vigor Hypothesis. The Plant Stress Hypothesis suggests that plants under abiotic stress will be more susceptible to insect herbivores due to depressed plant defenses and increases in leaf nitrogen: carbon ratios, which can make the plant more palatable to insects (White 1984). This theory implies that under drought conditions, plants are likely to experience synergistic negative effects from the combination of stresses, although it has been shown in some systems that plants can exhibit higher levels of defense compounds under drought stress (Haugen et al. 2008). By contrast, the Plant Vigor Hypothesis suggests that insects will preferentially feed on healthy fast-growing plants when given a choice (Price 1991, Hull-Sanders and Eubanks 2005). This can be due to a smaller investment in plant defenses by fast-growing plants (Price 1991), or because stressed plants will usually have reduced carbohydrate synthesis and reduced water uptake, resulting in reduced nutritional content. This hypothesis implies that abiotically stressed plants will experience either no increase, or even decreased damage, due to insect herbivory. These conflicting hypotheses perpetuate the ambiguity that exists in predictions relating to the effects of climate change on species interactions, and it is not yet clear whether any hypothesis exists that will be applicable to all plants and plant systems (Schoonhoven et al. 2005, Gutbrodt et al. 2011). Therefore, it is important to understand under what conditions these hypotheses make accurate predictions, especially in agricultural plants.

One approach to understanding how plants will respond to abiotic and biotic stresses is to identify those phenotypic traits that mediate a plant's interactions with its environment. Crop breeders have taken a lead in this regard by creating new varieties, such as plants that exhibit increased tolerance to drought (Carter et al. 2004, James et al. 2008, Du et al. 2009a, Du et al. 2009c, Manavalan et al. 2009) or increased resistance to herbivores (Carter et al. 2004, Du et al. 2009b, Yesudas et al. 2010). The genetically based phenotypic variation generated through crop breeding can also provide a powerful tool to identify additional plant traits that correlate with, and potentially even cause, a plant to exhibit greater or less resistance to stresses like drought and insect damage. Non-crop ecological genetics studies frequently utilize this approach, and they have successfully identified many traits involved in plant responses to drought and herbivory (Johnson and Agrawal 2005, James et al. 2008, Carmona et al. 2011). For instance, phenotypic differences in stomatal conductance, water potential and leaf water content have been correlated to genotypic variation in response to drought (Levi et al. 2009, Farooq et al. 2010), and reproductive phenology, plant size and trichome density have been correlated with resistance to herbivores (Agrawal et al. 2002, Johnson and Agrawal 2005, Sletvold et al. 2010). We would like to know if any plant phenotypic traits exist that can be correlated with predictions for both drought and insect herbivory among varying plant genotypes. If so, then crop breeders can select varieties possessing these traits that will remain viable under continued environmental change.

Here we seek to investigate how drought and simulated insect herbivore population outbreaks will influence plant-herbivore interactions, and if plant traits exist that can predict plant responses to both drought and herbivory in a model crop system, soybean (*Glycine max* (L.) Merr.). We conducted a field experiment where we asked the following questions: (i) How do abiotic (drought) and biotic (insect herbivory) stresses affect the performance of soybean? (ii) How does drought impact soybean resistance to insect herbivores? (iii) Can we identify genetic variability in phenotypic traits that predicts plant performance or susceptibility to herbivores under environmental stress?

METHODS

Study system

Soybean (*Glycine max* (L.) Merr.) is a leguminous herbaceous plant that offers a good system to test the effects of abiotic and biotic stresses on plants and plant-insect interactions. Soybean breeding has resulted in a wide array of phenotypic traits among current varieties, including increased resistance to drought and herbivory (Carter et al. 2004, Charlson et al. 2009). We made use of this variation to choose 16 phenotypically diverse genotypes (Appendix A) to answer our research questions. These genotypes represented a broad range of genetically based phenotypic variation identified by breeders and quantified in our previous experiments (see Chapter 2). These genotypes were also selected to represent the four major maturity groups (maturity groups 4, 5, 6 and 7) grown in temperate North

America. (Maturity group is a categorical descriptor of the time it takes a variety to flower and produce mature fruits, where the lower the maturity group, the faster the variety will reach reproductive maturity, and the more north it will likely be planted.)

Field experiment overview and treatments

On June 8, 2010, we planted seed of 16 genotypes of soybean at the Lake Wheeler Road Field Laboratory of North Carolina State University (NCSU) on an Appling fine sandy loam soil. The field was arranged into 16 blocks that were laid out into four rows and four columns with 3-4 m between both rows and columns (for field layout, see Appendix D). Within each treatment block we planted the 16 genotypes into individual 1.83 m rows arranged in a 4x4 grid (Fig. 3.1A, Appendix D); individual rows served as our unit of replication in analyses. Each maturity group was represented by four different replicate genotypes and all the genotypes within one maturity group were planted as adjacent row sets to minimize asymmetric competitive effects. The spatial position of maturity groups within a block, and the position of genotypes within a maturity group were fully randomized, and the experimental treatments applied to blocks (see below) were also randomized in a Latin Square design. We assigned one of four treatment combinations to each block: i) drought and herbivory, ii) drought and no herbivory, iii) ambient water and herbivory, iv) ambient water and no herbivory (Appendix D). We reduced edge effects by planting border beans on the outer rows of the row sets; the maturity



Figure 3.1 Treatment block set up and drought design, where **A** shows block set up with 4 rows down representing 4 maturity groups, and 6 rows across with experimental genotype rows in center and border beans in the two outside rows, **B** shows Dripperline attached to PVC headers at top of blocks, **C** shows Dripperline in U-formation at bottom of blocks, and **D** shows 7 strips of plastic covering one treatment block.

groups of these borders were chosen to match the neighboring maturity group. We also planted soybean en masse around our experimental blocks to deter deer and other mammalian herbivory, which we attempted to exclude with a 2.1m electrified fence. We thinned plants to 8-9 plants per 0.3 m (48-54 plants per row) 18 days after planting.

Two drought treatments, either the presence or absence of drought, were applied to entire blocks using irrigation and plastic. Irrigation was delivered by running PVC from a water tank with a gas water pump to individual blocks. We controlled the delivery of water to each treatment block with an independent valve that allowed us to control which blocks were watered during watering events. Water was delivered from the PVC headers to individual plant rows using Netafim™ Dripperline (Techline emitter spacing 30.5 cm which dispenses water at a flow rate of 3.41 L/hr) (Netafim, Fresno, CA, USA). As the Dripperline emitters were standardized, we were able to ensure that watered blocks received the same relative amount of water by timing the length of watering events. The Dripperline was run in long U-formation around each row, including border beans, such that six Dripperline U's were used to water the entire treatment block (Fig. 3.1B,C). The emitters were staggered such that rows received water every 15 cm. Over the irrigation, we laid 6 mil (industry standard unit referring to thickness of sheeting where 1 mil = 0.001 in or 0.0254 mm) polyethylene white plastic sheeting, cut into strips 11 m x 1 m, and laid alongside each row such that 7 strips were used to completely cover each treatment block (Fig. 3.1D) including a 1 m border of plastic extended out from the edges of

each treatment block. This effectively diverted most natural precipitation away from our blocks. As the field was on a slight slope, we dug trenches (25cm wide, 13cm deep) along the upslope side of each block that received drought to divert water runoff.

We initiated the drought by ceasing to water plants in mid-July (37 days after planting) and we laid plastic 9 days later. We ended the drought in early-October, 11 weeks (122 days after planting) after we started the drought by removing the plastic. This timing corresponded with a very heavy rain and many plants were approaching reproductive maturity. Our drought treatment was facilitated by the fact that our experiment was conducted during the second hottest summer on record in Raleigh since 1944, which caused extended dry periods at our field site (for temperature and precipitation data throughout the experiment, see Appendix E). We confirmed that our treatment was successful by measuring soil water in mid-August, at which time we observed a 35% reduction in the gravimetric water content of the soil ($8.9 \pm 0.31\%$ water in irrigated soil versus $5.75 \pm 0.31\%$ in water-stressed soil; $F_{1,14} = 29.65$, $p < 0.001$; Appendix F). Visible signs of wilting of soybeans were also observed and our physiological measurements of stomatal conductance showed that plants were responding to our manipulation of water (Appendix F).

We manipulated insect damage by removing natural herbivores from herbivory control plots and by simulating an herbivore outbreak in herbivory-treated blocks. We reduced natural herbivory by spraying control blocks (non-herbivory) with Baythroid® XL (Bayer CropScience, Research Triangle Park, NC, USA) at the

manufacturer's recommended rate (118mL per acre) every other week starting in late-June. We increased the spray frequency to every week from mid-July to mid-August because of intense herbivory during this time, and we added an extra spray in mid-Sept, again, to address a high level of herbivore presence. We simulated an herbivore outbreak in mid-July (44 days after planting), by introducing ~300 *Helicoverpa zea* Boddie (Noctuidae) 1st instar larvae to each 1.8m experimental row of herbivory treated blocks; this corresponded to ~5 additional caterpillars per plant. *H. zea* is a common generalist herbivore of soybean in the southeast U.S., where this study was conducted.

When deer started to penetrate our fence in mid-September, we took steps to alleviate the damage. We constructed a second deer fence outside of the electric fence that already existed and hung yellow and green flagging tape streamers around both fences. Also, in mid-Oct., we applied Miller[®] Hot Sauce (Miller Chemical, Hanover, PA, USA) to the field. However, these actions were only temporarily successful, and the potential to lose yield to deer damage influenced some of our harvest timing.

In mid-late Sept., we began harvesting rows as they became mature. To harvest, we cut the middle 1.2 m of the row close to the soil surface, allowed the plants to dry at ambient temperatures in a greenhouse for a minimum of 2 weeks, and threshed them to harvest seed. We dried the seed in paper bags at ambient room temperature in a lab for at least two weeks, then weighed the seed. Harvest was completed on Oct. 30, 2010 (day 145, 20.5 weeks after planting).

Trait measurements related to drought

A major objective of our research was to understand how variation in plant traits mediated a plant's response to abiotic and biotic stresses. We measured stomatal conductance four times throughout the drought period, ranging from 2 to 8 weeks into the treatment, with a Delta-T AP4 Porometer (Delta-T Devices, Burwell, Cambridge, UK) on a haphazardly selected, fully expanded leaf at the top of the canopy of each experimental row, facing the sun. Eight weeks into the treatment, we also measured water potential with a Scholander Pressure Chamber (PMS Instrument Company, Albany, OR, USA) on the terminal leaflet used to measure stomatal conductance for each row. These measurements provided insight into the variation between genotypes in their responses to drought, and we attempted to correlate these traits to plant tolerance of drought.

Three weeks into the drought treatment, we measured pre-dawn percent leaf water content to evaluate whether there was phenotypic variation among the genotypes in the effects of drought on water content of above-ground tissues. We collected a round hole punch 3.8 cm in diameter from a random leaf at the top of the canopy of each experimental row with a Marvy[®] Uchida LVEJCP craft punch (Uchida of America, Corp., Torrance, CA, USA). We measured the wet weight of the leaf punches immediately following collection, dried the leaf punches in a dryer cabinet at approximately 38°C for one day, and then measured the dry weight. From this

measurement, we also calculated specific leaf area (SLA: ratio of leaf surface area/dry weight).

Trait measurements related to herbivory

To assess the effects of drought on herbivory we quantitatively measured plant damage from insects on a subset of each experimental row. During weeks 6, 10, and 15 of the experiment, damage was visually estimated as percent leaf area removed by herbivores on five haphazardly selected leaves from each of three plants per experimental row, and averaged to estimate the amount of damage in the row. In addition to testing for the effects of drought on plant susceptibility to herbivores, these data showed that our insecticide treatment was effective at reducing insect damage (effect of insecticide: $p < 0.05$ for all dates; Appendix F) by 27-63%, depending on the date of measurement. The damage observed in our herbivory blocks fell within the natural range of herbivory damage observed in North Carolina. This was confirmed by measuring herbivory on soybean in late August at 10 soybean fields in the region. Herbivory in these fields ranged between 2.9% and 15.6% (mean: 7.1%, std. error: 3.99%); the mean level of herbivory in our herbivory blocks at this same time was 6.7% (std. error: 3.01%). It is unknown if the regional fields were supplied with insecticide.

In an attempt to identify traits correlated with plant susceptibility to herbivores, we measured leaf trichome densities. In early-mid July, we collected the 2nd fully expanded trifoliate leaf down from the apical meristem of a random plant per

experimental row. We punched a 7 mm diameter hole between major veins near the base of the terminal leaflet, and counted the number of trichomes on one half of both upper and lower surfaces (approximately 77 mm² per side).

We assessed the effect of drought on susceptibility to herbivores using a no-choice bioassay. Six weeks after drought began, two 3.8 cm round holes were punched from haphazardly selected leaves at the top of the canopy of each row with a hole punch. We placed these leaf punches individually into 60 x 15mm polystyrene petri dishes lined with moistened filter paper. We then placed newly hatched caterpillars of *H. zea* and *Spodoptera exigua* Hübner (Noctuidae) (Benzon Research, Inc., Carlisle, PA, USA), individually on punched leaves, such that we assayed resistance of each row to both insect species. The insects were allowed to feed on a leaf punch in the petri dish for one week, at which point we removed the insects and placed them in a 1.5 mL centrifuge tube. They were allowed to void their gut contents for approximately 24 hours and then were frozen. We then weighed them on a Mettler AT20 FACT microbalance digital scale (Mettler-Toledo, Inc., Columbus, OH, USA).

Trait measurements related to life history

In addition to using row seed yield as a measure of plant performance, we also measured plant growth rate (calculated as a change in plant height per day through time, which I refer to as "plant growth rate" hereafter). We measured the height of two plants per experimental row 3 times throughout the experiment from

the soil surface to the primary shoot apical meristem. The two plants were tagged during the first measurement so that we measured the same plants for each round of growth measurements. From this data we calculated relative growth rates for early (day 14-29, June) and mid (day 30-50, July) growth rates.

To understand how drought and herbivory affected the reproductive phenology of the genotypes, we measured a number of traits. We measured flowering phenology as the day the first flower was fully opened, fruit phenology as the first day 2-3 mm of a fruit could be observed emerging from the senescing flower, and maturity date as the day when 95% of pods in the row were brown, or the day the row was harvested (it was necessary to vary harvest timing here because there was a potential for some rows to either disperse seed before 95% of the pods were brown, or suffer excessive levels of deer damage). We calculated fruit maturity rate as the length of time between first fruit date and maturity date.

Statistical analysis

We examined the effects of drought, herbivory, plant genotype and maturity group using general linear mixed models in Proc GLM of SAS (SAS Institute, Cary, NC, USA). The statistical models employed reflected the blocking and nesting structure of the experimental design where a single row of plants was used as the unit of replication in all analyses. When analyzing plant traits as the response variable (e.g. seed yield, physiological and phenological traits), we used the following model: $\text{Plant trait} = \text{Mean}_{\text{overall}} + \text{Block} (\text{Drought} \times \text{Herbivory}) + \text{Water} +$

Herbivory + Drought X Herbivory + Maturity Group + *Genotype(Maturity Group)* + Herbivory X Maturity Group + Drought X Maturity Group + *Drought X Genotype(Maturity Group)* + *Herbivory X Genotype(Maturity Group)* + *Drought X Herbivory X Genotype(Maturity Group)* + Drought X Herbivory X Maturity Group + Error. All effects in italics were treated as random and the correct denominator expression and degrees of freedom were determined using the “test” statement in Proc GLM. To decrease the parameterization of the model and to increase statistical power, we removed non-significant interactions (cutoff of $p > 0.15$), and the effect of block and maturity group using a sequential stepwise backwards procedure. This procedure was applied to each response variable by starting at the highest-order interaction term, and proceeding with simpler terms until we arrived at the “reduced model”. We always retained the Drought X Herbivory interaction terms in the reduced model because we were interested in testing for the presence/absence of this interaction a priori. We examined the residuals of the untransformed data for each variable separately to determine whether assumptions of homogeneity of variance or normality were violated. In such cases we examined whether log- or square-root transformations of the data improved the residuals.

We used a similar approach to analyze effects of genotype and treatments on herbivore susceptibility, but we used a simpler model because these models only included blocks where herbivores were allowed to naturally colonize, i.e. unsprayed plots. In the analysis of herbivory on plants in the field, we used the full model:

$$\text{Herbivory} = \text{Mean}_{\text{overall}} + \textit{Block}(\textit{Drought}) + \text{Drought} + \text{Maturity Group} +$$

Genotype(Maturity Group) + Drought X Maturity Group + Drought X Genotype(Maturity Group) + Error. As before, all effects in italics were treated as random and denominator terms and degrees of freedom were stipulated according to the “test” statement in Proc GLM.

We also analyzed herbivore susceptibility according to the weight gain and survival of caterpillars in a no-choice petri dish assay. When analyzing weight gain we started with the same full model as described above. For caterpillar survival we used generalized linear mixed models with a binomial error distribution and logit link function, where genotype was designated as a random effect. In analyses involving *H. zea*, the likelihood statistics would not converge, so we employed Proc Glimmix to investigate the effects of Drought and Maturity Group in one analysis (Model 1), and Proc Genmod to examine the effects of Drought and Genotype in a second analysis (Model 2).

We plotted pairwise correlations in JMP 8.0 (SAS) based on genotypic least squares means for phenotypic traits to determine how phenotypic traits predicted plant performance and resistance to herbivory.

RESULTS

Plant performance

Our first research question sought to understand how abiotic (drought) and biotic (herbivory) stresses affect soybean performance. Overall, drought had the largest effect, causing a 37.5% reduction in seed yield (Fig. 3.2; for all experiment

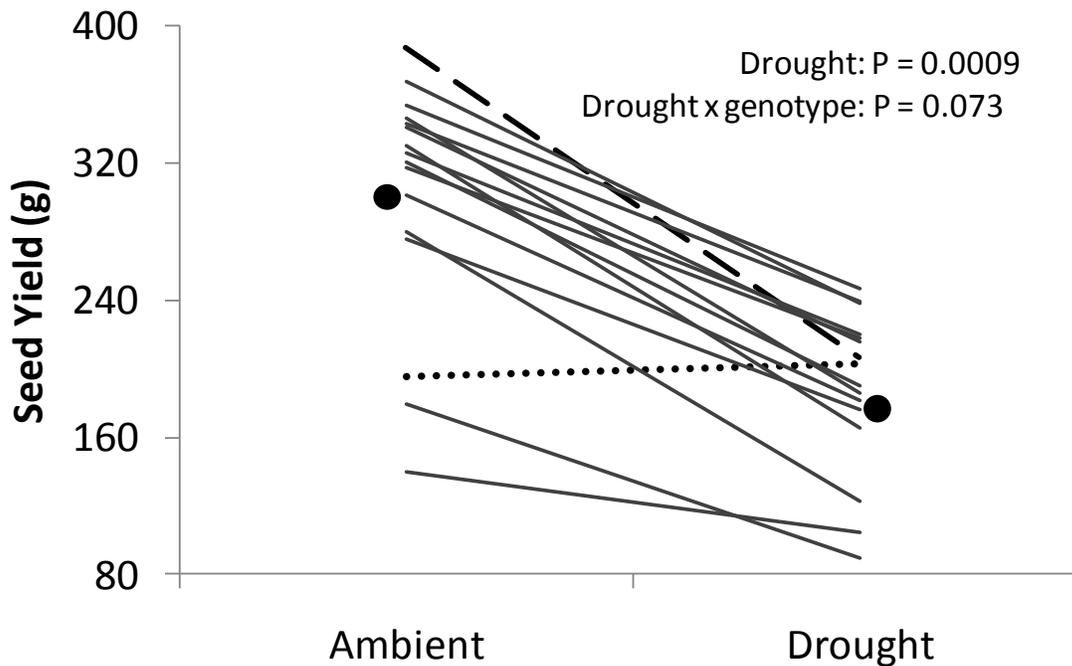


Figure 3.2 Effects of drought treatment x plant genotype on soybean seed yield. Here, each line represents the least squares mean (LSM) for a soybean genotype for both ambient water and drought treatments. For these graphs, the slope of each line implies directionality and magnitude of the variation between the ambient and drought means for the genotype. For example, the genotype (line) shown as dashes indicates that the droughted plants of this genotype produced approximately 180 g less seed yield than the ambient plants, and the genotype (line) shown as dots indicates that the droughted plants of this genotype produced approximately 10 g more seed yield than the ambient plants. The p-value for these means is represented as Drought x genotype. The large black dots outside the genotype lines represent overall means for all genotypes for both ambient and drought treatments and their p-value is represented as Drought.

means see Appendix A), and this effect was relatively consistent among plant genotypes (D x G, $F = 1.61$, $p = 0.07$; Table 3.1). Drought resulted in a 7.4% reduction in final height ($F = 4.20$, $p = 0.06$; Fig. 3.3), although the magnitude of this effect varied among genotypes (D x G, $F = 1.77$, $p = 0.04$; Table 3.2). Drought also reduced mid-season (July) plant growth rate by 9.7%, although this was not a significant reduction ($F = 2.36$, $p = 0.15$; Table 3.3, Fig. 3.4). Drought significantly sped up fruit phenology and maturation (Tables 3.4-3.6, Fig. 3.5), such that plants experiencing drought fruited and matured faster than control plants, although never by more than two days. There was no interaction between drought and herbivory for any performance measurement (Tables 3.1-3.7).

Insect herbivory had a weaker effect than drought on plant performance. There was no effect of herbivory on seed yield (Table 3.1) or on final height (Table 3.2). Herbivory reduced early (June) and mid (July) growth rates by 9.4% and 10.2%, respectively, but this effect significantly varied among plant genotypes (Table 3.3, Fig. 3.6). For example, in June our herbivory treatment reduced growth by as little as 0% on some genotypes to as much as 25% on other genotypes. Herbivory slowed rates of fruit phenology and maturation (Tables 3.4-3.6, Fig. 3.7), such that plants experiencing herbivory fruited and matured later than plants receiving insecticide, although never by more than three days.

Table 3.1 Effects of experimental treatments and soybean genotype on seed yield. We include results from analysis of variance (ANOVA) for soybean seed yield.

Seed yield				
	df	SS	F	P
Block (drought x herbivory) ¹	12	497901	7.45	<0.001
Drought ²	1	807296	17.98	0.0009
Herbivory ²	1	185.7	0	0.95
Maturity group ³	3	297671	2.76	0.0884
Genotype (maturity group) ⁴	12	432027	4.01	0.0066
Drought x herbivory ²	1	450.19	0.01	0.92
Drought x genotype (maturity group) ¹	15	134651	1.61	0.0728
Herbivory x maturity group ¹	3	39435	2.36	0.0726

¹ Denominator for testing these effects was MS (error); ² Denominator was MS block (drought x herbivory); ³ Denominator was MS (genotype (maturity group)); ⁴ Denominator was MS (drought x genotype (maturity group)).

Table 3.2 Effects of experimental treatments and soybean genotype on final plant height. We include results from analysis of variance (ANOVA) for soybean final height.

Final height				
	df	SS	F	P
Block (drought x herbivory) ¹	12	0.93	10.22	<.001
Drought ²	1	0.35	4.2	0.06
Herbivory ³	1	0.006	0.08	0.78
Genotype ⁴	15	4.95	24.59	<.001
Drought x herbivory ³	1	0.06	0.71	0.41
Drought x genotype ¹	15	0.2	1.77	0.04

¹ Denominator for testing these effects was MS error; ² Denominator was MS block (drought x herbivory) + MS drought x genotype - MS error; ³ Denominator was MS block (drought x herbivory); ⁴ Denominator was MS drought x genotype.

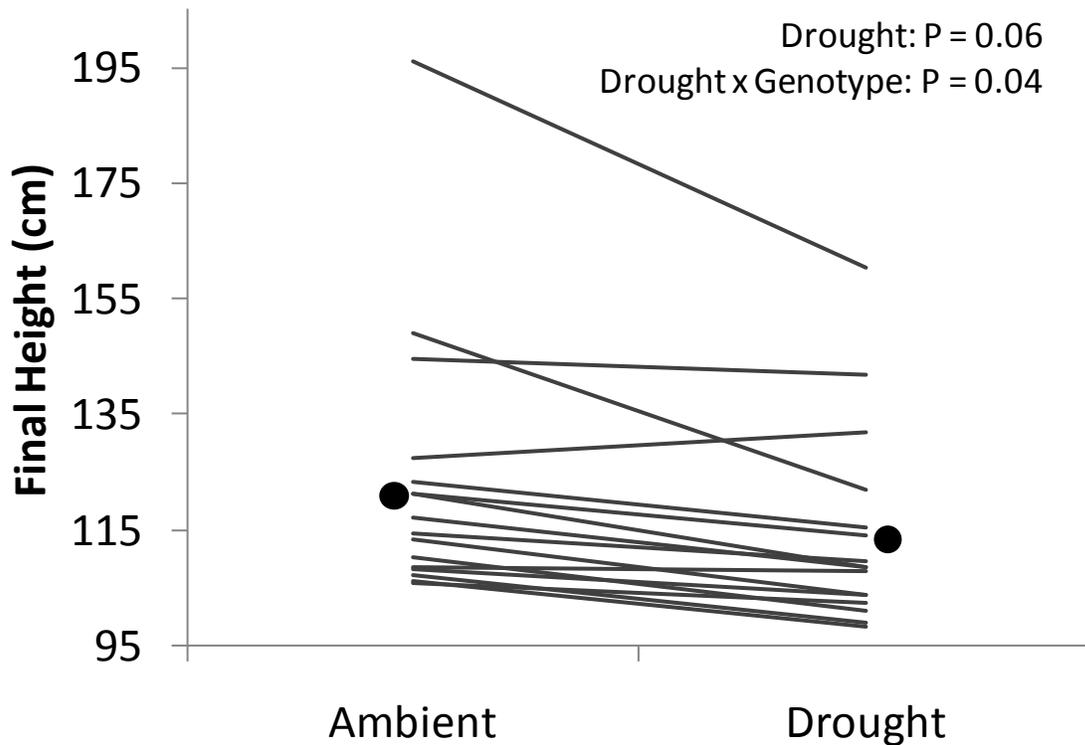


Figure 3.3 Effects of drought treatment x plant genotype on final soybean height. Here, each line represents the least squares mean for a soybean genotype for both ambient and drought treatments. The p-value for these means is represented as Drought x genotype. The large black dots outside the genotype lines represent overall means for all genotypes for both ambient and drought treatments and their p-value is represented as Drought.

Table 3.3 Effects of experimental treatments and soybean genotype on plant growth rates. We include results from analysis of variance (ANOVA) for early (June) and mid (July) growth rates.

Early growth rate (June)				
	df	SS	F	P
Block (drought x herbivory) ¹	12	1.45	6.84	<.0001
Drought ²	1	0.02	0.14	0.71
Herbivory ³	1	0.56	3.89	0.07
Genotype ⁴	15	6.37	10.71	<.0001
Drought x herbivory ²	1	0.0001	0.01	0.94
Herbivory x genotype ¹	15	0.59	2.24	0.006
Mid growth rate (July)				
	df	SS	F	P
Block (drought x herbivory) ¹	12	3.7	34.12	<0.001
Drought ⁵	1	0.73	2.36	0.15
Herbivory ⁶	1	0.65	2.06	0.18
Maturity group ⁷	3	4.81	3.83	0.04
Genotype (maturity group) ⁸	12	5.02	27.55	<.0001
Drought x herbivory ⁵	1	0.04	0.13	0.72
Drought x maturity group ¹	3	0.09	3.37	0.0195
Herbivory x maturity group ⁸	3	0.25	5.48	0.01
Herbivory x genotype (maturity group) ¹	12	0.18	1.68	0.073

¹ Denominator for testing these effects was MS error; ² Denominator was MS block (drought x herbivory); ³ Denominator was Block (drought x herbivory); ⁴ Denominator was MS herbivory x genotype; ⁵ Denominator was MS (block (drought x herbivory)); ⁶ Denominator was MS (block (drought x herbivory)) + MS (herbivory x genotype (maturity group)) - MS error; ⁷ Denominator was MS (genotype (maturity group)); ⁸ Denominator was MS (herbivory x genotype (maturity group)).

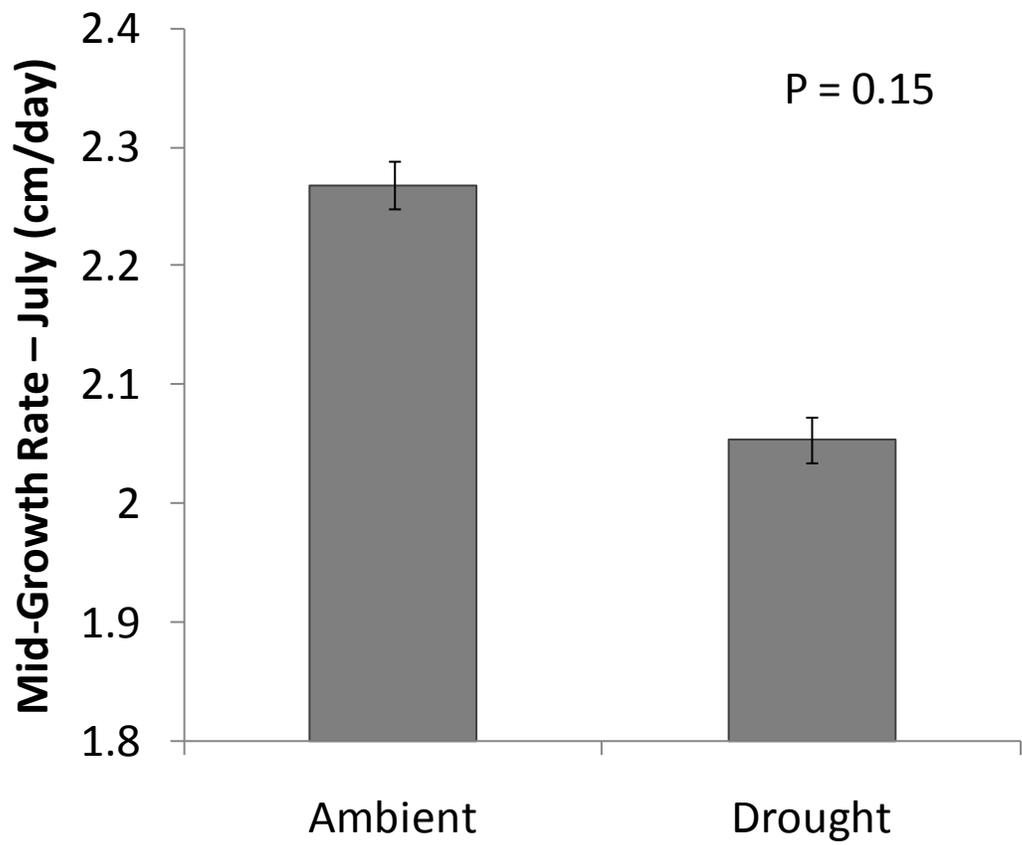


Figure 3.4 Effect of drought on mid growth rate. Here, data represents overall least squares means with standard error bars for ambient water and drought treatments for mid-growth rate (July).

Table 3.4 Effects of experimental treatments and soybean genotype on date of first fruits. We include results from analysis of variance (ANOVA) for the date that the first soybean fruits in the row were observed emerging 2-3 mm from senescing flower).

Date of first fruits				
	df	SS	F	P
Block (drought x herbivory) ¹	12	27.6	0.95	0.49
Drought ²	1	25.77	11.2	0.006
Herbivory ³	1	77.75	16.81	0.002
Maturity group ⁴	3	4087.79	9.16	0.002
Genotype (maturity group) ⁵	12	1785.99	31.42	<0.001
Drought x herbivory	1	0.62	0.27	0.61
Herbivory x genotype (maturity group) ¹	15	71.05	1.97	0.02

¹ Denominator for testing these effects was MS error; ² Denominator was Block (drought x herbivory);
³ Denominator was MS block (drought x herbivory); ⁴ Denominator was Genotype (maturity group);
⁵ Denominator was MS herbivory x genotype (maturity group).

Table 3.5 Effects of experimental treatments and soybean genotype on fruit mature date. We include results from analysis of variance (ANOVA) for the date that 95% of fruits in the soybean row were mature.

Date fruit mature				
	df	SS	F	P
Block (drought x herbivory) ¹	12	265.36	3.58	<.0001
Drought ²	1	270.19	9.59	0.007
Herbivory ³	1	531.88	24.05	0.0004
Maturity group ⁴	3	28973	16.38	0.0002
Genotype (maturity group) ⁵	12	7075.08	48.2	<.0001
Drought x herbivory	1	19.69	0.89	0.36
Drought x genotype (maturity group) ¹	15	183.5	1.98	0.018
Herbivory x maturity group ¹	3	36.17	1.95	0.12

¹ Denominator for testing these effects was MS error; ² Denominator was MS (block (drought x herbivory)) + MS drought x genotype - MS error); ³ Denominator was Block (drought x herbivory);
⁴ Denominator was MS genotype (maturity group); ⁵ Denominator was MS drought x genotype.

Table 3.6 Effects of experimental treatments and soybean genotype on fruit maturation rate. We include results from analysis of variance (ANOVA) for the soybean fruit maturation rate (time between date of first fruits and date fruit mature).

Fruit maturation rate				
	df	SS	F	P
Block (drought x herbivory) ¹	12	211.86	1.87	0.04
Drought ²	1	127.47	5.7	0.03
Herbivory ³	1	200.47	11.36	0.006
Maturity group ⁴	3	11595	11.98	0.001
Genotype (maturity group) ³	12	3871.64	22.8	<.001
Drought x herbivory ³	1	13.5	0.76	0.4
Drought x genotype (maturity group) ¹	15	212.28	1.5	0.11

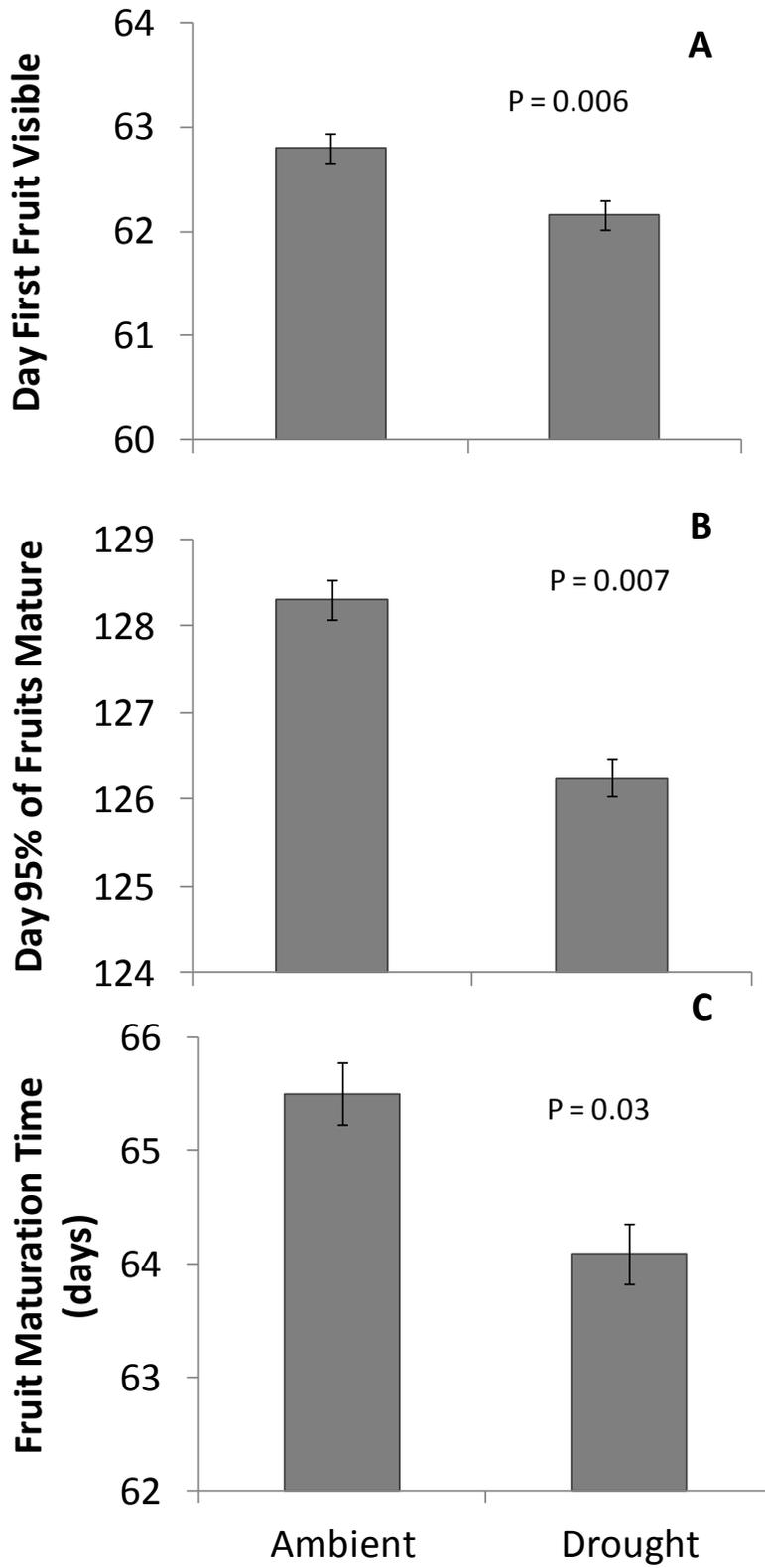
¹ Denominator for testing these effects was MS error; ² Denominator was MS block (drought x herbivory); ³ Denominator was MS drought x genotype; ⁴ Denominator was MS genotype (maturity group).

Table 3.7 Effects of experimental treatments and soybean genotype on date of first flowers. We include results from analysis of variance (ANOVA) for the date that the first soybean flowers in the row were fully opened.

Date of first flowers				
	df	SS	F	P
Block (drought x herbivory) ¹	12	46.53	2.21	0.01
Drought ²	1	2.64	0.68	0.43
Herbivory ²	1	9	2.32	0.15
Maturity group ³	3	1833.63	6.63	0.007
Genotype (maturity group) ¹	12	1105.81	52.5	<.001
Drought x herbivory ²	1	1.89	0.49	0.5
Drought x maturity group ¹	3	10.3	1.96	0.12

¹ Denominator for testing these effects was MS error; ² Denominator was MS block (drought x herbivory); ³ Denominator was MS genotype (maturity group).

Figure 3.5 Effects of drought on fruit phenology. Here, data represents overall least squares means with standard error bars for ambient water and drought treatments for **A** the day first fruits were visible (day first fruit of the soybean row was observed emerging 2-3 mm from senescing flower), **B** the day fruits were mature (day 95% of fruits in soybean row were matured), and **C** fruit maturation rate (time in days between day first fruits visible and day fruits were mature).



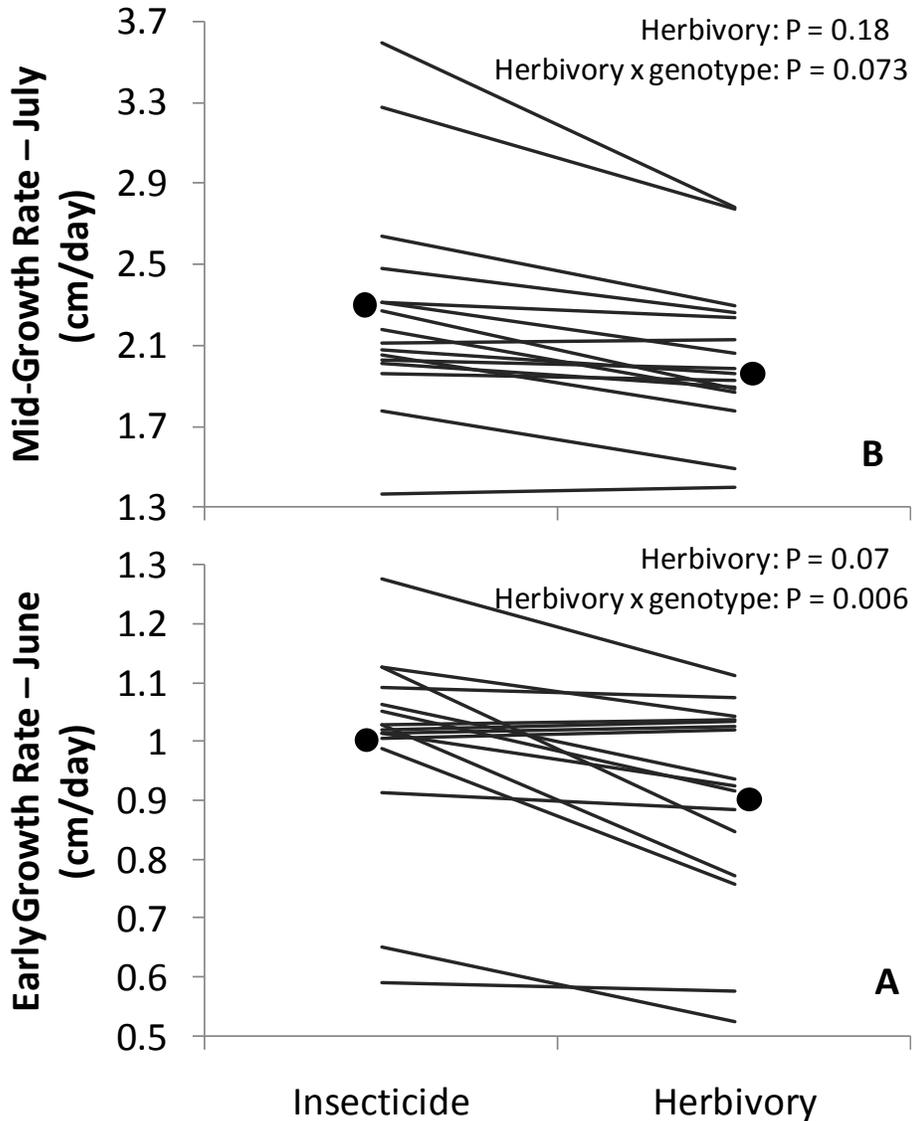
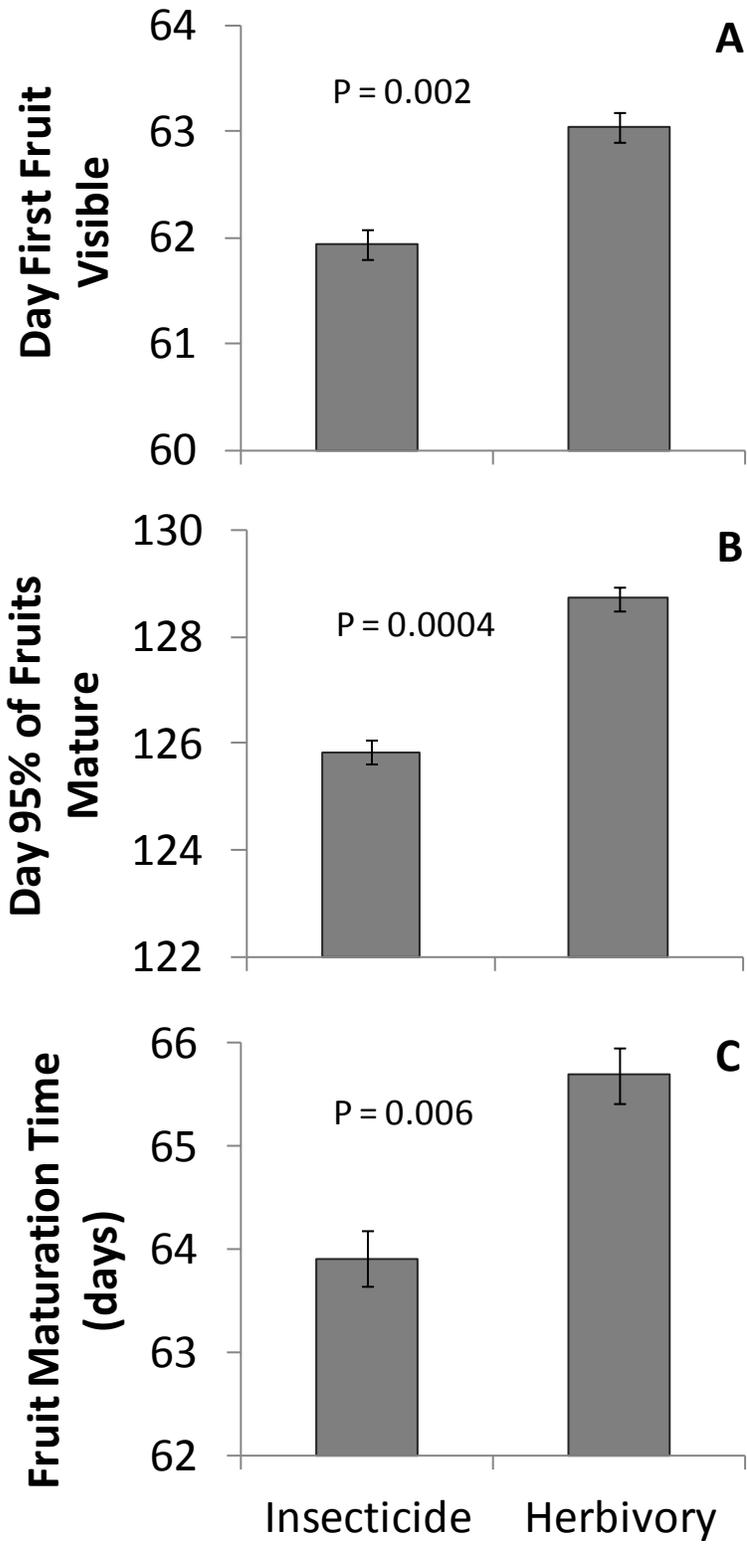


Figure 3.6 Effects of herbivory treatment x plant genotype on early and mid growth rates. Here, each line represents the least squares mean for a soybean genotype for insecticide and herbivory treatments for **A** early growth rate (June) and **B** mid-growth rate (July). The p-value for these means is represented as Herbivory x genotype. The large black dots outside the genotype lines represent overall means for all genotypes for both insecticide and herbivory treatments, and their p-value is represented as Herbivory. Note that the range of early growth rates showed no overlap with the range of mid-growth rates.

Figure 3.7 Effects of herbivory on fruit phenology. Here, data represents overall least squares means with standard error bars for insecticide and herbivory treatments for **A** the day first fruits were visible (day first fruit of the soybean row was observed emerging 2-3 mm from senescing flower), **B** the day fruits were mature (day 95% of fruits in soybean row were matured), and **C** fruit maturation rate (time in days between day first fruits visible and day fruits were mature).



Resistance to herbivory

Our second research question asked whether drought affected soybean resistance to insect herbivores. We found that on average drought was associated with a 28% reduction in herbivory by late-summer, an effect that was weakly significant (Table 3.8). When evaluated among genotypes, however, there was large variation in how drought modified a genotype's susceptibility to herbivores, where the range of genotypic responses ranged from a 12% increase in herbivory with drought, to a 42% decrease (Table 3.8, Fig. 3.8), suggesting that the insect preference will depend on both the combination of a soybean's genotype and the degree of drought.

Our no-choice bioassay suggested that the strong drought x genotype interactions observed for levels of herbivory are largely driven by differences in herbivore choice rather than herbivore performance. Drought had no clear effect on the performance or survival of either *H. zea* or *S. exigua* (Table 3.9). Genotypes did vary in their resistance to *H. zea*, but not to *S. exigua* (Table 3.9). It does seem that soybean genotype played a role in the insect's performance, although any evidence of this is largely overshadowed by the role of the insecticide treatment (Table 3.10), which was the dominant factor in the variation within both insect performance and survival.

Traits that predict plant performance and resistance to herbivory

Our third research question focused on identifying genetically variable

Table 3.8 Effects of experimental treatments and soybean genotype on insect herbivory. We include results from analysis of variance (ANOVA) for 3 measures of % insect damage to each soybean row (mid-July, mid-Aug., and mid-Sept.).

Herbivory mid-July				
	df	SS	F	P
Block (drought) ¹	6	11.17	2.51	0.03
Drought ²	1	0.01	0.01	0.94
Genotype (maturity group) ¹	15	53.65	4.82	<.001
Herbivory mid-August				
	df	SS	F	P
Block (drought) ¹	6	41.71	4.47	0.0005
Drought ²	1	2.07	0.3	0.61
Genotype (maturity group) ¹	15	45.62	1.96	0.03
Herbivory mid-September				
	df	SS	F	P
Block (drought) ¹	6	86.28	2.42	0.03
Drought ³	1	174.69	4.26	0.0558
Maturity group ⁴	3	727.14	4.3	0.0281
Genotype (maturity group) ⁵	12	676.54	1.73	0.1572
Drought x genotype (maturity group) ¹	15	489.01	5.5	<0.0001

¹ Denominator for testing these effects was MS error; ² Denominator was MS block (drought);

³ Denominator was MS Block (drought) + MS drought x genotype - MS error; ⁴ Denominator was MS genotype (maturity group); ⁵ Denominator was MS drought x genotype (maturity group).

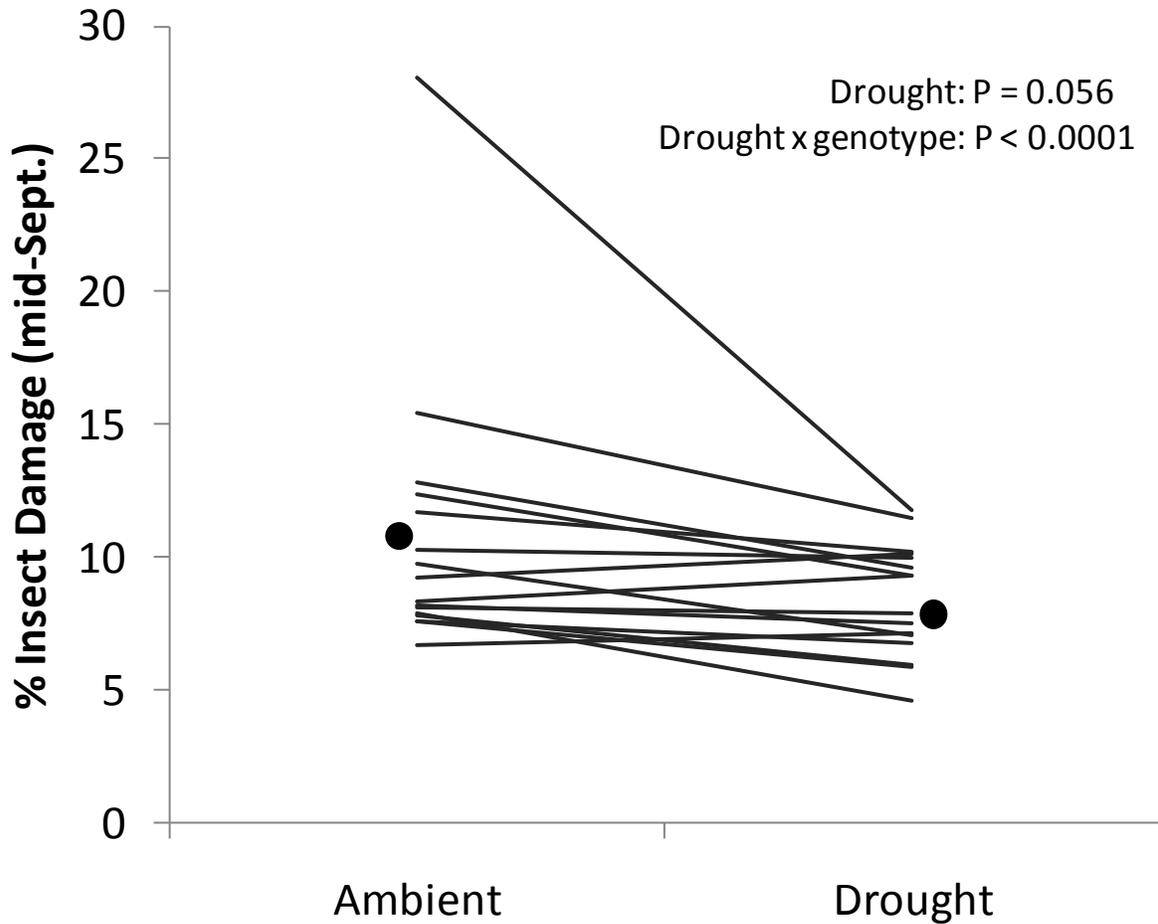


Figure 3.8 Effects of drought treatment x plant genotype on percent herbivore damage. Here, each line represents the least squares mean of % herbivore damage on the soybean rows for a soybean genotype for ambient water and drought treatments. The p-value for these means is represented as Drought x genotype. The large black dots outside the genotype lines represent overall means for all genotypes for both ambient and drought treatments, and their p-value is represented as Drought.

Table 3.9 Effects of experimental treatments and soybean genotype on a bioassay. We include results from analysis of variance (ANOVA) for a no-choice feeding assay using generalist neonate caterpillars to test soybean susceptibility to herbivory by *Helicoverpa zea* and *Spodoptera exigua*, where *ndf* represents numerator degrees of freedom and *ddf* represents denominator degrees of freedom.

Bioassay

***H. zea* mass**

	df	SS	F	P
Block (drought) ¹	6	8.11	2.69	0.02
Drought ²	1	0.1	0.07	0.79
Genotype ¹	15	14.05	1.87	0.03

***H. zea* survival**

Model 1:	df	SS	F	P
Drought	1	6	1.25	0.31
Maturity group	3	117	2.1	0.1

Model 2:	df	X ²	P
Drought	1	2.13	0.14
Genotype	15	26.29	0.0351

***S. exigua* mass**

	df	SS	F	P
Block (drought) ¹	6	97.99	4.96	0.0002
Drought ²	1	2.88	0.18	0.6894
Genotype ¹	15	36.99	0.75	0.7294

***S. exigua* survival**

	ndf	ddf	F	P
Drought	1	6	0.13	0.73
Maturity group	3	105	0.22	0.89
Genotype (maturity group)	12	105	0.96	0.49

¹ Denominator for testing these effects was MS error; ² Denominator was Block (drought).

Table 3.10 Effects of insecticide treatment on bioassay insects. We include results from analysis of variance (ANOVA) for effects of insecticide spray in the soybean field on the generalist neonate caterpillars of our no-choice feeding assay, where we tested soybean susceptibility to herbivory by *Helicoverpa zea* and *Spodoptera exigua*.

Effect of insecticide on neonates

***H. zea* biomass**

	df	SS	F	P
Block (herbivory)	14	126.94	2.79	0.0007
Herbivory	1	189.52	20.9	0.0004
Error	240	780.9		

***H. zea* survival**

	df	F	P
Herbivory	1,14	16.76	0.0011

***S. exigua* biomass**

	df	SS	F	P
Block (herbivory)	14	146.32	4.37	<.0001
Herbivory	1	45.26	4.33	0.0563
Error	240	574.19		

***S. exigua* survival**

	df	F	P
Herbivory	1,14	11.56	0.0043

phenotypic traits that predicted plant performance or susceptibility to herbivores. The strongest predictors of seed yield were plant growth rate, some fruit phenology, and herbivory (Table 3.11). An increase in growth rate was negatively genetically correlated with a decrease in seed yield (Fig. 3.9A) in both ambient ($r = -0.48$, $p = 0.06$) and drought ($r = -0.30$, $p = 0.25$) treatments, although it was only significant in ambient plants. Fruit maturation was weakly positively associated with yield in ambient plants (Table 3.11). An increase in herbivore damage correlated negatively with a decrease in yield (Fig. 3.9B), and the correlation was stronger in ambient plants ($r = -0.66$, $p = 0.005$) than in droughted plants ($r = -0.37$, $p = 0.015$). While only significant in ambient plants (ambient: $r = +0.62$, $p = 0.01$; drought: $r = +0.24$, $p = 0.37$), water potential was positively correlated with seed yield, and this effect was weakened with drought. There were no other performance-related response variables that showed any significant correlations.

The strongest predictor of susceptibility to herbivores was plant growth rate (Table 3.11, Fig. 3.9C). Plant growth rate genetically correlated with the amount of herbivory received, but the effect was stronger in ambient plants ($r = +0.71$, $p = 0.002$) than in droughted plants ($r = +0.59$, $p = 0.015$). Early reproductive phenologies showed a weak negative association with herbivory (Table 3.11), more so in droughted plants than in ambient plants. No other traits show a clear relationship with herbivory.

Table 3.11 Correlation coefficients for traits that predict plant performance and resistance to herbivory. We include results from Pearson's correlation coefficient for the soybean genotype least squares means of soybean traits that can potentially predict plant performance and resistance to herbivory across ambient and drought treatments.

Traits that predict plant performance and resistance to herbivory

Plant performance

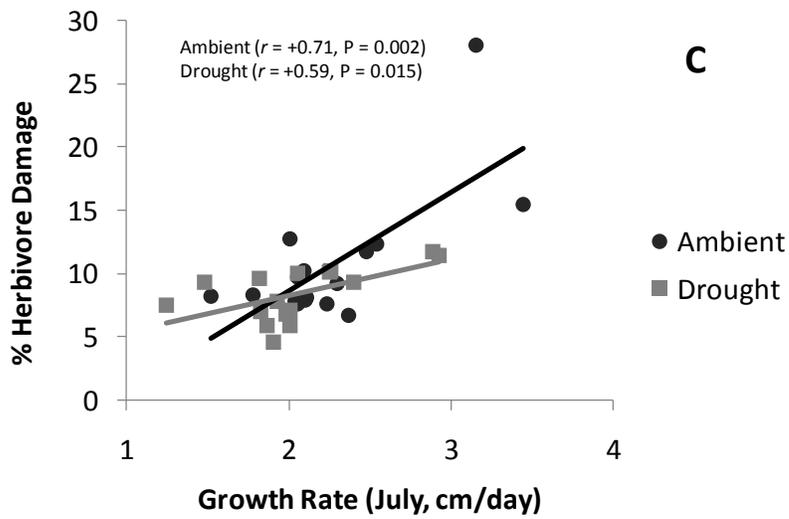
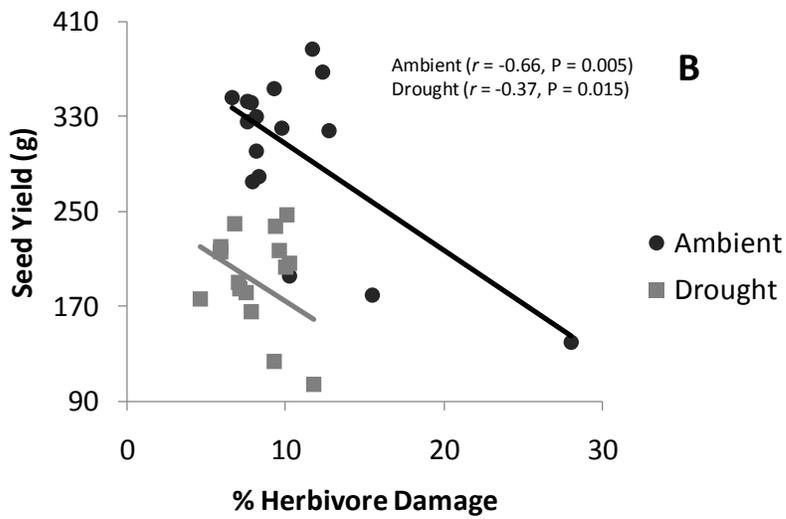
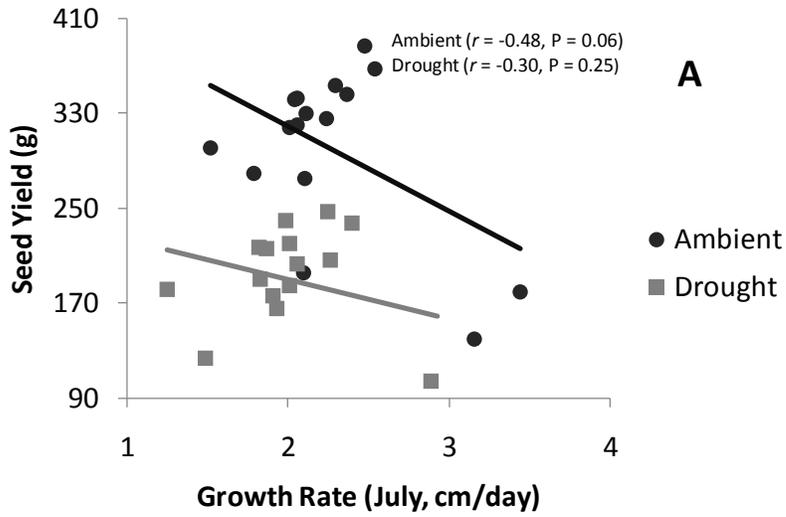
Predictor	Response variable	Ambient		Drought	
		r	P	r	P
Growth Rate (mid)	Seed Yield	-0.48	0.060	-0.30	0.249
Date First Flower Open	Seed Yield	+0.39	0.134	+0.03	0.915
Date First Fruit Emerged	Seed Yield	+0.40	0.119	-0.00	0.977
Date 95% Fruits Mature	Seed Yield	+0.49	0.056	+0.10	0.688
Fruit Maturation Rate	Seed Yield	+0.48	0.057	+0.17	0.532
% Herbivore Damage	Seed Yield	-0.66	0.005**	-0.37	0.146
Trichome #	Seed Yield	-0.04	0.881	-0.05	0.828
Specific Leaf Area	Seed Yield	+0.10	0.714	+0.26	0.323
% Leaf Water	Seed Yield	-0.22	0.403	+0.09	0.727
Stomatal Conductance	Seed Yield	-0.41	0.112	+0.14	0.585
Water Potential	Seed Yield	+0.62	0.012**	+0.24	0.370

Resistance to herbivory

Predictor	Response variable	Ambient		Drought	
		r	P	r	P
Growth Rate (mid)	% Herbivore Damage	+0.71	0.002**	+0.59	0.015*
Date First Flower Open	% Herbivore Damage	-0.46	0.075	-0.50	0.050*
Date First Fruit Emerged	% Herbivore Damage	-0.48	0.059	-0.57	0.021*
Date 95% Fruits Mature	% Herbivore Damage	-0.40	0.130	-0.37	0.150
Fruit Maturation Rate	% Herbivore Damage	-0.30	0.265	-0.22	0.387
Trichome #	% Herbivore Damage	+0.20	0.467	+0.10	0.780
Specific Leaf Area	% Herbivore Damage	-0.05	0.830	-0.26	0.327
% Leaf Water	% Herbivore Damage	+0.22	0.426	+0.14	0.633
Stomatal Conductance	% Herbivore Damage	+0.46	0.078	-0.10	0.663
Water Potential	% Herbivore Damage	-0.47	0.067	-0.14	0.571

* Significant at the 0.05 level; ** Significant at the 0.01 level

Figure 3.9 Traits that predict plant performance and resistance to herbivory. Here, we present regressions for **A** soybean seed yield as a function of mid-growth rate (July), **B** soybean seed yield as a function of % herbivore damage, and **C** % herbivore damage as a function of mid-growth rate (July). Each point represents the least squares mean for each soybean genotype for either ambient or drought treatments. Trend lines have been added.



DISCUSSION

We found that overall, drought was the dominant stressor affecting soybean performance, whereas herbivory had relatively weak and transient effects on plants. Specifically, drought impacted most measures of plant performance, including seed yield, final height and fruit phenology (Tables 3.1-3.2, 3.4-3.7). By contrast, insect herbivory only affected early (vegetative) growth rate and fruit phenology (Tables 3.3, 3.4-3.7). Drought also decreased susceptibility of plants to herbivores (Fig. 3.8), consistent with the Plant Vigor Hypothesis, which posits that fast growing plants will be most susceptible to herbivores (Price 1991). Life-history traits, such as growth rate and phenology were the traits that most strongly predicted these responses (Fig. 3.7, 3.9). Below we consider effects of abiotic and biotic stress on plant performance, effects of drought on susceptibility to herbivory, traits that predict plant performance and resistance to herbivory, and whether abiotic or biotic stress is more important in agriculture.

Effects of abiotic and biotic stress on plant performance

A primary objective of our study was to understand whether abiotic and biotic stresses have additive or non-additive effects on plant performance. Drought and herbivory, when significant, impacted plant performance independently of one another, indicating their effects were additive. These results show similarities with our controlled growth experiments (see Chapter 2), where there were similarly no interactions between drought and herbivory. The lack of interactions in both the

controlled growth experiments and field experiment strongly suggest that the combined effects of drought and herbivory are unlikely to produce dramatically decreased performance in soybeans due to synergistic negative effects. Our results further imply that the effects of these stressors can be studied independently of one another in the context of predicting the effects of climate change.

Numerous studies support our general finding that soybeans exhibit clear decreases in performance due to drought (Hoogenboom et al. 1987, Ray and Sinclair 1998, Liu et al. 2003, James et al. 2008). Also, many studies report negative impacts on soybean due to herbivory (Smelser and Pedigo 1992, Felton et al. 1994, Kamalay et al. 1997, Terry et al. 1999, Rypstra and Marshall 2005, Costamagna et al. 2007). However, few studies have investigated the responses of soybean to combinations of abiotic and biotic stress (Smelser and Pedigo 1992, Rogers et al. 1994). Smelser and Pedigo (1992) found that drought and herbivory from bean leaf beetles (pod feeders) did not result in increased loss of yield, but instead, droughted plants saw less pod loss to herbivory than healthy plants. These results are consistent with our finding that water stressed soybeans are less susceptible to herbivores.

In other contexts, the combined effects of abiotic and biotic stress can lead to synergistic negative effects on the performance of organisms. For example, Relyea (2003, 2004, 2005) demonstrated that amphibians simultaneously exposed to pesticides and risk of predation experience 2-46 times greater mortality than amphibians only exposed to pesticides. Deegan et al. (2007) showed that nutrient

loading in salt marshes and the removal of a predator caused synergistic increases on benthic microalgae biomass. More relevant to crop studies, drought led to increased susceptibility to herbivores in rice, and together drought and the added insect damage caused non-additive synergistic losses in yield (Boling et al. 2004, Litsinger et al. 2005, Litsinger et al. 2011). Therefore, it is clear that the combination of multiple stressors can have wide ranging effects on the performance of organisms, from additive to synergistic negative effects. Discerning under what conditions and in what systems a particular outcome will be realized can only be determined through direct experimentation.

Effects of drought on susceptibility to herbivores

There were some disparities between our indoor experiments (see Chapter 2) and our field experiment in the effects of drought on insect herbivory. In controlled environments, drought typically led to increased performance of three generalist lepidopteran herbivore species, although this result varied greatly between soybean genotypes in both magnitude and directionality. In our field experiment, we observed the opposite result, where drought resulted in decreased levels of insect herbivory. Potentially important differences between these experiments were the availability of choice and the ability of the insects to move from one plant to another, as well as the fact that drought and herbivory were not concurrent in our indoor experiments as they were in the field. In our indoor experiments and our bioassays, insects were not given any choice between either treatment or plant genotype, and they were not able

to move from one plant to another. This deemed that the insect eat what it was given or nothing, and insect performance measurements may be convoluted by instinct to survive. In the field, not only did the insects have a choice between plant genotype within their immediate vicinity, but they also had the ability to move to nearby soybean blocks receiving varying treatment combinations. This availability of choice and movement more clearly indicates insect preferences to plant drought stress levels and plant genotype than our indoor experiments and bioassays allowed. It is unknown how this hypothesis would apply in the field if widespread and severe drought were to occur over a large area, however.

Abiotic stresses like drought have long been predicted to affect the susceptibility of plants to herbivores. Our findings of lower herbivory on plants subjected to drought, as well as the positive genotypic correlation between plant growth rate and herbivory, support the Plant Vigor Hypothesis (Price 1991). This is contrary to the Plant Stress Hypothesis which suggests that abiotic stress, such as drought, will cause an increase in insect herbivory (White 1984), although Price (1991) was clear in pointing out that one hypothesis does not necessarily negate the other. He suspects, rather, that the two hypotheses are more likely extremes of a larger continuum involving stress, resistance traits and the specificity of the insect herbivore. This idea was recently supported by Gutbrodt et al. (2011) who found that herbivore responses to drought depended on insect feeding preferences and specificity on *Alliaria petiolata* (Brassicaceae).

Traits that predict plant performance and resistance to herbivory

We found that susceptibility to herbivores depended highly on soybean genotype. Variation in resistance to herbivores is well known from soybean (Underwood 1998, Terry et al. 1999, James et al. 2008) and a common phenomenon in most plant systems (Horner and Abrahamson 1992, Agrawal et al. 1999, McGuire and Johnson 2006, Johnson 2008). This result was also demonstrated in Chapter 2, where the magnitude and directionality of herbivore resistance varied with simulated drought conditions, meaning that, while some soybeans showed mild to extreme susceptibility to herbivores following a drought, others remained mildly to highly resistant. Variations in resistance among different genotypes are important because they directly relate to phenotypic traits that may assist breeders in their discovery and selection of stress-resistance plants.

One way ecology can provide a more general predictive framework to understand how climate change will influence plants and their interactions with other species is to understand the plant traits that mediate their interactions with the environment (McGill et al. 2006). An examination of genetically controlled variation in phenotypic traits (i.e. morphological, physiological, etc.) may help us understand the ecological workings of the community studied so that we can better predict community responses in light of environmental changes to come. Our results show that soybean growth rate, seed yield and insect herbivory are all genetically correlated with each other, such that high growth rates were correlated with lower seed yields and higher levels of insect herbivory, and higher levels of herbivory were

correlated with lower seed yields. We speculate that soybeans that invest a great deal of resources in early growth and development expend valuable resources at the cost of allocation to fruit and seed production later in the season, and that generalist insects appear to prefer these types of soybean. This speculation may allow researchers to explore predictions about soybean responses to climate change and resistance to stresses in the context of variation in traits rather than direct pairwise comparisons with specific insect species.

It has long been the dogma that plant metabolic compounds and physical defense traits (i.e. trichomes, latex) dictate plant resistance to herbivores. However, this dogma is coming into question as more studies emerge showing the importance of other plant traits not traditionally implicated in plant defense (i.e. growth rate, stomatal conductance), yet still having large effects on the resistance or susceptibility to herbivores (Pilson 2000, Agrawal 2004, Johnson 2008). In a meta-analysis of ecological genetics studies, Carmona et al. (2011) found that life-history traits and plant gross morphology were consistently genetically correlated with herbivore resistance, even more so than secondary metabolites. Our results also show that higher growth rate and lower seed yield may predict higher levels of herbivory, although the importance of secondary compounds was not investigated here. These correlations are consistent across most of our ambient and droughted plants, and there is evidence that drought-stressed plants in other systems may actually contain higher levels of defense compounds (Haugen et al. 2008), suggesting that the importance of secondary compounds may need to be re-

evaluated. This supports the idea that phenotypic traits not traditionally implicated in resistance may be as important as, and perhaps even more important than, secondary compounds in predicting a plant's resistance to herbivory.

Is abiotic or biotic stress more important in agriculture?

Our results suggest that abiotic stress, namely drought, is an important stress affecting soybean performance. While biotic stress, namely insect herbivory, will be important to watch, it appears that generalist herbivores were less impactful in this study. Our plants receiving herbivory treatment experienced no loss of seed yield compared to the plants receiving insecticide spray, despite the introduction of ~5 additional young caterpillars to each plant early in the season and relatively high levels of herbivore damage on these plants ($\approx 10\%$ on average). This result implies that average field herbivory had no discernible effect on soybean seed yield, the most economically relevant measure of plant performance, in this 2010 study. This may mean that utilizing management resources to protect soybean from common field herbivory should be carefully evaluated. And, in general, we believe that drought will be the primary stress that farmers should focus on mitigating.

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REFERENCES

- Agrawal, A. A. 2004. Plant defense and density dependence in the population growth of herbivores. *American Naturalist* **164**:113-120.
- Agrawal, A. A., J. K. Conner, M. T. J. Johnson, and R. Wallsgrave. 2002. Ecological genetics of an induced plant defense against herbivores: Additive genetic variance and costs of phenotypic plasticity. *Evolution* **56**:2206-2213.
- Agrawal, A. A., P. M. Gorski, and D. W. Tallamy. 1999. Polymorphism in plant defense against herbivory: Constitutive and induced resistance in *Cucumis sativus*. *Journal of Chemical Ecology* **25**:2285-2304.
- Andrewartha, H. G. and L. C. Birch. 1954. *The Distribution and Abundance of Animals*. The University of Chicago Press, Chicago & London.
- Boling, A., T. P. Tuong, S. Y. Jatmiko, and M. A. Burac. 2004. Yield constraints of rainfed lowland rice in Central Java, Indonesia. *Field Crops Research* **90**:351-360.
- Carmona, D., M. J. Lajeunesse, and M. T. J. Johnson. 2011. Plant traits that predict resistance to herbivores. *Functional Ecology* **25**:358-367.

- Carter, T.E., R. L. Nelson, C. Sneller, and Z. Cui, 2004. Genetic Diversity in Soybean. Soybeans: Improvement, Production, and Uses, Third Edition (Agronomy). Boerma, H. R. and J. E. Specht (Ed.). American Society of Agronomy, Madison, WI. 303-416.
- Charlson, D. V., S. Bhatnagar, C. A. King, J. D. Ray, C. H. Sneller, T. E. Carter, and L. C. Purcell. 2009. Polygenic inheritance of canopy wilting in soybean *Glycine max* (L.) Merr. Theoretical and Applied Genetics **119**:587-594.
- Cook, E. R., R. Seager, M. A. Cane, and D. W. Stahle. 2007. North American drought: Reconstructions, causes, and consequences. Earth-Science Reviews **81**:93-134.
- Costamagna, A. C., D. A. Landis, and C. D. Difonzo. 2007. Suppression of soybean aphid by generalist predators results in a trophic cascade in soybeans. Ecological Applications **17**:441-451.
- Deegan, L. A., J. L. Bowen, D. Drake, J. W. Fleeger, C. T. Friedrichs, K. A. Galvan, J. E. Hobbie, C. Hopkinson, D. S. Johnson, J. M. Johnson, L. E. Lemay, E. Miller, B. J. Peterson, C. Picard, S. Sheldon, M. Sutherland, J. Vallino, and R. S. Warren. 2007. Susceptibility of salt marshes to nutrient enrichment and predator removal. Ecological Applications **17**:S42-S63.
- Dermody, O., B. F. O'Neill, A. R. Zangerl, M. R. Berenbaum, and E. H. DeLucia. 2008. Effects of elevated CO₂ and O₃ on leaf damage and insect abundance in a soybean agroecosystem. Arthropod-Plant Interactions **2**:125-135.
- Du, W. J., M. Wang, S. X. Fu, and D. Y. Yu. 2009a. Mapping QTLs for seed yield and drought susceptibility index in soybean (*Glycine max* L.) across different environments. Journal of Genetics and Genomics **36**:721-731.
- Du, W. J., D. Y. Yu, and S. X. Fu. 2009b. Analysis of QTLs for the trichome density on the upper and downer surface of leaf blade in soybean *Glycine max* (L.) Merr. Agricultural Sciences in China **8**:529-537.
- Du, W. J., D. Y. Yu, and S. X. Fu. 2009c. Detection of quantitative trait loci for yield and drought tolerance traits in soybean using a recombinant inbred line population. Journal of Integrative Plant Biology **51**:868-878.
- Easterling, D. R., G. A. Meehl, C. Parmesan, S. A. Changnon, T. R. Karl, and L. O. Mearns. 2000. Climate extremes: Observations, modeling, and impacts. Science **289**:2068-2074.

- Farooq, M., N. Kobayashi, O. Ito, A. Wahid, and R. Serraj. 2010. Broader leaves result in better performance of indica rice under drought stress. *Journal of Plant Physiology* **167**:1066-1075.
- Felton, G. W., C. B. Summers, and A. J. Mueller. 1994. Oxidative responses in soybean foliage to herbivory by bean leaf beetle and 3-cornered alfalfa hopper. *Journal of Chemical Ecology* **20**:639-650.
- Gutbrodt, B., K. Mody, and S. Dorn. 2011. Drought changes plant chemistry and causes contrasting responses in lepidopteran herbivores. *Oikos* **000**:1-9.
- Hamilton, J. G., O. Dermody, M. Aldea, A. R. Zangerl, A. Rogers, M. R. Berenbaum, and E. H. DeLucia. 2005. Anthropogenic changes in tropospheric composition increase susceptibility of soybean to insect herbivory. *Environmental Entomology* **34**:479-485.
- Hammond, R. B., P. Bierman, E. Levine, and R. L. Cooper. 2001. Field resistance of two soybean germplasm lines, HC95-15MB and HC95-24MB, against bean leaf beetle (Coleoptera : Chrysomelidae), western corn rootworm (Coleoptera : Chrysomelidae), and Japanese beetles (Coleoptera : Scarabidae). *Journal of Economic Entomology* **94**:1594-1601.
- Haugen, R., L. Steffes, J. Wolf, P. Brown, S. Matzner, and D. H. Siemens. 2008. Evolution of drought tolerance and defense: Dependence of tradeoffs on mechanism, environment and defense switching. *Oikos* **117**:231-244.
- Hoogenboom, G., M. G. Huck, and C. M. Peterson. 1987. Root-growth rate of soybean as affected by drought stress. *Agronomy Journal* **79**:607-614.
- Horner, J. D. and W. G. Abrahamson. 1992. Influence of plant genotype and environment on oviposition preference and offspring survival in a gallmaking herbivore. *Oecologia* **90**:323-332.
- Hull-Sanders, H. M. and M. D. Eubanks. 2005. Plant defense theory provides insight into interactions involving inbred plants and insect herbivores. *Ecology* **86**:897-904.
- Intergovernmental Panel on Climate Change Fourth Assessment Report. 2007. *Climate Change 2007: Synthesis Report*. Allali, A., R. Bojariu, S. Diaz, I. Elgizouli, D. Griggs, D. Hawkins, O. Hohmeyer, B. P. Jallow, L. Kajfež-Bogataj, N. Leary, H. Lee, and D. Wratt (Ed.). Cambridge University Press, Cambridge.

- James, A. T., R. J. Lawn, and M. Cooper. 2008. Genotypic variation for drought stress response traits in soybean. I. Variation in soybean and wild *Glycine* spp. for epidermal conductance, osmotic potential, and relative water content. *Australian Journal of Agricultural Research* **59**:656-669.
- Johnson, M. T. J. 2008. Bottom-up effects of plant genotype on aphids, ants, and predators. *Ecology* **89**:145-154.
- Johnson, M. T. J. and A. A. Agrawal. 2005. Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). *Ecology* **86**:874-885.
- Kamalay, J. C., J. H. Barger, and P. E. Pierson. 1997. Survival and development of gypsy moths (Lepidoptera: Lymantriidae) on corn and soybean foliage. *Journal of Economic Entomology* **90**:147-153.
- Levi, A., A. Paterson, V. Barak, D. Yakir, B. Wang, P. Chee, and Y. Saranga. 2009. Field evaluation of cotton near-isogenic lines introgressed with QTLs for productivity and drought related traits. *Molecular Breeding* **23**:179-195.
- Levine, M. T. and K. N. Paige. 2004. Direct and indirect effects of drought on compensation following herbivory in scarlet gilia. *Ecology* **85**:3185-3191.
- Li, Y. P., W. Ye, M. Wang, and X. D. Yan. 2009. Climate change and drought: A risk assessment of crop-yield impacts. *Climate Research* **39**:31-46.
- Litsinger, J. A., J. P. Bandong, and B. L. Canapi. 2011. Effect of multiple infestations from insect pests and other stresses to irrigated rice in the Philippines: II. Damage and yield loss. *International Journal of Pest Management* **57**:117-131.
- Litsinger, J. A., J. P. Bandong, B. L. Canapi, C. G. Dela Cruz, P. C. Pantua, A. L. Alviola, and E. H. Batay-An. 2005. Evaluation of action thresholds for chronic rice insect pests in the Philippines. I. Less frequently occurring pests and overall assessment. *International Journal of Pest Management* **51**:45-61.
- Liu, F. L., M. N. Andersen, and C. R. Jensen. 2003. Loss of pod set caused by drought stress is associated with water status and ABA content of reproductive structures in soybean. *Functional Plant Biology* **30**:271-280.
- Manavalan, L. P., S. K. Guttikonda, L. S. P. Tran, and H. T. Nguyen. 2009. Physiological and molecular approaches to improve drought resistance in soybean. *Plant and Cell Physiology* **50**:1260-1276.

- McGill, B. J., B. J. Enquist, E. Weiher, and M. Westoby. 2006. Rebuilding community ecology from functional traits. *Trends in Ecology & Evolution* **21**:178-185.
- McGuire, R. J. and M. T. J. Johnson. 2006. Plant genotype and induced responses affect resistance to herbivores on evening primrose (*Oenothera biennis*). *Ecological Entomology* **31**:20-31.
- Parmesan, C. and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**:37-42.
- Pilson, D. 2000. Herbivory and natural selection on flowering phenology in wild sunflower, *Helianthus annuus*. *Oecologia* **122**:72-82.
- Portmann, R. W., S. Solomon, and G. C. Hegerl. 2009. Spatial and seasonal patterns in climate change, temperatures, and precipitation across the United States. *Proceedings of the National Academy of Sciences of the United States of America* **106**:7324-7329.
- Price, P. W. 1991. The plant vigor hypothesis and herbivore attack. *Oikos* **62**:244-251.
- Ray, J. D. and T. R. Sinclair. 1998. The effect of pot size on growth and transpiration of maize and soybean during water deficit stress. *Journal of Experimental Botany* **49**:1381-1386.
- Relyea, R. A. 2003. Predator cues and pesticides: A double dose of danger for amphibians. *Ecological Applications* **13**:1515-1521.
- Relyea, R. A. 2004. Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. *Environmental Toxicology and Chemistry* **23**:1080-1084.
- Relyea, R. A. 2005. The lethal impacts of roundup and predatory stress on six species of North American tadpoles. *Archives of Environmental Contamination and Toxicology* **48**:351-357.
- Rogers, H. H., G. B. Runion, and S. V. Krupa. 1994. Plant-responses to atmospheric CO₂ enrichment with emphasis on roots and the rhizosphere. *Environmental Pollution* **83**:155-189.
- Rypstra, A. L. and S. D. Marshall. 2005. Augmentation of soil detritus affects the spider community and herbivory in a soybean agroecosystem. *Entomologia Experimentalis Et Applicata* **116**:149-157.

- Schoonhoven, L. M., J. J. A. van Loon, and M. Dicke. 2005. Insect-plant biology. Second edition. Oxford University Press, Oxford, New York.
- Seager, R., A. Tzanova, and J. Nakamura. 2009. Drought in the southeastern United States: Causes, variability over the last millennium, and the potential for future hydroclimate change. *Journal of Climate* **22**:5021-5045.
- Sletvold, N., P. Huttunen, R. Handley, K. Karkkainen, and J. Agren. 2010. Cost of trichome production and resistance to a specialist insect herbivore in *Arabidopsis lyrata*. *Evolutionary Ecology* **24**:1307-1319.
- Smelser, R. B. and L. P. Pedigo. 1992. Bean leaf beetle (Coleoptera, Chrysomelidae) herbivory on leaf, stem, and pod components of soybean. *Journal of Economic Entomology* **85**:2408-2412.
- Terry, L. I., K. Chase, J. Orf, T. Jarvik, L. Mansur, and K. G. Lark. 1999. Insect resistance in recombinant inbred soybean lines derived from non-resistant parents. *Entomologia Experimentalis Et Applicata* **91**:465-476.
- Underwood, N. C. 1998. The timing of induced resistance and induced susceptibility in the soybean Mexican bean beetle system. *Oecologia* **114**:376-381.
- White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. *Oecologia* **63**:90-105.
- Williams, J. W. and S. T. Jackson. 2007. Novel climates, no-analog communities, and ecological surprises. *Frontiers in Ecology and the Environment* **5**:475-482.
- Willis, C. G., B. Ruhfel, R. B. Primack, A. J. Miller-Rushing, and C. C. Davis. 2008. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences of the United States of America* **105**:17029-17033.
- Yesudas, C. R., H. Sharma, and D. A. Lightfoot. 2010. Identification of QTL in soybean underlying resistance to herbivory by Japanese beetles (*Popillia japonica*, Newman). *Theoretical and Applied Genetics* **121**:353-362.

CHAPTER 4

Soybean, Herbivory and Climate Change Conclusions

Three major conclusions can be made from this research. The first conclusion is that drought appears to be a more dominant stress on soybean than temperature or insect herbivory. This conveys the importance of water in all plant processes, especially growth and development. The primary concern in protecting the health and productivity of soybean for predicted climate change should focus on imparting tolerance of and resistance to drought. The utilization of resources to eradicate generalist insect herbivores in a first-year soybean plot should be evaluated carefully, as the circumstances of this study produced no overall loss of yield to generalist insect herbivory. It appears that temperature will not be a major stress to soybean at a global increase of 2°C.

The second, and most interesting conclusion of this research is that the stresses tested here never interacted to impact plant performance or each other. This means that drought and temperature will not increase insect herbivory, on average, for the soybean genotypes tested here (Appendix A). It also means that if increasing global temperature does result in increased drought and herbivorous insect population outbreaks, that soybean will not be dramatically more affected through synergistic negative effects. This implies that the testing of these stresses in future soybean research may effectively be tested independently.

The third, and over-arching conclusion is that genotypic differences arise in response to drought, temperature and herbivory. Not surprising, soybean genotype resulted in variations in most measured traits and stress effects. The genotypic variations seen in this study were often both in magnitude and directionality, suggesting that, while some genotypes will remain viable and resilient in light of future climate change, others may be deficient in their ability to withstand more than one environmental stress. These genotypes that appear resilient may be able to aid breeders in their search for mechanisms behind stress tolerance.

It has been emphasized that phenotypic traits may be good predictors of soybean resistance to stress. Phenotypic traits that genetically predicted soybean yield were growth rate and herbivore damage (both negative correlations). The trait that genetically predicted overall herbivore damage was growth rate (positive correlation). It is re-emphasized that the search for predictive phenotypic traits will ultimately lead to better understanding of resistance and tolerance mechanisms (Sadok and Sinclair 2010, Sinclair et al. 2010). An understanding of these mechanisms and the sequencing of the soybean genome (Kim et al. 2010) will provide much needed clarity in this field, and will bring us closer to ensuring future food security in the inevitable event of environmental change.

REFERENCES

- Kim, M. Y., S. Lee, K. Van, T. H. Kim, S. C. Jeong, I. Y. Choi, D. S. Kim, Y. S. Lee, D. Park, J. Ma, W. Y. Kim, B. C. Kim, S. Park, K. A. Lee, D. H. Kim, K. H. Kim, J. H. Shin, Y. E. Jang, K. Do Kim, W. X. Liu, T. Chaisan, Y. J. Kang, Y. H. Lee, J. K. Moon, J. Schmutz, S. A. Jackson, J. Bhak, and S. H. Lee. 2010. Whole-genome sequencing and intensive analysis of the undomesticated soybean (*Glycine soja* Sieb. and Zucc.) genome. *Proceedings of the National Academy of Sciences of the United States of America* **107**:22032-22037.
- Sadok, W. and T. R. Sinclair. 2010. Transpiration response of 'slow-wilting' and commercial soybean (*Glycine max* (L.) Merr.) genotypes to three aquaporin inhibitors. *Journal of Experimental Botany* **61**:821-829.
- Sinclair, T. R., C. D. Messina, A. Beatty, and M. Samples. 2010. Assessment across the United States of the benefits of altered soybean drought traits. *Agronomy Journal* **102**:475-482.

APPENDICES

Appendix A – Soybean Genotypes and Means

Soybean genotypes used in experiments and their calculated trait means for Experiment 3 (Chapter 2; Tables A.1-A.2) and the field experiment (Chapter 3; Tables A.3-A.4). *Genotype number* is the experimental code for each genotype used in data collection and the codes remain the same for each genotype across experiments.

Table A.1 Soybean genotypes used in Experiment 3 (Chapter 2). *Maturity group* is the growth classification representing length of time from planting until pods have matured. The lower the maturity group numerically, the shorter the time until maturity and, generally, the more north the cultivar will be planted. *Entry* is the soybean name or breeder code for each cultivar line. *Source* is the source of the genotype (i.e., breeder, supplier or accession number). *Rationale for inclusion* is the trait that the genotype is thought or known to possess that is relevant to this study or which deems it a genotype of interest.

Genotype number	Maturity group	Entry	Source	Rationale for inclusion
1	4	PI243540	Rouf Mian, USDA, Wooster OH	resistance to soybean aphid
2	4	PI567301B	Rouf Mian, USDA, Wooster OH	resistance to soybean aphid
3	4	PI567321A	Rouf Mian, USDA, Wooster OH	resistance to soybean aphid
4	4	PI567324	Rouf Mian, USDA, Wooster OH	resistance to soybean aphid
5	4	PI567336A	Rouf Mian, USDA, Wooster OH	resistance to soybean aphid
6	4	PI567352B	Rouf Mian, USDA, Wooster OH	resistance to soybean aphid
7	2L	Wyandot	Rouf Mian, USDA, Wooster OH	group 4 control
8	2-3	Wyandot BC4 Aphid Res.	Rouf Mian, USDA, Wooster OH	aphis resistance
9	4	KY 03-dp-130	2007 TCLP 306	dense pubescence
10	4	KY 98-2932	2007 TCLP 306	dense pubescence
11	4	Stressland	TC08INC 1	group 4 control
12	4	Manokin	JWB08INC	soybean cyst nematode resistance
13	4	PI 471931	TC07INC201	partially slow wilting/flood tolerant PI from Nepal
14	5	Fowler	TC08INC14	soybean cyst nematode resistance
15	5	N02-7002	TC08INC6	soybean cyst nematode resistance
16	5	N94-7784	TC07INC210	slow wilting type from Egypt
17	5	5601T	Daryl Bowman 2008	group 5 control

(Appendix A cont'd.)

Table A.1 (cont'd.)

Genotype number	Maturity group	Entry	Source	Rationale for inclusion
18	5	PI 471938	TC08INC22	slow wilting PI
19	5	PI 416937	TC08INC21	slow wilting PI from Japan, Mexican Bean Beetle resistance
20	5	Vance	TC08INC9	small seeded type for natto
21	5	R01-416F	TCLP403 Remnant 2009 (Chen '08)	sustained N fixation under drought, derived from Jackson
22	5	R01-581F	TCLP403 Remnant 2009 (Chen '08)	sustained N fixation under drought, derived from Jackson
23	5	R01-52F	TCLP403 Remnant 2009 (Chen '08)	sustained N fixation under drought?
24	5	93705-50	TC07INC205	slow wilter from Jackson x KS4895
25	5	N98-7265	TC08INC19	slow wilting progeny of PI 471938
26	6	Boggs	TC08INC42	soybean cyst resistance
27	6	N04-9646	TC08INC27	drought tolerance
28	6	N01-10974	TC08INC30	large seed type for tofu, high protein in seed
29	6	N93-110-6	TC08INC32	slow wilting derivative of PI 416937
30	6	NC-Roy	TC08INC36	group 6 control
31	6	NTCPR94-5157	TC08INC37	slow wilting breeding line
32	7	Jackson	TC06INC230	old cultivar with sustained N fixation under drought
33	7	G00-3209	2008 Foundation Seed (GA)	root knot resistance, soybean cyst resistance, 25% pedigree from PI 416937
34	7	N01-11771	TC08INC73	slow wilt, 25% pedigree derived from PI416937
35	7	N01-11136	TC08INC66	slow wilt, 25% pedigree derived from PI416937; 50% pedigree derived from NTCPR94-5157
36	7	N01-11777	TC08INC69	slow wilt, 25% pedigree derived from PI416937
37	7	NC-Raleigh	TC08INC80	group 7 control
38	7	N7002	TC08INC72	25% of pedigree derived from PI 416937
39	7	Benning	TC08INC45	group 7 control
40	7	G04-Ben229IR-G	Dr. Boerma, Univ. of Georgia 2005	corn ear worm resistance (and resistance to other insects)
41	7	G04-Ben229IR-H	Dr. Boerma, Univ. of Georgia 2005	corn ear worm resistance (and resistance to other insects)
42	7	G04-Ben229IR-M	Dr. Boerma, Univ. of Georgia 2005	corn ear worm resistance (and resistance to other insects)
43	7	G05-Ben229IR-MGH	Dr. Boerma, Univ. of Georgia 2005	corn ear worm resistance (and resistance to other insects)
44	7	G05-6493	Dr. Boerma, Univ. of Georgia 2005	corn ear worm resistance (and resistance to other insects)
45	7	N05-7452	TC08INC60	25% pedigree derived from PI 416937
46	7	N05-7462	TC08INC78	25% pedigree derived from PI 416937

(Appendix A cont'd.)

Table A.1 (cont'd.)

Genotype number	Maturity group	Entry	Source	Rationale for inclusion
47	7	N06-7564	TC08INC63	12.5% pedigree derived from PI 416937
48	7	Cook	TC08INC77	group 8 control
49	8	Hyuuga	TC06INC246	resistant to soybean rust
50	8	N05-7432	TC08INC85	25% pedigree from PI 416937;25% pedigree from PI 471938
51	5	93705-36	TC08INC12	slow wilter from Jackson x KS4895

Table A.2-1 Soybean genotype trait least squares means for Experiment 3 (Chapter 2). *W* is all watered plants, or drought controls; *D* is all droughted plants; *Growth rate* is rate of change of plant height (cm/day); *Mass* is plant aboveground dry biomass.

Genotype number	W Growth rate	D Growth rate	W Mass	D Mass	Drought tolerance (= D Mass - W Mass)	Wilt avoidance (days until critical wilt; D only)
1	1.13	1.15	16.85	9.62	-7.24	7.75
2	0.69	0.70	12.44	7.32	-5.13	10.48
3	1.20	1.82	10.96	4.95	-6.01	9.07
4	1.22	1.12	12.54	7.87	-4.67	10.55
5	1.03	1.12	5.32	4.82	-0.50	12.23
6	1.15	1.10	3.98	2.18	-1.80	10.25
7	1.03	0.92	17.35	10.54	-6.80	8.09
8	0.81	0.83	16.38	10.10	-6.28	8.21
9	0.86	0.66	15.83	8.77	-7.06	11.24
10	1.15	1.12	8.94	4.76	-4.19	11.39
11	0.90	1.10	15.81	10.96	-4.85	9.56
12	1.04	1.03	10.47	8.98	-1.49	11.63
13	0.94	0.79	18.67	11.50	-7.16	8.78
14	0.99	0.90	12.27	5.61	-6.66	11.33
15	1.39	1.00	10.09	9.98	-0.11	8.88
16	0.86	0.96	15.47	8.91	-6.56	9.98
17	1.27	1.11	17.49	9.14	-8.35	8.99
18	1.18	1.07	18.53	12.22	-6.31	9.94
19	0.88	1.07	15.01	8.83	-6.18	5.91
20	0.78	0.76	15.83	6.16	-9.67	11.42
21	0.92	0.87	16.95	9.37	-7.58	8.91
22	0.81	0.81	14.48	7.97	-6.50	10.34
23	1.02	0.98	20.09	13.10	-6.99	7.97
24	1.31	1.03	14.97	8.86	-6.11	9.35
25	1.03	1.43	15.44	13.77	-1.66	9.83
26	0.94	1.32	19.25	8.95	-10.30	9.69
27	0.89	0.77	17.85	13.24	-4.61	10.69
28	1.11	1.33	17.39	12.03	-5.35	7.48
29	1.06	0.97	20.45	12.67	-7.78	8.36

(Appendix A cont'd.)

Table A.2-1 (cont'd.)

Genotype number	W Growth rate	D Growth rate	W Mass	D Mass	Drought tolerance (= D Mass - W Mass)	Wilt avoidance (days until critical wilt; D only)
30	0.94	0.98	18.69	13.55	-5.14	9.05
31	0.86	0.76	16.56	11.11	-5.46	9.02
32	1.19	1.09	11.57	5.41	-6.16	8.84
33	0.99	1.50	12.30	4.73	-7.58	11.29
34	0.76	0.75	17.88	7.25	-10.63	9.67
35	0.90	0.70	13.32	11.49	-1.83	9.46
36	0.50	0.64	11.35	6.49	-4.86	13.33
37	0.85	0.76	15.66	6.96	-8.70	8.27
38	1.18	1.13	13.51	8.30	-5.21	12.39
39	0.83	0.85	21.54	10.86	-10.68	9.32
40	0.98	0.83	18.29	8.21	-10.08	11.01
41	0.86	0.99	12.75	11.62	-1.13	8.58
42	1.05	0.77	10.80	5.83	-4.97	10.76
43	0.73	0.69	17.02	6.85	-10.17	10.31
44	0.94	1.04	22.24	12.20	-10.05	7.83
45	1.12	1.04	9.28	8.46	-0.82	11.23
46	1.74	1.85	10.85	10.48	-0.37	7.82
47	0.92	0.79	8.99	6.78	-2.22	9.22
48	1.08	1.14	14.94	6.83	-8.11	8.66
49	0.86	1.05	16.75	10.16	-6.60	7.99
50	1.36	0.94	13.04	10.09	-2.96	9.16
51	0.72	0.96	17.95	15.14	-2.82	9.18

Table A.2-2 Soybean genotype trait least squares means continued for Experiment 3 (Chapter 2). *W* is all watered plants, or drought controls; *D* is all droughted plants; [*insect species*] are the means for insect wet biomass feeding on given plant genotype in a no-choice feeding assay.

Genotype number	W <i>H. virescens</i>	D <i>H. virescens</i>	W <i>H. zea</i>	D <i>H. zea</i>	W <i>S. exigua</i>	D <i>S. exigua</i>
1	2.83	9.63	1.67	2.62	1.48	2.95
2	6.15	3.69	2.37	1.88	2.18	0.88
3	4.26	5.18	2.50	2.77	1.36	1.57
4	5.55	7.45	1.35	1.72	1.74	1.33
5	5.65	4.79	2.37	1.56	1.19	1.10
6	4.02	3.40	2.75	1.19	1.82	0.52
7	4.13	7.22	2.59	3.75	1.80	2.28
8	3.23	10.07	1.02	2.88	0.57	2.39
9	2.56	5.47	1.27	1.30	1.63	0.96

(Appendix A cont'd.)

Table A.2-2 (cont'd.)

Genotype number	<i>W H. virescens</i>	<i>D H. virescens</i>	<i>W H. zea</i>	<i>D H. zea</i>	<i>W S. exigua</i>	<i>D S. exigua</i>
10	3.70	3.83	1.82	1.67	1.19	1.42
11	5.24	6.80	1.40	2.30	1.77	1.92
12	3.53	2.81	1.68	1.54	1.21	0.48
13	4.77	9.62	1.48	3.08	1.53	2.43
14	4.63	4.89	1.40	1.87	1.91	1.41
15	2.48	3.52	1.00	1.97	1.56	2.01
16	1.04	6.26	0.70	1.61	0.41	0.98
17	6.10	4.10	1.88	1.83	1.33	1.03
18	3.74	5.16	2.13	1.86	1.20	1.22
19	4.61	2.58	0.61	1.50	1.21	1.47
20	6.41	5.52	1.12	2.07	1.17	1.34
21	3.59	6.27	1.10	2.01	0.68	1.39
22	4.67	2.14	0.98	2.12	1.69	1.38
23	4.96	5.63	1.78	2.14	0.88	0.93
24	7.97	6.67	3.37	2.66	1.91	1.51
25	1.81	1.79	1.16	1.64	0.81	1.02
26	4.58	3.33	1.10	1.51	0.74	0.97
27	4.78	3.15	1.46	1.04	0.78	1.72
28	4.01	3.20	1.31	1.14	0.45	1.23
29	6.94	4.27	1.05	2.49	1.94	0.94
30	2.80	5.34	1.32	2.32	0.31	2.12
31	3.10	6.16	1.09	2.04	0.33	1.13
32	3.48	4.90	2.20	1.54	1.59	0.88
33	3.79	2.42	1.39	1.73	0.89	1.36
34	2.95	6.04	1.00	2.06	0.67	1.34
35	5.25	6.48	1.79	1.46	0.84	1.03
36	3.98	6.01	1.75	0.97	0.88	0.74
37	1.76	2.96	1.32	2.08	0.67	3.01
38	2.26	6.00	1.54	2.95	1.63	0.66
39	3.31	6.07	1.09	2.18	1.12	4.05
40	4.46	4.53	2.34	1.53	1.74	1.57
41	6.16	6.01	2.00	2.53	1.80	1.06
42	2.40	3.16	1.78	1.41	1.14	0.86
43	4.58	4.43	1.13	1.75	2.06	0.67
44	4.47	5.45	1.56	1.73	1.19	1.16
45	6.51	3.44	1.37	1.23	1.71	0.94
46	4.83	5.09	1.24	2.33	1.13	1.62
47	3.46	2.49	1.12	0.86	0.30	0.57
48	2.46	7.20	0.85	2.17	0.60	1.58
49	3.36	4.33	1.50	1.80	1.41	1.27
50	3.41	4.88	1.33	1.40	0.84	2.53
51	3.31	6.42	1.35	0.99	1.67	0.90

Appendix A (cont'd.)

Table A.3 Soybean genotypes used in field experiment (Chapter 3). “BB” under *Genotype number* represents border beans, which were used to eliminate edge effects. *Maturity group* is the growth classification representing length of time from planting until pods have matured. The lower the maturity group numerically, the shorter the time until maturity and, generally, the more north the cultivar will be planted. *Entry* is the soybean name or breeder code for each cultivar line. *Source* is the source of the genotype (i.e., breeder, supplier or accession number). *Rationale for inclusion* is the trait that the genotype is thought or known to possess that is relevant to this study or which deems it a genotype of interest.

Genotype number	Maturity group	Entry	Source	Rationale for inclusion
2	4	PI567301B	Rouf Mian, USDA, Wooster OH	resistance to soybean aphid
6	4	PI567352B	Rouf Mian, USDA, Wooster OH	resistance to soybean aphid
11	4	Stressland	Dr. Boerma, Univ. of Georgia 2005	group 4 control
13	4	PI 471931	TC08INC78	partially slow wilting/flood tolerant PI from Nepal
16	5	N94-7784	Daryl Bowman 2008	slow wilting type from Egypt
17	5	5601T	TC08INC6	group 5 control
24	5	93705-50	TC08INC37	slow wilter from Jackson x KS4895
27	6	N04-9646	Dr. Boerma, Univ. of Georgia 2005	drought tolerance
28	6	N01-10974	TC08INC36	large seed type for tofu, high protein in seed
29	6	N93-110-6	JWB08INC	slow wilting derivative of PI 416937
30	6	NC-Roy	Dr. Boerma, Univ. of Georgia 2005	group 6 control
38	7	N7002	Rouf Mian, USDA, Wooster OH	25% of pedigree derived from PI 416937
39	7	Benning	TC08INC12	group 7 control
43	7	G05-Ben229IR-MGH	Rouf Mian, USDA, Wooster OH	corn ear worm resistance (and resistance to other insects)
47	7	N06-7564	Rouf Mian, USDA, Wooster OH	12.5% pedigree derived from PI 416937
51	5	93705-36	Rouf Mian, USDA, Wooster OH	slow wilter from Jackson x KS4895
BB	4	RA452		
BB	5	Hutcheson		
BB	6	Dillon		
BB	7	NC-Raleigh		

Appendix A (cont'd.)

Table A.4-1 Soybean genotype trait least squares means for field experiment (Chapter 3). *S* is all plants sprayed with insecticide; *B* is all plants receiving natural herbivory (or bugs); *W* is all watered plants, or drought controls; *D* is all droughted plants; *Growth rate* is rate of change of plant height (cm/day) for early (June) and mid (July) growth rates.

Genotype number	S Early growth rate	B Early growth rate	W Mid growth rate	D Mid growth rate	S Mid growth rate	B Mid growth rate	W Final height	D Final height
2	1.03	0.77	3.44	2.93	3.59	2.78	144.63	141.94
6	1.09	1.08	3.15	2.89	3.27	2.77	196.19	160.38
11	0.99	0.76	2.09	2.06	2.27	1.88	107.06	98.75
13	1.02	1.03	2.48	2.26	2.48	2.26	105.63	102.50
16	0.91	0.88	2.10	1.90	2.03	1.98	149.13	121.88
17	1.01	1.03	2.30	2.25	2.31	2.24	108.56	107.69
24	1.13	1.04	2.54	2.40	2.64	2.30	127.44	131.94
27	0.59	0.58	1.52	1.24	1.37	1.40	106.00	98.38
28	1.28	1.11	2.37	2.01	2.32	2.06	121.13	113.88
29	1.01	0.93	2.06	1.83	1.96	1.93	110.19	100.81
30	1.00	1.02	2.24	2.01	2.11	2.13	121.38	108.50
38	1.05	0.92	2.11	1.93	2.18	1.87	108.19	103.69
39	1.13	0.85	2.01	1.82	2.05	1.78	123.38	115.25
43	0.65	0.53	1.78	1.48	1.78	1.49	113.31	103.56
47	1.03	1.04	2.04	1.87	2.01	1.90	117.13	108.50
51	1.06	0.94	2.06	1.98	2.08	1.96	114.25	109.50

Table A.4-2 Soybean genotype trait least squares means continued for field experiment (Chapter 3). *O* is overall means across treatments; *W* is all watered plants, or drought controls; *D* is all droughted plants; *Seed yield* is the dry seed weight harvested from the rows; *First flower* is the day the first flower was observed fully opened in the row; *First fruit* is the day the first fruit of the row was observed emerging 2-3 mm from the senescing flower.

Genotype number	O Seed yield	W Seed yield	D Seed yield	O First flower	W First flower	D First flower	W First fruit	D First fruit
2	134.38	179.35	89.41	50.44	50.50	50.38	59.50	59.75
6	122.27	139.90	104.64	47.88	48.25	47.50	56.63	55.88
11	199.08	195.62	202.54	44.50	44.50	44.50	51.75	51.25
13	296.65	387.18	206.13	50.50	50.50	50.50	59.75	59.00
16	225.78	275.15	176.41	53.19	53.50	52.88	63.88	62.50
17	300.18	353.37	246.99	50.50	50.50	50.50	56.50	55.38
24	302.77	367.78	237.76	50.63	50.63	50.63	61.25	60.50
27	241.13	300.88	181.39	57.19	57.50	56.88	69.40	67.50
28	265.81	346.40	185.23	51.56	51.75	51.38	64.00	62.50
29	255.08	320.57	189.60	51.88	52.00	51.75	63.88	63.50
30	272.61	325.43	219.79	54.31	55.38	53.25	67.13	65.88

Appendix A (cont'd.)

Table A.4-2 (cont'd.)

Genotype number	O Seed yield	W Seed yield	D Seed yield	O First flower	W First flower	D First flower	W First fruit	D First fruit
38	247.94	330.23	165.66	52.75	53.00	52.50	65.13	65.13
39	267.45	317.62	217.28	53.94	53.75	54.13	64.75	65.88
43	201.34	279.53	123.16	57.94	57.63	58.25	70.50	69.00
47	278.71	341.23	216.19	57.44	57.25	57.63	67.50	67.00
51	290.82	342.46	239.19	52.13	51.75	52.50	63.38	64.00

Table A.4-3 Soybean genotype trait least squares means continued for field experiment (Chapter 3). *S* is all plants sprayed with insecticide; *B* is all plants receiving natural herbivory (or bugs); *W* is all watered plants, or drought controls; *D* is all droughted plants; *O* is overall means across treatments; *First fruit* is the day the first fruit of the row was observed emerging 2-3 mm from the senescing flower; *Fruit mature* is the day 95% of pods in row were mature; *Maturity rate* is the difference in days between *First fruit* and *Fruit mature*.

Genotype number	S First fruit	B First fruit	W Fruit mature	D Fruit mature	O Maturity rate	W Maturity rate	D Maturity rate
2	59.50	59.75	121.50	119.50	60.88	62.00	59.75
6	54.88	57.63	114.13	107.75	54.69	57.50	51.88
11	51.25	51.75	100.75	99.88	48.81	49.00	48.63
13	59.50	59.25	120.75	118.50	60.25	61.00	59.50
16	63.00	63.38	118.25	113.88	52.88	54.38	51.38
17	55.75	56.13	121.75	120.88	65.38	65.25	65.50
24	60.75	61.00	126.13	123.50	63.94	64.88	63.00
27	67.50	69.30	139.75	137.75	70.32	70.38	70.25
28	63.50	63.00	128.75	125.25	63.75	64.75	62.75
29	63.00	64.38	129.63	127.88	65.06	65.75	64.38
30	65.50	67.50	136.00	135.38	69.19	68.88	69.50
38	64.38	65.88	142.25	141.88	76.94	77.13	76.75
39	64.38	66.25	142.50	139.00	75.44	77.75	73.13
43	69.00	70.50	142.50	142.75	72.88	72.00	73.75
47	67.00	67.50	140.38	138.38	72.13	72.88	71.38
51	62.00	65.38	127.88	127.88	64.19	64.50	63.88

Appendix A (cont'd.)

Table A.4-4 Soybean genotype trait least squares means continued for field experiment (Chapter 3). *S* is all plants sprayed with insecticide; *B* is all plants receiving natural herbivory (or bugs); *O* is overall means across treatments; *W* is all watered plants, or drought controls; *D* is all droughted plants; *Stomatal conduct* is stomatal conductance measured with a porometer on one haphazardly chosen leaf at the top of the canopy of each row, facing the sun; [(#)] represents the measurement in series, i.e. there were four different occasions where conductance was measured, and series means not presented were not calculated.

Genotype number	S Stomatal conduct (1)	B Stomatal conduct (1)	O Stomatal conduct (2)	O Stomatal conduct (4)	W Stomatal conduct (4)	D Stomatal conduct (4)
2	1328.75	801.88	735.19	394.88	655.25	134.50
6	816.63	966.88	967.41	551.31	804.88	297.75
11	1182.50	986.25	1030.09	421.99	531.50	312.48
13	1383.75	842.63	810.11	287.23	408.75	165.70
16	814.38	1400.00	783.06	441.48	630.75	252.20
17	839.00	588.25	631.43	350.98	493.50	208.46
24	921.25	756.25	705.68	508.83	759.75	257.90
27	865.75	835.00	817.18	340.75	452.88	228.63
28	666.63	1008.13	706.32	308.70	401.88	215.53
29	1064.63	906.00	842.37	330.92	418.38	243.46
30	1045.63	903.75	813.71	358.88	440.88	276.88
38	1052.88	735.88	867.67	249.56	363.00	136.13
39	800.63	1022.50	617.41	172.09	216.06	128.11
43	2098.75	1070.63	845.70	347.47	510.88	184.06
47	1112.50	785.75	736.18	470.02	783.38	156.66
51	1339.75	1016.75	902.29	352.50	454.63	250.38

Table A.4-5 Soybean genotype trait least squares means continued for field experiment (Chapter 3). *O* is overall means across treatments; *W* is all watered plants, or drought controls; *D* is all droughted plants; *Water potential* is the leaf water potential measured on the terminal leaflet used to measure the 4th series of stomatal conductance; % *Leaf water* is the pre-dawn percentage of water weight in one leaf punch of the row; *SLA* is specific leaf area (area of leaf punch/ dry weight of leaf punch).

Genotype number	O Water potential	W Water potential	D Water potential	O % Leaf water	W % Leaf water	D % Leaf water	O SLA	W SLA	D SLA
2	11.80	11.56	12.03	68.90	79.35	58.45	228.17	275.29	181.04
6	9.81	11.00	8.63	66.26	68.86	63.66	184.74	200.30	169.18
11	12.94	11.31	14.56	63.01	71.99	54.03	259.00	358.67	159.33
13	13.67	13.75	13.59	74.03	72.10	75.97	282.19	262.71	301.68
16	13.53	14.44	12.63	66.89	70.20	63.57	280.82	311.43	250.22

Appendix A (cont'd.)

Table A.4-5 (cont'd.)

Genotype number	O Water potential	W Water potential	D Water potential	O % Leaf water	W % Leaf water	D % Leaf water	O SLA	W SLA	D SLA
17	14.09	15.59	12.59	66.64	71.34	61.94	219.72	239.23	200.22
24	15.73	15.38	16.09	66.42	65.18	67.67	301.46	382.21	220.72
27	12.56	13.38	11.75	62.11	52.09	72.13	172.83	154.41	191.26
28	15.08	15.94	14.22	59.56	69.76	49.36	274.11	377.66	170.55
29	14.78	14.53	15.03	58.71	65.37	52.05	201.67	249.08	154.25
30	11.33	11.00	11.66	68.16	73.40	62.92	303.95	257.07	350.83
38	15.23	14.50	15.97	71.43	76.63	66.22	264.49	339.99	189.00
39	11.09	10.09	12.09	69.38	78.57	60.20	292.42	428.32	156.52
43	13.14	12.72	13.56	66.76	68.36	65.15	216.06	199.36	232.75
47	14.38	15.03	13.72	51.11	54.42	47.80	132.45	129.78	135.12
51	11.75	13.31	10.19	69.80	63.25	76.34	275.56	239.85	311.27

Table A.4-6 Soybean genotype trait least squares means continued for field experiment (Chapter 3). *O* is overall means across treatments; *W* is all watered plants, or drought controls; *D* is all droughted plants; *Herbiv* is the visual estimation of % insect damage to the leaves of the row; *Trichomes* is the measure of the number of trichomes found on 77 mm² of a leaf per row; *H. zea* is the wet mass of the insects of that species feeding on given genotype following a no-choice feeding assay; [(#)] represents the measurement in series, i.e. there were three different occasions where herbivory was measured.

Genotype number	O Herbiv (1)	O Herbiv (2)	W Herbiv (3)	D Herbiv (3)	O Trichomes	W Trichomes	D Trichomes	O <i>H. zea</i>
2	2.89	3.73	15.45	11.43	83.63	80.31	86.94	0.06
6	2.73	3.78	28.08	11.77	209.25	201.25	217.25	1.46
11	2.95	2.86	10.25	10.00	120.69	128.69	112.69	1.48
13	3.44	4.03	11.72	10.23	92.47	93.38	91.56	2.54
16	1.63	3.13	7.90	4.62	104.52	91.69	117.34	2.37
17	2.62	2.95	9.25	10.12	159.45	175.63	143.28	2.49
24	3.46	4.56	12.37	9.33	175.22	179.00	171.44	2.18
27	1.60	3.29	8.17	7.50	173.00	179.63	166.38	3.60
28	3.48	3.55	6.65	7.13	59.31	58.06	60.56	1.89
29	3.66	5.24	9.73	7.03	119.09	104.81	133.38	2.24
30	2.49	3.68	7.60	5.87	162.84	163.06	162.63	1.92
38	2.88	3.99	8.13	7.85	184.75	192.75	176.75	1.29
39	3.92	3.73	12.78	9.60	175.67	180.19	170.75	4.04
43	2.18	3.23	8.30	9.27	203.27	224.94	181.59	2.88
47	2.66	4.32	7.83	5.92	169.77	167.44	172.09	0.65
51	2.56	3.91	7.60	6.77	134.97	136.50	133.44	3.75

Appendix B – Greenhouse Layout, Experiment 1

Greenhouse layout for Experiment 1. Green circles each represent one soybean plant/pot (80 total). Other plants grown in greenhouse during experiment are labeled, as are the main greenhouse features.

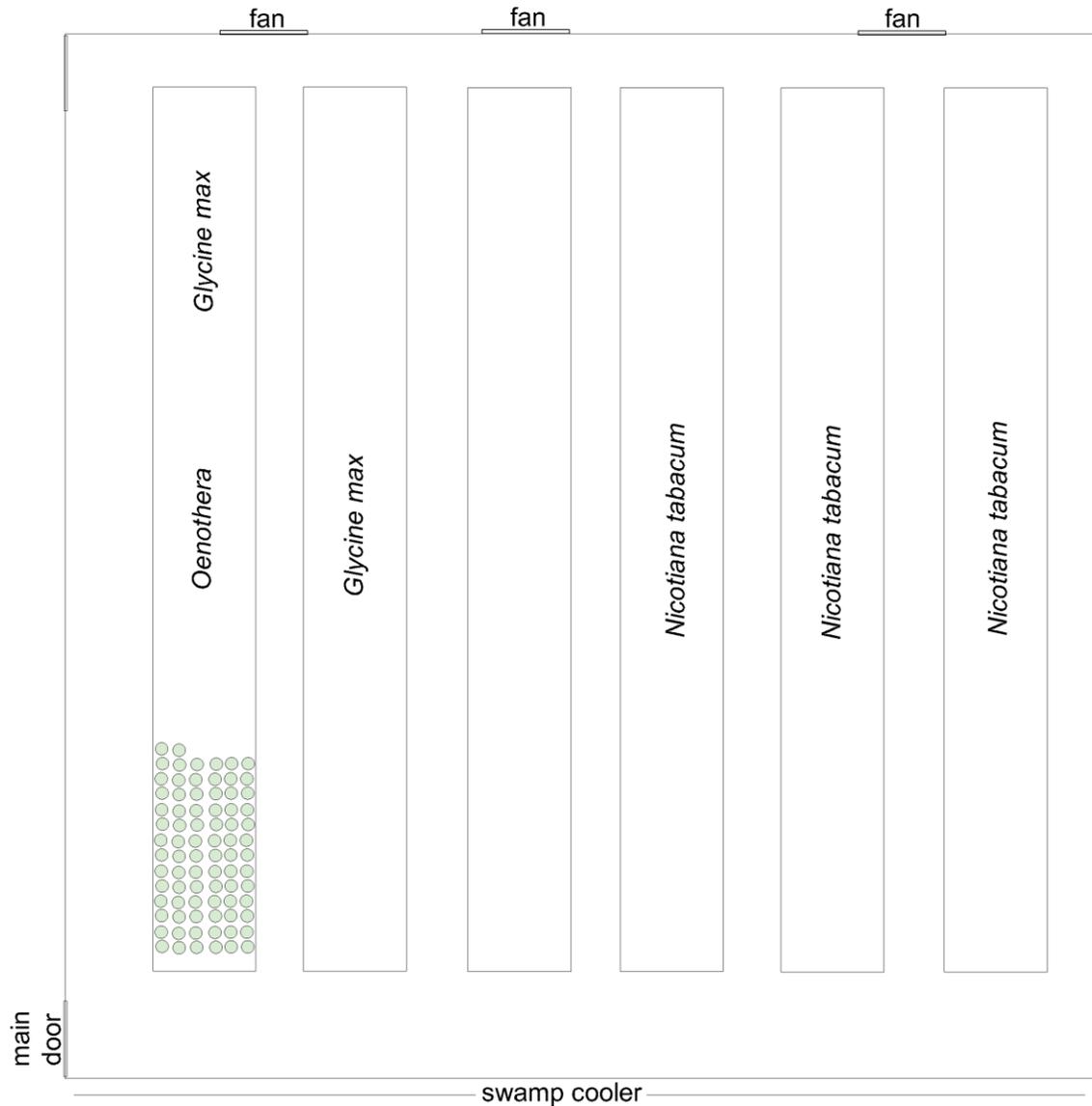


Figure B.1 Greenhouse layout, indoor Experiment 1.

Appendix C – Greenhouse Layout, Experiment 3

Greenhouse layout for Experiment 3. Green circles each represent one soybean plant/pot (510 total). Other plants grown in greenhouse during experiment are labeled, as are the main greenhouse features.

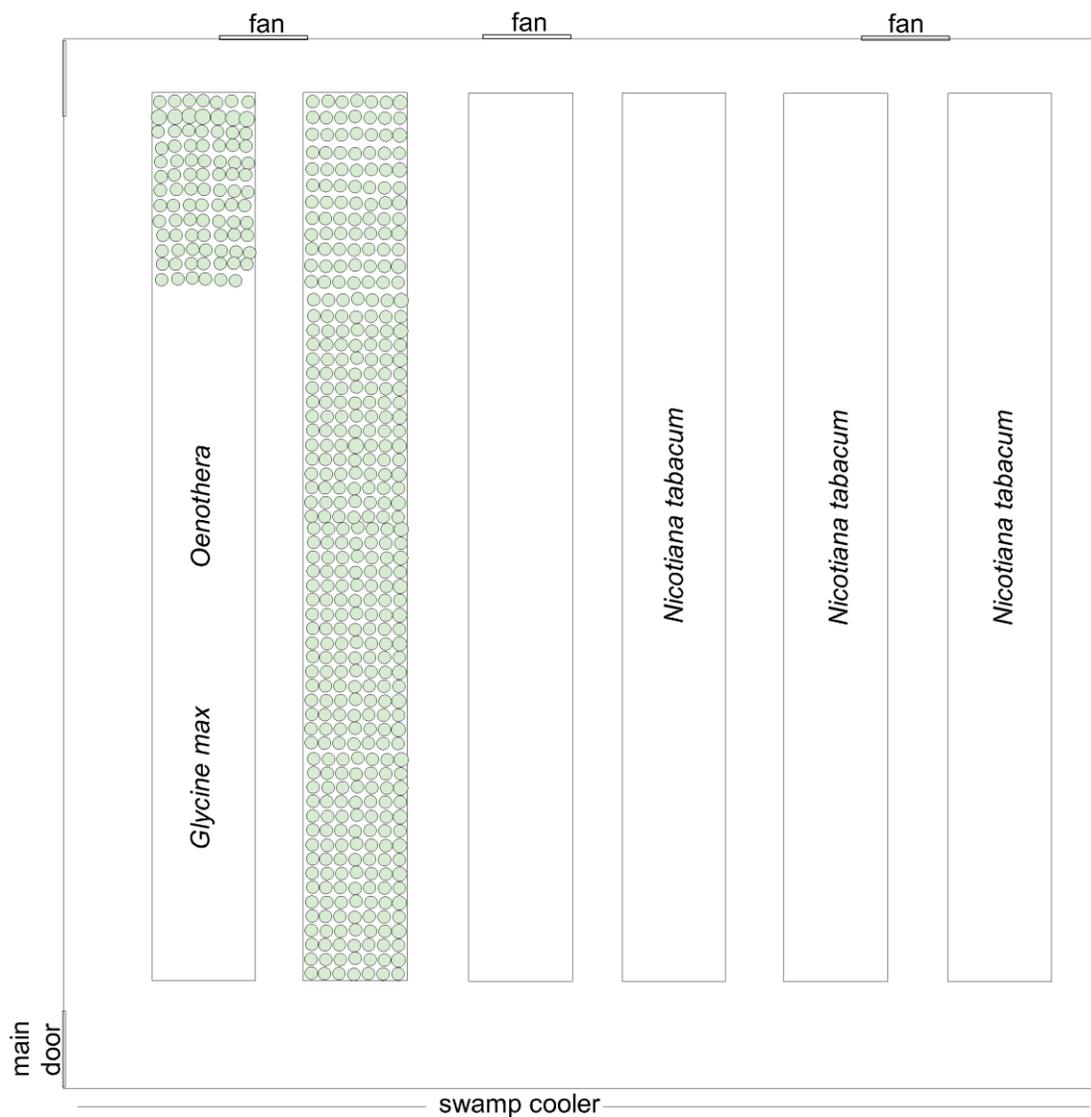


Figure C.1 Greenhouse layout, indoor Experiment 3.

Appendix D – Field Layout

Organizational layout of the field experiment. The *General Field Layout* (Fig. D.1) illustrates the overall layout of the field, including dimensions (not necessarily pictured to scale here), field features, and spatial distribution. It also illustrates the layout of border bean rows vs. experimental rows. *Experimental Block Layout* (Fig. D.2) illustrates the experimental treatments, i.e. drought and herbivory.

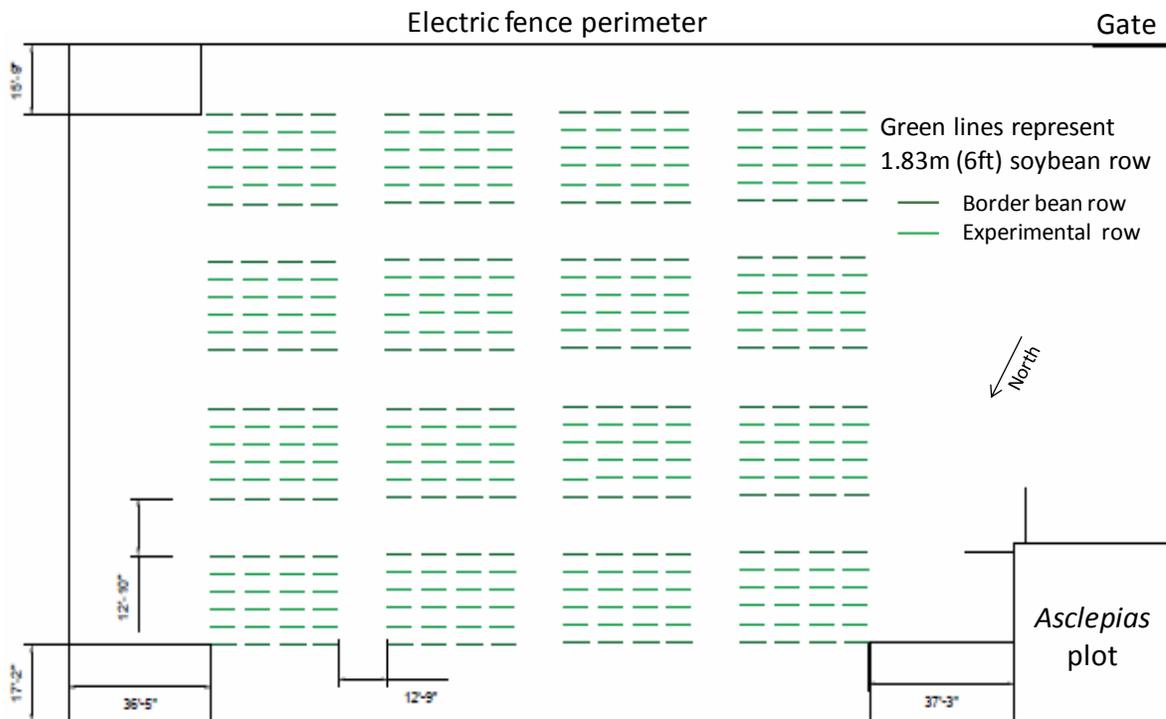
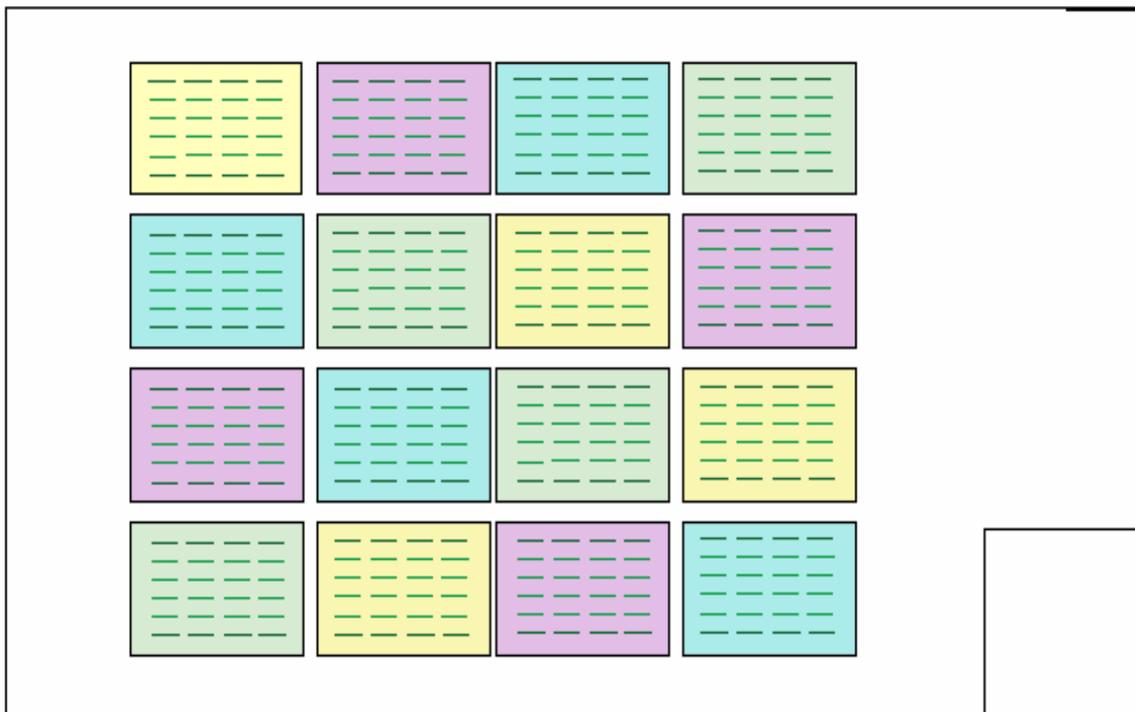
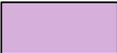


Figure D.1 General layout, field experiment. Experimental soybean rows are shown in bright green, border bean rows are shown in dark green. Dimensions (not necessarily pictured to scale here), field features, and spatial distribution are also shown.

(Appendix D cont'd.)



 Ambient water / insecticide spray

 Drought / insecticide spray

 Ambient water / herbivory

 Drought / herbivory

Figure D.2 Experimental layout, field experiment. Experimental treatments for soybean treatment blocks (16 total) are shown as follows: ambient water, insecticide spray in purple; drought, insecticide spray in yellow; ambient water, herbivory in blue; drought, herbivory in green.

Appendix E – Climate Data, Field Experiment

Temperature and precipitation data for the location of this experiment. All data was obtained from the National Climatic Data Center of the National Oceanic and Atmospheric Administration (NOAA) and the National Environmental Satellite, Data, and Information Service (NESDIS) (<http://www.ncdc.noaa.gov/oa/ncdc.html>). All data presented in this Appendix was obtained at the NOAA station: Raleigh State University located on the campus of North Carolina State University (Elevation: 121.9m above s/l; Coordinates: 35°48'N / 78°42'W). This station was the closest active station to the experiment during the experimental time period and is approximately 7 km from the field site.

Table E.1 Monthly station data 2011, temperature (°C), for the duration of the field experiment (June-November). Measurements presented include monthly averages for the weather station (max, min, overall), departure from the normal temperature for that month, and the highest and lowest temperatures recorded for the month and their respective dates.

TEMPERATURE (°C)								
Month	Average Max	Average Min	Overall Average	Departure From Normal	Highest Recorded	Date of Highest	Lowest Recorded	Date of Lowest
JUNE	33.33	21.50	27.44	3.72	37.22	24	15.00	8
JULY	34.06	21.33	27.72	1.78	38.33	24	13.33	4
AUG	33.00	21.61	27.33	2.34	37.22	11	14.44	29
SEPT	31.78	17.44	24.61	2.95	36.11	4	13.89	12+
OCT	24.33	10.17	17.28	1.95	31.67	12	1.11	30
NOV	17.56	4.83	11.22	0.39	23.33	1	-2.78	29

(Appendix E cont'd.)

Table E.2 Monthly station data 2011, precipitation (cm), for the duration of the field experiment (June-November). Measurements presented include monthly precipitation totals received at the weather station, departure from the normal for each month, greatest amount of precipitation received in one 24 hour period and the respective date for this period, and number of days for the month receiving 0.25, 1.25, and 2.5 cm or more.

Monthly Station Data 2011

Month	PRECIPITATION (cm)						
	Total	Departure From Normal	Greatest 24 Hours	Date of 24 Hrs	NO. OF DAYS		
					.25 or More	1.25 or More	2.5 or More
JUNE	7.9	-2.4	2.8	2	5	3	1
JULY	4.0	-7.0	1.5	19	4	2	0
AUG	11.0	0.1	3.1	6	8	3	1
SEPT	19.8	9.0	10.0	30	4	3	2
OCT	5.2	0.0	1.8	29	4	3	0
NOV	2.5	-5.2	1.2	17	2	0	0

Table E.3 Daily precipitation 2011, for the duration of the field experiment (June-November). Shown is the monthly total precipitation received (cm) at the weather station and amounts of precipitation received for each date of each month. Empty fields represent days where no precipitation was recorded.

Daily Precipitation (cm) 2011

Month	Total	DAY OF MONTH									
		1	2	3	4	5	6	7	8	9	10
JUNE	7.9		2.8	2.4				0.4			
JULY	4.0										
AUG	11.0		0.8		2.0		3.1				
SEPT	19.8										
OCT	5.2										
NOV	2.5				0.1	1.2					

(Appendix E cont'd.)

Table E.3 (cont'd.)
Daily Precipitation (cm) 2011

Month	DAY OF MONTH										
	11	12	13	14	15	16	17	18	19	20	21
JUNE				1.0							
JULY			0.9	0.3					1.5		
AUG								1.0	1.2		
SEPT											
OCT					1.5						0.3
NOV							1.2				

Table E.3 (cont'd.)
Daily Precipitation (cm) 2011

Month	DAY OF MONTH									
	22	23	24	25	26	27	28	29	30	31
JUNE									1.3	
JULY							1.3			
AUG		0.3	1.4	1.2						
SEPT						6.9	2.0	1.0	10.0	
OCT					1.5			1.8		
NOV										

(Appendix E cont'd.)

Table E.4 Daily temperature 2011, for the duration of the field experiment (June-November). Shown is the monthly average max and min temperatures (°C) recorded at the weather station and max and min temperatures recorded for each date of each month. Empty fields represent no data available.

Daily Temperatures (°C) 2011

Month	Max/ Min	Avg.	DAY OF MONTH									
			1	2	3	4	5	6	7	8	9	10
JUNE	MAX	33.33	28.89	31.11	31.11	32.22	34.44	35.00	29.44	28.33	29.44	33.33
JUNE	MIN	21.50	20.00	19.44	21.11	21.67	21.67	22.78	18.33	15.00	17.78	20.56
JULY	MAX	34.06	28.89	28.33	28.89	30.00	32.22	35.56	36.67		35.56	31.67
JULY	MIN	21.33	17.78	17.22	15.56	13.33	15.56	16.11	18.33	20.00	22.22	21.11
AUG	MAX	33.00	25.56	31.11	34.44	35.56	36.67	32.78	33.33	33.89	35.00	36.11
AUG	MIN	21.61	21.11	21.67	21.11	23.33	23.89	22.22	21.11	22.78	23.33	23.33
SEPT	MAX	31.78	35.00	33.33	31.11	36.11	29.44	32.78	33.33	34.44	31.67	28.89
SEPT	MIN											
OCT	MAX	24.33	24.44	25.00	22.78	21.67	20.00	20.00	21.11	26.67	25.56	28.33
OCT	MIN	10.17	16.67	10.56	8.89	10.00	8.33	6.67	8.89	9.44	9.44	12.78
NOV	MAX	17.56	23.33	15.56	15.00	12.78	14.44	11.67	11.11	17.78	21.67	20.56
NOV	MIN	4.83	6.67	4.44	6.67	9.44	5.56	6.11	-2.22	-0.56	3.33	5.00

(Appendix E cont'd.)

Table E.4 (cont'd.)
Daily Temperatures (°C) 2011

Month	Max/ Min	DAY OF MONTH										
		11	12	13	14	15	16	17	18	19	20	21
JUNE	MAX	32.22	34.44	35.00	35.56	32.22	32.78	33.89	32.22	33.89	36.11	35.00
JUNE	MIN	20.00	20.00	21.67	23.89	23.33	21.67	22.22	22.78	20.00	20.56	21.67
JULY	MAX	33.89	35.00	31.11	35.00	34.44	33.89	33.33	33.89	32.78	36.11	37.22
JULY	MIN	23.33	20.56	22.78	22.78	22.22	23.33	22.78	22.22	23.89	23.33	22.78
AUG	MAX	37.22	35.56	34.44	31.11	32.22	33.33	36.11	34.44	28.89	32.22	31.67
AUG	MIN	24.44	23.89	23.33	23.89	23.33	22.78	23.89	23.89	23.89	21.11	20.00
SEPT	MAX	29.44	30.00	30.56	31.67	32.78	32.78	33.89	31.11	33.89	33.89	31.67
SEPT	MIN											
OCT	MAX	28.89	31.67	31.11	25.56	22.22	19.44	20.56	24.44		27.78	20.00
OCT	MIN	13.89	13.33	15.00	12.22	7.22	6.11	5.00	7.78	11.11	12.22	8.33
NOV	MAX	18.89	17.78	18.89	20.00	21.11	19.44	18.33	17.78	16.11	18.33	20.00
NOV	MIN	6.11	3.33	0.56	1.11	4.44	7.22	11.11	5.00	3.33	3.33	2.78

Table E.4 (cont'd.)
Daily Temperatures (°C) 2011

Month	Max/ Min	DAY OF MONTH									
		22	23	24	25	26	27	28	29	30	31
JUNE	MAX	36.11	36.67	37.22	35.00	33.89	36.11	36.67	33.33	27.78	
JUNE	MIN	21.67	22.78	23.33	26.11	23.89	22.78	23.89	23.33	21.11	
JULY	MAX	37.22	37.78	38.33	37.78	36.11	31.67	32.22	34.44	35.00	32.78
JULY	MIN	23.89	24.44	25.00	25.00	23.89	21.67	22.22	23.33	23.89	20.00
AUG	MAX	32.22	31.67	30.00	32.78	33.33	30.56	30.00	30.56	34.44	36.11
AUG	MIN	21.11	23.33	20.00	20.56	21.11	18.89	17.22	14.44	17.22	18.33
SEPT	MAX	33.89	35.56	33.89	35.00	35.00	26.67	27.78	24.44	23.89	
SEPT	MIN										
OCT	MAX	23.33	20.00	22.78	25.56	26.67	27.78	30.00	26.67	21.11	19.44
OCT	MIN	7.22	4.44	7.22	13.33	17.22	19.44	18.89	8.33	1.11	4.44
NOV	MAX	21.11	22.22	19.44	16.11	18.33	15.56	13.89	15.00	13.89	
NOV	MIN	5.56	10.00	11.67	10.00	10.56	6.11	-2.22	-2.78	2.78	

Appendix F – Field Experiment ANOVA Tables

For all tables we include results from analysis of variance (ANOVA) for effects of experimental treatments and plant genotype on measured phenotypic traits of soybean and experimental conditions.

Table F.1 Effects of experimental treatments and soybean genotype on water potential.

Water potential				
	df	SS	F	P
Block (drought) ¹	12	1082.15	5.06	<0.0001
Drought ²	1	6.81	0.08	0.79
Herbivory ²	1	0.01	0	0.99
Genotype ¹	15	688.99	2.58	0.001
Drought x herbivory ²	1	44.93	0.5	0.49

¹ Denominator for testing these effects was MS error; ² Denominator was MS block (drought x herbivory).

Table F.2 Effects of experimental treatments and soybean genotype on leaf trichome number / 77mm² leaf tissue (averaged from upper and lower surfaces).

Trichomes				
	df	SS	F	P
Block (drought x herbivory) ¹	12	1.31	1.8	0.049
Drought ²	1	0.01	0.1	0.76
Herbivory ²	1	0.2	1.79	0.21
Genotype ¹	15	30.51	33.49	<.001
Drought x herbivory ²	1	0.17	1.58	0.23

¹ Denominator for testing these effects was MS error; ² Denominator was Block (drought x herbivory).

(Appendix F cont'd.)

Table F.3 Effects of experimental treatments and soybean genotype on pre-dawn % leaf water content.

% Leaf water	df	SS	F	P
Block (drought x herbivory) ¹	12	60858	13.02	<.001
Drought ²	1	2674.18	0.51	0.49
Herbivory ³	1	21779	4.29	0.0604
Genotype ⁴	15	7558.88	0.92	0.56
Drought x herbivory ³	1	6839.53	1.35	0.27
Drought x genotype ¹	15	8192.9	1.4	0.15

¹ Denominator for testing these effects was MS error; ² Denominator was MS block (drought x herbivory) + MS (drought x genotype) - MS error; ³ Denominator was MS block (drought x herbivory); ⁴ Denominator was MS drought x genotype.

Table F.4 Effects of experimental treatments and soybean genotype on specific leaf area (log transformed).

Specific leaf area	df	SS	F	P
Block (drought x herbivory) ¹	12	68.71	13.33	<0.001
Drought ²	1	4.99	0.84	0.38
Herbivory ³	1	35.42	6.19	0.03
Genotype ⁴	15	11.79	0.34	0.34
Drought x herbivory ³	1	2.82	0.49	0.5
Drought x genotype ¹	15	9.46	1.47	0.12

¹ Denominator for testing these effects was MS error; ² Denominator was MS block (drought x herbivory); ³ Denominator was MS block (drought x herbivory); ⁴ Denominator was MS drought x genotype.

(Appendix F cont'd.)

Table F.5 Effects of experimental treatments and soybean genotype on stomatal conductance (late-July).

Stomatal conductance (late-July)				
	df	SS	F	P
Block (drought x herbivory) ¹	12	29.64	9.39	<.001
Drought ²	1	8.28	3.35	0.09
Herbivory ³	1	0.19	0.07	0.8
Genotype ⁴	15	5.73	0.66	0.78
Drought x herbivory ²	1	5.18	2.1	0.17
Herbivory x genotype ¹	15	8.66	2.2	0.008

¹Denominator for testing these effects was MS error; ²Denominator was MS block (drought x herbivory); ³Denominator was MS block (drought x herbivory) + MS (herbivory x genotype) - MS error; ⁴Denominator was MS (herbivory x genotype).

Table F.6 Effects of experimental treatments and soybean genotype on stomatal conductance (mid-August).

Stomatal conductance (mid-August)				
	df	SS	F	P
Block (drought x herbivory) ¹	12	2818.33	8.25	<.001
Light* ¹	1	110.97	3.9	0.05
Drought ²	1	7467.77	30.88	0.0001
Herbivory ²	1	37.56	0.16	0.7
Maturity group ³	3	157.82	0.83	0.5
Genotype (maturity group) ¹	12	765.9	2.24	0.01
Drought x herbivory ²	1	15.68	0.07	0.8
Drought x maturity group ¹	3	126.11	1.48	0.22
Herbivory x maturity group ¹	3	43.83	0.51	0.67
Drought x herbivory x maturity group ¹	3	166.68	1.95	0.12

*The intensity of light ($\mu\text{mol m}^{-2} \text{s}^{-1}$) was included as a covariate. ¹Denominator for testing these effects was MS error; ²Denominator was MS block (drought x herbivory); ³Denominator was MS genotype (maturity group).

(Appendix F cont'd.)

Table F.7 Effects of experimental treatments and soybean genotype on stomatal conductance (late-August).

Stomatal conductance (late-August)				
	df	SS	F	P
Block (drought x herbivory) ¹	12	24.27	5.94	<.001
Drought ²	1	8.91	4.41	0.06
Herbivory ²	1	0.48	0.24	0.63
Maturity group ³	3	6.17	4.05	0.03
Genotype (maturity group) ¹	12	6.09	1.49	0.13
Drought x herbivory ²	1	0.31	0.15	0.7

¹ Denominator for testing these effects was MS error; ² Denominator was MS block (drought x herbivory); ³ Denominator was MS genotype (maturity group).

Table F.8 Effects of experimental treatments and soybean genotype on stomatal conductance (early-September).

Stomatal conductance (early-September)				
	df	SS	F	P
Block (drought x herbivory) ¹	12	140.21	27.15	<.001
Drought ²	1	58.08	4.97	0.046
Herbivory ²	1	0.93	0.08	0.78
Maturity group ³	3	5.5	2.46	0.11
Genotype (maturity group) ¹	12	9.43	1.83	0.046
Drought x herbivory ²	1	0.56	0.05	0.83
Drought x maturity group ¹	3	1.47	1.14	0.34
Herbivory x maturity group ¹	3	3.23	2.5	0.06
Drought x herbivory x maturity group ¹	3	3.79	2.93	0.03

¹ Denominator for testing these effects was MS error; ² Denominator was MS (block (drought x herbivory)); ³ Denominator was MS genotype (maturity group).

(Appendix F cont'd.)

Table F.9 Effects of experimental treatments and soybean genotype on the date of harvest for each soybean row.

Harvest date				
	df	SS	F	P
Block (drought x herbivory) ¹	12	89.8	1.97	0.029
Drought ²	1	56.68	2.81	0.11
Herbivory ²	1	13.59	1.82	0.2
Maturity group ³	3	19709	39.08	<0.001
Genotype (maturity group) ⁴	12	2033.82	10.15	<0.001
Drought x herbivory ²	1	14.31	1.92	0.19
Drought x genotype (maturity group) ¹	15	250.64	4.39	<0.001
Herbivory x maturity group ¹	3	20.9	1.83	0.14

¹ Denominator for testing these effects was MS (error); ² Denominator was MS (block (drought x herbivory)); ³ Denominator was MS (genotype (maturity group)); ⁴ Denominator was MS (drought x genotype (maturity group)).

Table F.10 Effects of experimental treatments on soil moisture during drought.

Soil moisture (early-August)				
	df	SS	F	P
Block (drought) ¹	14	162.05	3.47	0.001
Drought ²	1	29.05	2.51	0.14

Soil moisture (mid-August)				
	df	SS	F	P
Block (drought) ¹	14	74.72	1.79	0.07
Drought ²	1	158.25	29.65	<.001

¹ Denominator for testing these effects was MS error; ² Denominator was Block (drought).

(Appendix F cont'd.)

Table F.11 Effects of insecticide on herbivory treatment.

Effect of insecticide on herbivory treatment

Mid-July

	df	SS	F	P
Block (herbivory)	14	21.1	1.61	0.0782
Herbivory	1	225.3	85.2	<0.0001
Error	240	225.3		

Mid-August

	df	SS	F	P
Block (herbivory)	14	56.9	2.6	0.002
Herbivory	1	143.77	45.43	<0.0001
Error	144	225.49		

Mid-September

	df	SS	F	P
Block (herbivory)	14	678.28	2.68	0.0016
Herbivory	1	165.38	4.41	0.0484
Error	144	2604.07		
