EFFECTS OF DROUGHT-STRESS ON COTTON (*GOSSYPIUM HIRSUTUM* L.) AND HOST-PLANT RESISTANCE TO WESTERN FLOWER THRIPS (*FRANKLINIELLA OCCIDENTALIS* PERGANDE)

A Dissertation

by

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ABSTRACT

Herbivory by Western Flower Thrips (*Frankliniella occidentalis* Pergande) (WFT) and drought-stress due to limited water availability are currently two major factors that can severely impact cotton (*Gossypium hirsutum* L.) production. This dissertation examines the effects of drought-stress on cotton and host-plant resistance (HPR) to WFT in laboratory conditions, and seeks to identify the physiological and morphological mechanisms that underlie drought-tolerance and HPR. A life-history systems-approach was developed that provides a new level of detail for understanding how environmental variation impacts adult female WFT. The approach was illustrated by investigating the combined effects of cotton genotype, periodic drought-stress, and prey availability on the adult female omnivorous thrips using a factorial design. Three treatment conditions were significantly different, none of which were predicted based on prevailing ecological-hypotheses. At the same time, the approach produced three novel insights about WFT life-history and reproductive strategy. The roles of negative photo-taxis and leaf biomechanical properties were investigated as potential mechanisms that influence WFT foraging-decisions on individual cotyledons. Results showed that WFT foraging-decisions could be considered adaptive, but there was limited support for either of the mechanisms investigated. The physiological responses to drought stress and drought recovery were investigated for three transgenic cotton cultivars and an untransformed wild-type (WT). At peak drought, ABA levels, stomatal area, and stomatal apertures in the transgenic isolone, AtRAV1-1 were 48% lower, 27.7%, and 16.3% smaller than WT. These results suggest that AtRAV1-1 was the most drought-tolerant and support the hypothesis that changes in stomatal morphology may have functionally contributed to drought-tolerance. Lastly, I investigated whether changes in phytohormone concentrations associated with periodic-drought stress in four cotton cultivars (three transgenic and WT) were correlated with WFT feeding, fitness and state-dependent reproductive responses (i.e., the relationship between initial weight and

reproduction). Results showed that JA-Ile and JA were positively correlated with state-dependent egg viability and fecundity, respectively, and negatively correlated with total egg viability and fecundity, respectively, supporting the hypothesis that JA and JA-Ile underlie the negative effects on WFT reproduction and the associated shift to state-dependent reproduction.

DEDICATION

I would like to dedicate my dissertation to Drs. Harris and Sword, both of whom had the biggest impact on this dissertation and my professional development while at Texas A&M University. While Dr. Sword joined the committee half way through my program, he immediately opened up his lab to me, and since then we have worked together on a nearly daily basis. Above all, Dr. Sword is a remarkable ecologist. He can go from genes to ecosystems and jump from kingdom to kingdom (e.g. plants, animals, and fungi) all without skipping a beat! I would pitch new ideas or my interpretation of data to Dr. Sword, and if it made sense to him then I knew that I was on to something. He had a knack for saying 'good job, keep it up' at times when I needed to hear it most.

I learned so much through talking with Dr. Harris. He co-taught Host-Plant Resistance in Spring 2010 and based on his lectures and recommended readings (i.e., Erhlich & Raven 1964 and Jermy 1984), I knew early on that he would have much to contribute as a committee member. I am most fond of our conversations about the epistemology of science. We have talked about the resistance Copernicus, Darwin, and E.O. Wilson faced in proposing new paradigms, and how society and interpersonal conflicts can play a big role in scientific advances. Dr. Harris puts students above everything else. When I would knock on his office door to talk, the impression I got was that whatever he was doing could wait and was not as important as talking with me.

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CHAPTER I

INTRODUCTION

In 2012, the human population reached a new milestone and exceeds over 7 billion people. Consequently, the need to cultivate and harvest basic resources to support the human population (i.e., food and fiber) is greater than ever. Traditionally, meeting these demands has involved expanding the amount of cultivated land and/or increasing chemical inputs. However, such approaches have negative impacts on both the quantity and quality of other, beneficial natural resources and services (e.g. uncontaminated drinking water, atmospheric CO_2 recycling, maintenance and promotion of biodiversity, etc). Thus, there is growing awareness of the need for crop management strategies based on biological and ecological principles that can either increase efficiency of cultivated land (more yield per hectare) and/or reduce the rate of chemical applications.

Enhancing crop production through an understanding of biological principles is not a novel approach. For instance, genetic improvement began millennia ago through careful selection and inadvertent directed breeding by keen farmers (Evans 1993). Over the past century with rediscovery of Mendel's work, systematic breeding efforts have been particularly successful in increasing the yield of a range of crops cultivars (eg: maize, cotton, rice, soybean) (Evans 1993). More recently, genomic and genetic analyses have been used to identify candidate genes associated with increased yield, drought-tolerance, and/or herbivore resistance. While advances have been made through these approaches, an understanding of the metabolic and physiological traits that functionally contribute to the tolerant/resistant phenotype remains limited. I would suggest that a functional understanding of what makes a selected cultivar resistant/tolerant is an extremely valuable information for four reasons: 1) it provides the opportunity to identify novel resistant/tolerant mechanisms that could potentially lead to 'stacking' different mechanisms into elite cultivars, 2) it can lead to more efficient

cultivar screening programs, 3) seed bank resources can be more effectively utilized, and 4) when/if the cultivar is no longer effective, rather than starting from scratch, it provides an immediate point for investigating what had changed, information which can be used to develop the next generation of cultivars. In short, knowing what makes a resistant-cultivar resistant is paramount to maintaining a proactive rather than reactive approach in the development of new elite cultivars.

The overall objective of this dissertation is to better characterize and ultimately identify potential mechanisms of HPR and drought-stress in cotton. The approach necessarily requires a more basic understanding of both plants and insects than is typical for most research concerned with agricultural crops. To provide an applied context for the reader, a short literature review is presented in Chapter 2 examining the relevance of drought-stress and herbivory by WFT on cotton production in the Texas High Plains. The subsequent chapters (3-6) comprise distinct experiments that investigate the effects of and potential mechanisms underlying HPR and drought-stress, the results of which are interpreted through a very basic perspective. Chapter 3 introduces a new approach (a tool) that can provide a more precise understanding of how environment and plant genetic variation impacts the feeding and performance of WFT. The sources of variation investigated in particular were cotton genotype, periodic drought-stress, and prey availability (spider mite eggs *Tetranychus urticae* Kock) as these are each relevant factors to cotton production on the Texas High Plains (see Chapter 2 for review). Chapter 4 investigates why WFT prefer to feed on the undersides of leaves with the goal of characterizing and ultimately identifying the plant trait that mediates this preference. While this study may seem esoteric, identifying variation in HPR is a necessary first step for studying mechanisms of HPR. As result, sources of variation at any scale can be useful, with small-scale studies particularly suited for controlled manipulations. Chapter 5 compared physiological responses to drought stress and drought recovery of three transgenic cotton isolines relative to the wild type (*Gossypium hirsutum* L. cv Coker312). Chapter 6 builds off the results of Chapter 5 and investigates how periodically drought-stressed cotton affects susceptibility to herbivores with an emphasis

on how the mechanism of HPR changes in well-watered vs. periodically drought-stressed cotton conditions. Chapter 7 summarizes the results produced from this dissertation as a whole.

CHAPTER II

LITERATURE REVIEW: REGIONAL AND APPLIED CONTEXT

The Texas High Plains (THP) plants ~ 2 million ha of cotton annually which produces 34.9 - 43% of the United States total cotton production (TASS 2010). Supplemental irrigation in this region has been provided by pumping water from the Ogallala aquifer, and for many years the rate of removal has exceeded the rate of recharge (Alen et al. 2005). Furthermore, recent severe droughts on the THP and throughout Texas (e.g. 2011) have compounded the challenges associated with limited water availability on cotton production in general and for dry-land farmers that rely on natural irrigation events (i.e., rain) in particular. Thus, the direct and indirect effect of diminishing water availability is a major concern to the long-term sustainability of cotton production on the THP. One approach currently being explored on the THP is whether drought-tolerance in cotton can be genetically engineered. In Chapter 5, I contribute to this question by investigating the physiological responses of three transgenic cotton isolines to drought-stress and recovery.

How will herbivorous arthropods respond to drought-stressed cotton plants? Previous evidence of the effects of host-plant drought-stress on herbivores have been mixed. As a result, Huberty & Denno (2004) conducted a meta-analysis in an attempt to reconcile the mixed support for the traditionally held view that herbivorous insects respond positively to drought-stressed host-plants. A key conclusion is that the type of drought-stress imposed varied among studies, with some investigating the effects of continuous drought-stress on herbivores and others the effects of periodic drought-stress (i.e., a period of drought followed by recovery). Consequently, they proposed the Pulsed-Stress Hypothesis, which posits that herbivores, particularly those in the sap-sucking functional feeding guild such as aphids, respond positively to periods of drought-stress and recovery (Huberty & Denno 2004). The premise is that drought-stressed plants have elevated levels of nitrogen-containing compounds, e.g., proline

(Verslues & Bray 2006), which can be accessed by sap-sucking insects only when the plant regains turgor pressure through drought-recovery. An alternative, but less widely considered explanation for the mixed evidence supporting the effects of drought-stress on herbivores is the possibility that cross-talk in phytohormone responses associated with drought alters resistance to herbivores, and that such effects vary depending on the host-plant. This possibility is explored in Chapter 6. Moreover, pest management strategies are needed that factor in the response of arthropod pests to periodic drought stress of crops, and in- depth evaluations of feeding and fitness responses by WFT to periodic drought stress is one of the key objectives of this dissertation (see Chapter 3 and 6).

WFT is an omnivorous insect that feeds on cotton (leaves and pollen) and the eggs of spider mites. Since the late 1970s, the distribution of WFT has spread from western North America to become one of the most important agricultural pests worldwide (Kirk & Terry 2003; Reitz 2009). Thrips can be significant economic pests of cotton in the U.S (Williams 2010) including in the THP (Kerns *et al*. 2010). While several thrips species have been identified, WFT comprises 75-95% of the local thrips community on the THP (Reed *et al*. 2010). Thrips damage to early season cotton can result in significant leaf area reduction, loss of apical dominance, poor root development, delayed maturity and decreased lint yield (Harp & Turner 1976, Johnson *et al*. 1996, Sadras & Wilson 1998). In regions with longer growing seasons, seedlings can outgrow damage and compensate for reduction in plant vigor (Ellington *et al*. 1984, Leigh 1985). However, the growing season in the THP is limited (bracketed) between when it is warm enough to plant (seed germination) in the spring and before it gets too cool in the fall for fiber development (both of them are 60^{o}F) (Boman *et al*. 2007). Thus, optimization of heat units for crop growth while mitigating the risk of weather damage to the crop is key to maximizing profits in THP, and WFT are a major threat to that goal. Recently, the Environmental Protection Agency conducted a risk assessment of the systemic insecticide, Temik®, which has been the gold-standard for thrips control for nearly 40 years, and concluded that the product has a high risk to humans and the environment and

should be phased out of use by cotton farmers by 2018 (EPA.gov 2012). Thus, a major issue on the THP and throughout the Cottonbelt is how to manage thrips populations in a post-Temik era.

The omnivorous feeding strategy of WFT involves feeding on herbivorous spider mite (Acarina: Tetranychidae: *Tetranychus spp.)* eggs (Wilson *et al*. 1991), and in Chapter 3 this interaction is included as a treatment. Spider mites feed on cotton in the THP and have been shown to increase in abundance when fed plants that were cultivated under certain conditions of drought stress (English-Loeb 1989, 1990). Management of spider mite in general can be problematic for farmers because of their resistance to a broad range of acaricides (Van Leeuwen *et al*. 2009), and the use of non-target insecticides kill natural enemies, which can potentially cause outbreaks of mite populations. Hence, pest management strategies that rely primarily on natural enemies of spider mites are increasingly preferred over chemical strategies.

CHAPTER III

A LIFE-HISTORY SYSTEMS-APPROACH FOR STUDYING THE EVOLUTIONARY ECOLOGY OF AN OMNIVOROUS THRIPS, WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* PERGANDE

Introduction

Adult organisms have many life-history characteristics (LHC) that can be affected by environmental variation such as somatic maintenance and growth (e.g., fat and protein reserves, immunity), and various components of reproduction (e.g., fecundity, egg viability, offspring quality). Evolutionary ecology studies commonly select one or a few LHCs as proxy indicators of fitness even though it is known that they are one of many inter-related components that affect an organism's performance. Theory and empirical evidence both suggest that the response of any one adult LHC can be dependent upon multiple factors including (i) resource acquisition and allocation to other LHCs (Cody 1966; see Stearns 1992 for over 40 LHC trade-offs), (ii) the organism's current physiological state (McNamera & Houston 1996; Marden *et al.* 2003), (iii) the response of other LHCs 'up-stream' from the focal LHC (eg: eggs laid, hatched egg), and (iii) the life-history and reproductive strategy of the focal organism (i.e., income vs capital breeding) (Edward & Chapman 2011).

The burgeoning field of systems biology examines how interactions among the components of a system account for the function and behavior of that system (Snoep & Westerhoff 2005). An ISI Web of Knowledge search of "Systems Biology" (28 August 2012) returned five publications prior to 2000, and over 8,400 since then. Many journals dedicated to systems biology have since been launched, with the approach overwhelmingly applied to the study of metabolic and cell-signaling networks. Of the more than 8,400 articles, only 78, 62, 5, and 1 included the words or phrase "ecology", "ecosystem", "life history traits", and "life history characteristics", respectively. Although the utility and value of systems-based approaches for studying ecology has

gained attention recently (see Evans *et al.* 2012 for special topics issue on "Predictive Ecology: Systems Approaches"), it is clear that key biological entities (i.e., individuals) and their component characteristics have yet to be integrated into a system-based framework.

A systems-based approach is presented to investigate how environmental variation affects the growth and reproduction of an individual adult organism. In this case, fitness can be viewed as an emergent property that is determined by the integrated responses of component life history characteristics. Specifically, path analysis was used to produce resource acquisition and allocation patterns (RAAP) (Fig. 3.1) that relate the effects of and interactions between body mass and resource consumption on four LHCs commonly used as fitness indicators that also potentially interact with one another. The approach integrates components of physiology (state-dependency), foraging (resource acquisition), life history and reproductive strategies (patterns of resource allocation), and evolution (fitness), and thus broadly synthesizes key sub-disciplines that encompass the field of evolutionary ecology. The approach is demonstrated the approach by investigating how three sources of variation (plant genotype, periodic drought-stress in plants, and prey availability) affected the RAAPs of an omnivorous thrips, WFT. Previous research has shown that reductions in plant quality can cause a functional shift from herbivory to predation by WFT (Agrawal *et al.* 1999; Janssen *et al.* 2003). These observations are built upon by investigating two potentially important sources of intraspecific variation in plants, genotypic variation (Soria & Mollema 1995) and phenotypic variation in response to periodic drought-stress (Huberty & Denno 2004).

Methods and materials

General procedures

Cotton plants (*Gossypium hirsutum* L.) were seeded individually in 400 ml pots and cultivated in a small room (2.25m x 2.75m x 2.25m) (under continuous light 13.1±5.2 μmol m^{-2} s^{-1}, 34.7±11.1^{o}C). From the time of planting the duration of the experiment was 13 days, with all plants during each trial cultivated simultaneously under

the aforementioned conditions. Adult female WFT were obtained from colonies maintained on bean (*Phaseolus vulgaris* L.) cotyledons under florescent lights (12:12 light:dark, 1.2±0.3 μmol m^{-2} s^{-1}, 25.0±3.3^{o}C).

Bioassay

A standardized Petri dish bioassay was used throughout the study to measure feeding and selected measures of WFT performance. Accordingly, an individual adult female WFT was first weighed (Mettler-Toledo Ch-8606, Laboratory & Weighing Technologies, Greifensec, Switzerland), and then sealed in a 90-mm-diameter Petri dish with an excised cotton cotyledon for 3d. After three days, thrips were removed, re-weighed and the total area of feeding scars (mm^2) on the cotyledon was quantified. Thrips produce feeding scars characterized as silvery depressions that occur on the leaf surface and along the leaf edges, which can be quantified using a dissecting microscrope and a metric ruler (mm^2). Next, cotyledons were re-sealed in the Petri dish (without adult female WFT) for an additional three days to allow viable thrips larvae to hatch from eggs laid within the cotyledon (thrips in suborder Terebrantia lay eggs in leaf tissue) (Lewis 1997). Three days later, the number of live immature thrips (hereafter referred to as 'immatures') on the cotyledon was quantified. Last, to quantify the number of eggs laid and hatched eggs, the cotyledons were stained with two solutions as per Backus *et al*. (1988). The first solution consisted of 0.2% acid fuchsin (the staining agent) mixed in 95% ethanol and glacial acetic acid (1:1 vol/vol). The second solution enhanced the cotyledons' transparency and consisted of distilled water, 99% glycerine, and 85% lactic acid (1:1:1 vol/vol/vol). Thrips eggs were visualized with a dissecting microscope and were identified as kidney-shaped with hatched-eggs having a red transparent appearance and unhatched eggs appearing dark-red. If the adult female thrips were missing, died, or did not feed during the course of the experiment, the replicate was omitted from the analysis.

Experimental design

The aforementioned bioassay was used to investigate the influence of prey availability, plant genotype, and periodic drought-stress (PDS) on the feeding and

performance of WFT. The specific genotypes were All-Tex® Atlas (All-Tex Seed Inc., Levelland, TX; PVP: 9200188; PI 561579) and 05-47-802, which is an unreleased experimental breeding line, developed by Texas A&M AgriLife Research Cotton Improvement Project. A factorial design (2x2x2) was employed and all treatment combinations were examined simultaneously during each of two trials (n=10-15, per treatment combination, trial).

Periodic drought-stress in the absence of prey

The goal of this experiment was to investigate the effect of periodic drought-stress of cotton plants (in the absence of prey) on the feeding and fitness of WFT, and to see how these effects varied between two cotton genotypes. Periodic drought-stress (PDS) in plants can be characterized by two major events, namely a period of drought-stress (limited water availability) followed by a period of drought-recovery (sufficient water availability). In this study, PDS and well-watered (WW) plants were given 125ml of water 1 and 5 days after planting (DAP). On 9-DAP, WW plants were given 125 ml of water and PDS plants were not. Plants in both treatments were then withheld water for three days (10-12 DAP) (hereafter referred to as 'drought-stress period'). On 12-DAP, PDS and WW plants received water, which marked the start of the drought-recovery period. One day later (13 DAP), cotyledons were harvested from plants and were used in the thrips bioassay. Summarizing, WW plants received 125ml of water 1, 5, 9, 12 DAP, and PDS on 1, 5, and 12 DAP with the thrips bioassay commencing on 13 DAP. Plants generally emerged from the soil 4 DAP and those that did not were omitted from this experiment.

To validate the PDS-treatment had caused plant responses consistent with drought-stress, we measured leaf surface temperatures for plants in WW and PDS on 9-DAP through 13-DAP. We predicted that withholding water from plants would cause a reduction in transpiration rates via stomatal closure, resulting in elevated leaf surface temperatures (LST). LST were measured on the adaxial surface of individual cotyledons ~2 cm from petiole using a Fluke 62 IR thermometer. Leaf measurements were taken 9-DAP prior to watering WW-plants (hereafter referred to as 'pre-drought'), and also prior

to watering all plants on 12-DAP. Three subsamples from each cotyledon were collected in rapid succession from the adaxial surface ~2 cm from the petiole. Thus, six subsamples were collected per plant, which were averaged to provide a single data-point per plant. The LSTs of each plant were measured in the same part of the room to minimize variation in measurements. As the drought treatment progressed some of the cotyledons were drooping. In this case, a pen was used to lift the cotyledon so that it was oriented parallel to the ground. The cotyledon was held in place for 30s prior to taking LSTs measurements. Leaf surface temperature data for each drought period met assumptions of normality (Wilks-Shapiro $P<0.05$). Pre-drought and drought-recovery LST were analyzed using an ANOVA, whereas a repeated measures ANOVA was used to analyze LST during the period of active drought-stress (10-12 DAP) with an error term indicating DAP nested within plant.

Three subsamples from each cotyledon were collected in rapid succession from the adaxial surface ~2 cm from the petiole. Thus, six subsamples were collected per plant, which were averaged to provide a single data-point per plant. The LSTs of each plant were measured in the same part of the room to minimize variation in measurements. As the drought treatment progressed some of the cotyledons were drooping. In this case, a pen was used to lift the cotyledon so that it was oriented parallel to the ground. The cotyledon was held in place for 30s prior to taking LSTs measurements.

Prey availability in the absence of periodic drought-stress

The aim of this experiment was to investigate how prey availability in the absence of PDS affected the feeding and fitness of WFT, and to see how these effects varied between two cotton genotypes. The eggs of spider mites (*Tetranychus urticae*) were used as prey-items for thrips, and five eggs were placed near petiole on the adaxial surface on cotyledons with prey. The spider mite colony was maintained on bean cotyledons (*Phaseolus vulgaris*), and eggs were collected as per Scriven & McMurty (1971), but modified for small-scale studies. In brief, mites were released in water suspension by washing mite-infested plants in a water/bleach solution. Water with mites

in suspension was funneled through a series (x3) of metal sieves that decrease in size resulting in mites being strained from water suspension and sorted according to life-history stage (eggs, larvae and adult males, and adult females, respectively).

Plant genotype, prey availability, and periodic drought-stress

The goal of this experiment was to investigate the feeding and fitness responses of WFT on two cotton genotypes that had been periodically drought-stressed and included prey-items. The set-up of this experiment was identical to the experiments conducted on PDS in the absence of prey, except that all leaves were inoculated with five spider mite eggs.

Path analysis

Path analysis was used to produce RAAPs of thrips under each treatment condition. Path analysis is a statistical method used to identify the structure of dependency among variables, and is particularly useful for studying the evolutionary ecology of adult organisms because it can account for the inter-dependent, hierarchical relationship among various life-history traits related to fitness. The method consists of multiple linear regression equations that describe hypothesized relationships among factors, and these equations are solved simultaneously using maximum likelihood methods. The global fit of the model can be evaluated using χ^2 goodness-of-fit (α = 0.05). The model fit was assessed with four common indices: NFI <0.95 is acceptable; CFI >0.95 is acceptable; NCP >0.95 is acceptable; RMSEA <0.05 is acceptable. Analyses were conducted using AMOS software (SPSS Inc.) (Arbuckle 2011).

Two types of RAAP were generated using path analyses which were identical in all respects except that one included the variable final weight whereas the other included weight gain (Fig. 3.1.A and B, respectively). Both measures of weight could not be examined in the model simultaneously because they are perfectly linearly dependent resulting in positive definite matrices. The general procedure used to test for differences between RAAPs was to first test measurement invariance between an unconstrained model for two models combined, and then for a model with the path coefficients and intercepts constrained to be equal between the groups. If the chi-square differences

statistic was not significant for the original and constrained models then these models had measurement invariance with respect to the path coefficients and intercepts. If the chi-square differences statistic was not significant for the original and constrained models then the path coefficients and intercepts were invariant between the models. If the chi-square differences statistic was significant for the constrained model implying non-invariance of path coefficients and intercepts between models, then post-hoc analysis were conducted to determine which of the specific path strengths and/or means were different. A Bonferroni correction was applied in some cases due to multiple comparisons (n=2, adjusted $\alpha = 0.025$).

Results

Validation of PDS treatment

Prior to initiating the periodic-drought stress (PDS) treatment, there were no differences in leaf surface temperatures (LST) for plants allocated to WW (Well-Watered) and PDS treatments ($F_{1,125}$=0.152, P =0.697) (Fig. 3.2; Table 3.1). Providing water to WW plants on 9 DAP resulted in a reduction in LST the following day (10 DAP) (Fig. 3.2). During the drought-stress period (10-12 DAP), repeated measures ANOVA indicated considerable variation between trials in response of LSTs to various treatments, which prevented pooling of data (Table 3.1). The interaction between days after planting (DAP) and PDS was significant ($F_{1,514}$=0.6.728, P =0.009) and consistent between trials ($F_{1,514}$=0.851, P =0.357). This effect was due to LST of PDS plants increasing during the drought-stress period while LST for WW were more variable.

Re-watering plants on 12 DAP caused a marked drop in LST for all plants on 13 DAP (drought-recovery), but particularly for PDS plants which resulted in no differences in LST between WW and PDS plants ($F_{1,125}$=0.001, P =0.989). Thus, withholding water from plants in the PDS-treatment resulted in elevated LSTs, and subsequent re-watering resulted in a complete recovery of LST to levels comparable to WW plants. Overall, results gleaned from LSTs indicate the PDS-treatment was successful.

Plant genotype, prey availability, and periodic drought-stress

In general, the treatment conditions caused considerable qualitative variation in RAAPs of thrips (Fig. 3.1A and B; Table 3.2). In fact, only two pathways were consistently significant across all treatment conditions (Total eggs→Hatched eggs; Hatched eggs→Immature). However, constraining the path coefficients and intercepts resulted in three cases of significantly different RAAPs: 1) the availability of prey in WW conditions had a different effect depending on the cotton genotype, 2) on the cotton genotype Atlas, the effect of prey availability was significantly different depending on whether the plants were WW or PDS; 3) on cotton genotype V05, the effect of PDS was significantly altered by the availability of prey (Table 3.3; Fig 3.1A and B). Prey consumption by thrips was not affected by any treatment condition, and thrips consumed on average at least two mite eggs (data not shown). The results of post-hoc analysis of significantly different RAAPs are presented in Table 3.3 and show the specific path coefficients or intercepts that were significantly different.

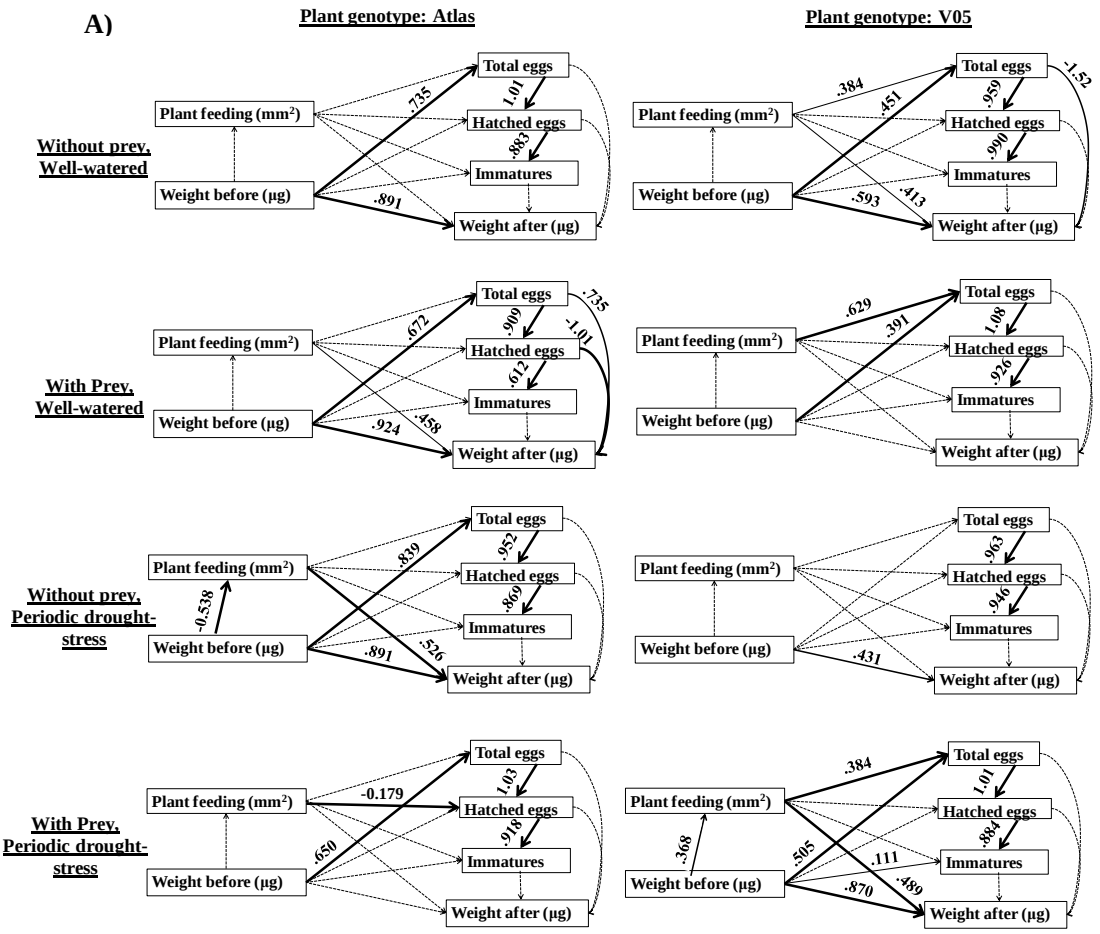

Figure 3.1. A life-history systems-approach for investigating the effects of cotton genotype (*Gossypium hirsutum*), periodic drought-stress, and prey availability on the performance of adult female Western Flower Thrips. Resource acquisition and allocation patterns of thrips were generated with path analysis for each of eight treatment conditions to illustrate the effects of initial weight and plant feeding on four measures of performance: total eggs laid, hatched eggs, alive immatures, and either A) final weight or B) weight gain. Standardized path coefficients are shown for significant pathways, and insignificant paths are show with dotted arrows. Arrows are bolded for significant pathways based on the degree of significance (light bold: $0.01<P<0.05$; heavy bold: $P<0.01$). See table 6.2 for details related to the fit of each model.

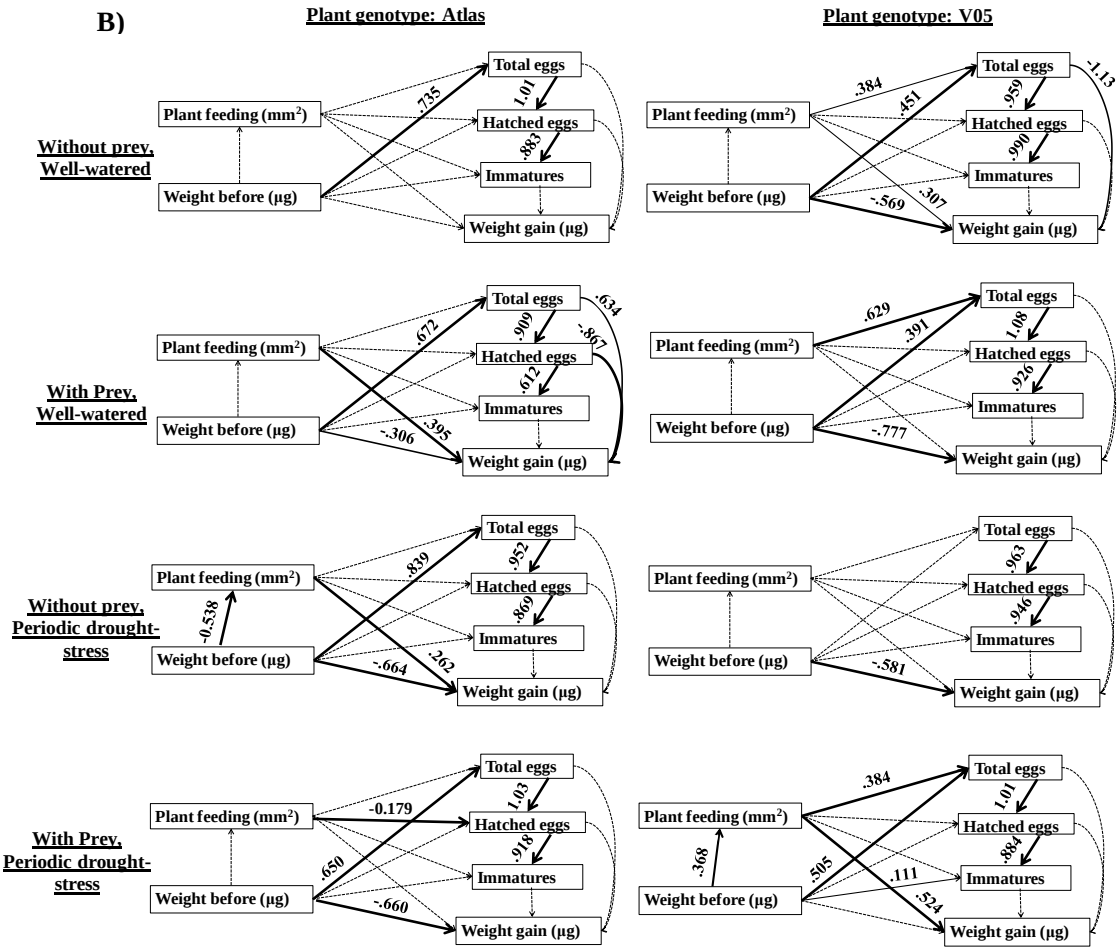

Figure 3.1. Continued.

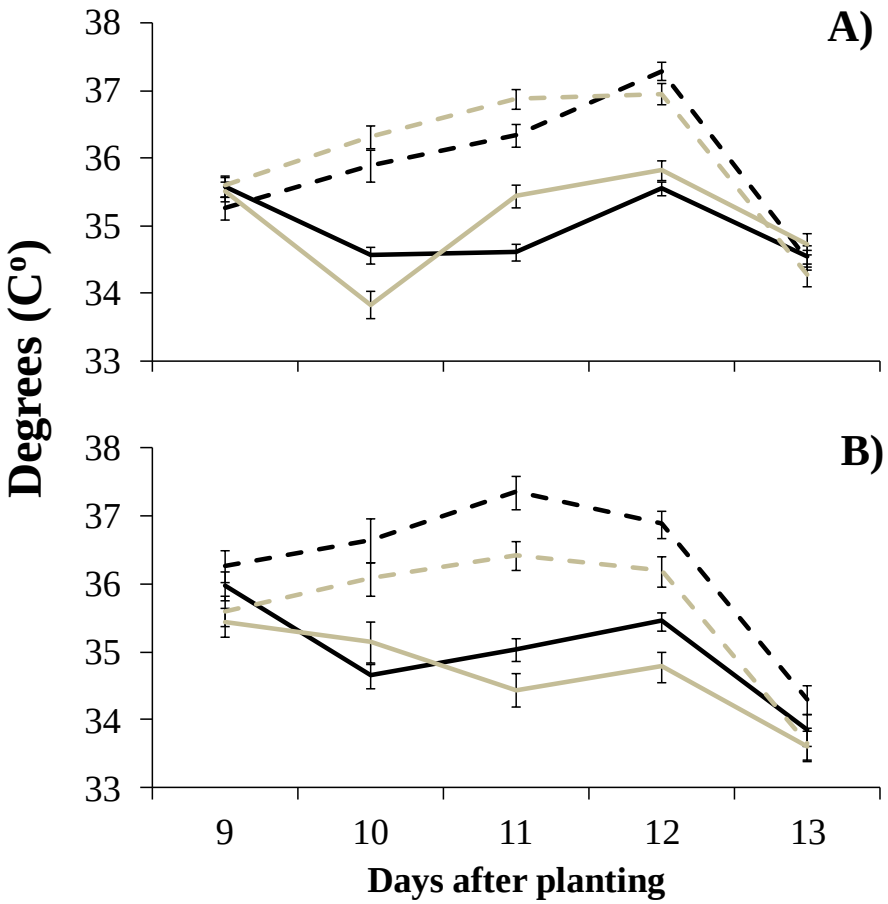

Figure 3.2. Results from two identical experiments ((A) Trial 1 and (B) Trial 2) during which leaf surface temperatures (LST) were collected from two cotton genotypes (*Gossypium hirsutum*) (Black= Atlas; Grey= V05). Variation between trials precluded the pooling of data. 'Well-watered' (solid lines) plants were given water 5-, 9-, and 12 DAP, whereas periodically drought-stress plants (dashed lines) were given water 5- and 12 DAP. (Note: LSTs were measured prior to watering on 9- and 12 DAP). A consistent effect across both trials was that withholding water from PDS plants resulted in a steady increase in LST from 9- though 12-DAP, and providing water resulted in decreased LSTs twenty-four hours later.

Table 3.1. Results of the effects of cotton genotype, periodic drought-stress, and days after planting on leaf surface temperatures at pre-drought, drought-stress, and drought-recovery.

Treatment	LST Pre-drought-stress (9 DAP)	Drought-stress (10-12 DAP)	Drought recovery (13 DAP)
Cotton Genotype (CG)	0.15	0.01	0.13
Periodic drought stress (PDS)	0.697	0.006	0.986
Days after planting (DAP)	NA	0.002	NA
Trial	0.077	<0.001	<0.001
CG:PDS	0.692	0.183	NA
CG:Trial	0.036	<0.001	0.238
PDS:Trial	0.346	0.002	0.129
DAP:CG	NA	0.299	0.225
DAP:PDS	NA	0.009	NA
DAP:Trial	NA	<0.001	NA
CG:PDS:Trial	0.43	0.012	0.995
CG:PDS:DAP	NA	0.412	NA
CG:DAP:Trial	NA	0.835	NA
DAP:PDS:Trial	NA	0.356	NA
CG:PDS:Trial:DAP	NA	0.193	NA

Table 3.2. Results for each of eight path analysis showing effects of predictor variables on response variables in each of path analyses (standardized path coefficient; *P* value), χ^2 goodness-of-fit, and three common indices assessing model fit (See Fig. 3.1A and B).

	Treatments								
Pathways	Atlas No Prey, No PDS	Atlas No Prey, PDS	Atlas Prey, No PDS	Atlas Prey, PDS	V05 No Prey, No PDS	V05 No prey, PDS	V05 Prey, No PDS	V05 Prey, PDS	Full model (treatments combined)
Initial wt (µg)--> Plant feeding (mm^2)	-0.009; 0.967	-0.538; 0.001	-0.175; 0.375	0.052; 0.80	-0.002; 0.993	-0.060; 0.759	-0.001; 0.995	0.368; 0.040	-0.058; 0.400
Plant feeding (mm^2) -->Eggs laid	0.155; 0.260	0.157; 0.295	0.134; 0.378	0.061; 0.694	0.384; 0.017	0.290; 0.122	0.629; <0.001	0.384; 0.006	0.300; <0.001
Initial wt (µg) -->Eggs laid	0.735; <0.001	0.839; <0.001	0.672; <0.001	0.650; <0.001	0.451; 0.005	0.050; 0.791	0.391; 0.003	0.505; <0.001	0.520; <0.001
Plant feeding (mm^2) -->Hatched eggs	-0.052; 0.418	-0.019; 0.740	0.003; 0.963	-0.179; 0.001	0.041; 0.335	-0.021; 0.713	-0.133; 0.061	-0.069; 0.342	-0.057; 0.007
Initial wt (µg) -->Hatched eggs	-0.71; 0.445	0.017; 0.836	0.055; 0.533	-0.113; 0.115	0.011; 0.803	0.054; 0.327	-0.109; 0.070	-0.028; 0.724	-0.012; 0.606
Eggs laid -->Hatched eggs	1.012; <0.001	0.952; <0.001	0.909; <0.001	1.034; <0.001	0.959; <0.001	0.963; <0.001	1.083; <0.001	1.006; <0.001	0.979; <0.001
Plant feeding (mm^2) -->Immatures	0.131; 0.123	0.061; 0.553	-0.021; 0.868	-0.081; 0.293	-0.057; 0.448	0.062; 0.201	0.024; 0.787	0.034; 0.521	0.015; 0.574
Initial wt (µg) -->Immatures	0.015; 0.898	0.065; 0.658	0.230; 0.170	-0.001; 0.995	-0.061; 0.426	0.070; 0.138	-0.027; 0.731	0.111; 0.053	0.686; 0.012
Hatched eggs -->Immatures	0.883; <0.001	0.869; <0.001	0.612; <0.001	0.918; <0.001	0.990; <0.001	0.946; <0.001	0.926; <0.001	0.884; <0.001	0.915; <0.001

Table 3.2. Continued.

Pathways including final weight (µg)	**Treatments** Atlas No Prey, No PDS	Atlas No Prey, PDS	Atlas Prey, No PDS	Atlas Prey, PDS	V05 No Prey, No PDS	V05 No prey, PDS	V05 Prey, No PDS	V05 Prey, PDS	Full model (treatments combined)
Plant feeding (mm^2)	0.307;	0.526;	0.458;	0.156;	0.413;	-0.033;	0.240;	0.489;	0.310;
-->Final wt (µg)	0.066	0.007	<0.001	0.474	0.015	0.850	0.345	0.001	<0.001
Initial wt (µg)-->	0.891;	0.505;	0.924;	0.342;	0.593;	0.431;	0.267;	0.870;	0.538;
Final wt (µg)	<0.001	0.075	<0.001	0.166	<0.001	0.011	0.213	<0.001	<0.001
Eggs laid-->	-0.560;	0.558;	0.735;	-0.033;	-1.516;	-0.891;	0.326;	-0.378;	-0.275;
Final wt (µg)	0.325	0.414	0.043	0.964	0.045	0.124	0.668	0.378	0.204
Hatched eggs -->	0.109;	-1.266;	-1.006;	0.458;	1.301;	0.878;	0.095;	0.535;	0.047;
Final wt (µg)	0.859	0.088	0.008	0.567	0.140	0.308	0.905	0.366	0.851
Immatures-->	0.037;	0.579;	-0.270;	-0.295;	-0.278;	0.125;	-0.389;	-0.787;	0.051;
Final wt (µg)	0.923	0.116	0.152	0.535	0.528	0.854	0.415	0.121	0.743

Table 3.2. Continued.

Pathways including Weight gain (µg)	**Treatments** Atlas No Prey, No PDS	Atlas No Prey, PDS	Atlas Prey, No PDS	Atlas Prey, PDS	V05 No Prey, No PDS	V05 No prey, PDS	V05 Prey, No PDS	V05 Prey, PDS	Full model (treatments combined)
Plant feeding (mm^2)	0.279;	0.262;	0.395;	0.142;	0.307;	-0.033;	0.170;	0.524;	0.253;
-->Wt gain (µg)	0.066	0.007	<0.001	0.474	0.015	0.850	0.345	0.001	<0.001
Initial wt (µg)-->	-0.38;	-0.664;	-0.306;	-0.660;	-0.569;	-0.581;	-0.777;	-0.258;	-0.587;
Wt gain (µg)	0.075	<0.001	0.032	0.003	<0.001	<0.001	<0.001	0.152	<0.001
Eggs laid-->	-0.51;	0.278;	0.634;	-0.030;	-1.127;	-0.881;	0.231;	-0.405;	-0.224;
Wt gain (µg)	0.325	0.414	0.043	0.964	0.045	0.124	0.668	0.378	0.204
Hatched eggs -->	0.099;	-0.630;	-0.867;	0.414;	0.967;	0.868;	0.067;	0.575;	0.039;
Wt gain (µg)	0.859	0.088	0.008	0.567	0.140	0.308	0.905	0.366	0.851
Immatures-->	0.033;	0.228;	-0.232;	-0.267;	-0.207;	0.123;	-0.276;	-0.843;	0.041;
Wt gain (µg)	0.923	0.116	0.152	0.535	0.528	0.854	0.415	0.121	0.743
Summary of model									
P value of χ^2	0.420	0.342	0.048	0.969	0.399	0.106	0.367	0.562	0.281
NFI	0.995	0.994	0.969	1	0.996	0.983	0.995	0.997	0.999
CFI	1	1	0.974	1	1	0.989	1	1	1.000
RMSEA	0	0	0.34	0	0	0.249	0	0	0.028
Group normality	1.189	-0.325	-0.37	-0.011	0.535	2.351	1.209	0.452	6.086
N (d.f.)	24 (1)	27 (1)	26 (1)	25 (1)	26 (1)	27 (1)	27 (1)	28 (1)	210 (1)

Standardized path coefficients are shown, which represent the change in the response variable given a standard deviation change in the predictor. NFI <0.95 is acceptable; CFI >0.95 is acceptable; RMSEA <0.05 is acceptable.

Table 3.3. Results of comparing selected path models that represent resource acquisition and allocation to fitness measures by adult female thrips under various ecological conditions.

Comparison	χ^2 goodness-of-fit test (*P* value) Unconstrained	Model with constrained path coefficients and means ($\pi_1=\pi_2$)	Specific pathways (P<0.05)
Between genotypes (Atlas vs. V05) (α=0.05)			
No Prey, WW	0.506	0.904	NA
Prey, WW	0.095	0.002	Feeding → Eggs laid; Initial wt → Final wt; Hatched eggs → Immatures; Final wt (V05 larger); Hatched eggs (V05 larger)
No Prey, PDS	0.172	.053	NA
Prey and PDS	0.755	0.153	NA
Within genotypes (α=0.025)			
Atlas			
Prey v. No Prey, WW	0.103	0.360	NA
PDS v. No Prey, WW	0.460	0.568	NA
Prey v. Prey + PDS	0.143	0.004	Feeding → Hatched eggs; Hatched eggs → Immatures; Hatched eggs (larger for Prey + PDS)
PDS v. Prey + PDS	0.637	0.301	NA
V05			
Prey v. No Prey, WW	0.466	0.431	NA
PDS v. No Prey, WW	0.190	0.260	NA
Prey v. Prey + PDS	0.502	0.579	NA
PDS v. Prey + PDS	0.204	0.024	Feeding → Final wt; Hatched eggs → Immatures; Initial wt → Eggs laid; Eggs laid (larger for PDS)

Discussion

Ecological factors

Resource acquisition and the physiological state of the organism can be two key factors that mediate the impact of environmental variation on adult organism's relative fitness. Furthermore, LHCs commonly used as indicators of relative fitness (total eggs laid, egg viability, offspring recruitment, weight gain or final weight) can interact in both positive and negative ways. Through use of path analysis, RAAPs of thrips were generated that account for the inter-dependent behavior of individual LHCs and can treat adult fitness as an emergent property. Results showed that thrips RAAPs on the genotype Atlas with prey available was significantly different than two other treatments conditions, both of which also had prey available. Thus, prey availability was a common feature among two of the treatments conditions significantly affecting RAAPs of thrips. Yet, in these cases, neither the amount of plant feeding (mm^2) nor the number of mite-eggs consumed varied among treatments, suggesting that aspects related to the quality of resources consumed, rather than the quantity, caused differences in RAAPs of thrips. Considering that the mite-eggs used in this study were from the same source 'batch' and their quality was assumed to be consistent among treatments, differences in resource quality was apparently due to variation in the quality of plant material. Previous investigations into the factors affecting patterns of omnivory by WFT found that a reduction in plant quality caused functional shifts from herbivory to predation (Agrawal *et al.* 1999; Janssen *et al.* 2003). Thus, plant quality can also impact the fitness of WFT without concurrent alterations in the extent of plant and prey consumption.

The combination of ecological factors that caused significantly different RAAP were not predicted based on prevailing ecological-hypotheses nor from previous evidence (Soria & Mollema 1995). Nitrogen has been considered a key property determining plant quality for herbivores (McNeill & Southwood 1978), and has been suggested as causal factor that promotes omnivory (White 1993) and outbreaks of herbivorous insects on periodic (or 'pulsed') drought-stressed plants (Huberty & Denno 2004). Such models would predict enhanced fitness of thrips when prey was available or

when confined with PDS-plants compared to control conditions, but the evidence in this study did not support these predictions. In fact, it is difficult to interpret these results in light of any one limiting factor (i.e., nitrogen) considering that multiple ecological factors were involved in cases of significantly different RAAPs (i.e., prey availability x plant genotype, and prey availability x periodic-drought-stress).

Evolutionary consequences

Because of the inter-dependent nature of adult LHCs, a major challenge with studying adult organisms is pinpointing which LHCs in particular were and were not affected by environmental variation. Post-hoc analyses of RAAPs provide such diagnostic capabilities, and this evidence in turn was used to interpret the overall effects on adult fitness. These results indicated that WFT had higher fitness when prey was available on genotype V05 than Atlas. For instance, WFT had more viable eggs on V05, indicating higher current reproductive success. Furthermore, for every hatched eggs a greater number of 1^{st} instars were recovered (Hatched eggs→ Immatures) and indicates higher progeny recruitment in the next generation of offspring and can be viewed as a second indicator of enhanced current reproductive success. Thrips on genotype V05 had significantly larger body mass at the end of the experiment, and also gained more weight than on Atlas. It is not known to what extent the weight gained by thrips can be attributed to either somatic growth or reproduction. Nonetheless, the larger size of thrips on V05 could have positive effects on future reproductive success because thrips that weighed more also laid more eggs (i.e., initial body mass was a strong predictor of eggs laid thrips on both V05 and Atlas). The significant positive relationship between the amount of plant feeding and the number of eggs laid on V05 (but not on Atlas) could be tentatively interpreted as an adaptive form of reproductive plasticity that couples the reproductive rate to the prevailing environmental conditions (Edward & Chapman 2011). Thus, indicators of both current and future reproductive success were enhanced on V05 compared to Atlas and consequently imply higher relative fitness of WFT on V05.

The second case of significantly different RAAPs was a within-genotype effect involving Atlas and was due to periodic drought-stress altering the effect of prey

availability. In this case, thrips performed better on PDS plants than on WW plants. Similar to the first case, thrips had higher quality eggs (hatched eggs) and higher offspring recruitment (hatched eggs → immatures) on PDS plants. Interestingly, there was a negative relationship between the amount of plant feeding (mm^2) and hatched eggs on PDS plants, which show as plant feeding (mm^2) increased there were fewer eggs to hatch. However, this effect did not ameliorate the overall larger number of hatched eggs in the PDS-condition.

The third instance of significantly different RAAPs was another within-genotype affect, but in this case involved the genotype V05 and was due to prey availability altering the effect of PDS. Considering there were no differences in plant consumption between treatments, and thrips consumed prey when available, it shows that more resources were consumed on PDS plants with prey available. Therefore, it was somewhat unexpected that thrips performed better on PDS plants without prey available when they consumed fewer total resources. Here, thrips had higher fecundity (more total eggs laid) and higher offspring recruitment (hatched eggs → immatures). That the initial weight was a stronger predictor of the number of eggs laid when prey was available, and at the same time there were fewer overall eggs in this treatment indicates that fecundity was state-dependent (McNamera & Houston 1996) and was a less successful of a strategy relative to conditions without prey where there was no state-dependency on reproduction.

An important conclusion is that no combinations of traits were consistently affected, highlighting the value of a systems-approach in understanding and teasing apart how adult organisms can be affected by ecological complexity. Offspring recruitment (Hatched eggs→ Immatures) was the only life-history trait consistently affected, whereas some measures of performance (e.g.: eggs laid, hatched eggs, final wt/wt gain) were variable in particular instances, and others were invariant (Eggs laid → Hatched eggs; Immatures). Additional research is needed to understand the proximate causes that result in some LHCs being more susceptible than others to the effects of various sources of ecological complexity.

Physiological trade-offs

Although life-history theory has predicted over 40 physiological trade-offs between aspects of reproduction and/or somatic growth, they can be difficult to detect (Stearns 1992). Such trade-offs have been shown in stressful environments while being absent in more favorable conditions (Marden *et al*. 2003), and this transient expression pattern may be why it has been notably difficult to identify physiological trade-offs (Stearns 1992; Flatt & Heyland 2012). In this study, evidence of two physiological trade-offs was fpimd. The first instance was when thrips were confined to the genotype Atlas with prey available. In this case, the physiological trade-off was between hatched eggs and final weight, which could be interpreted as a trade-off between either current reproduction and future reproduction (because larger thrips produce more eggs) or current reproduction and somatic growth, or some combination of both. It is not possible to explicitly differentiate these two possible physiological trade-offs because I did not dissect thrips to determine change in fat reserves nor did I collect the weight of individual eggs. Regardless, these results support the hypothesis that trade-offs are more likely to occur under stressful conditions as thrips performed relatively poor under these conditions (Stearns 1992). However, the second case was on the genotype V05 without prey available in WW conditions and involved a trade-off between eggs laid and final weight. In this case, we did not identify impacts on thrips performance, and yet a trade-off was still present. Taken together, these results provide mixed support for the hypothesis that trade-offs are more likely to manifest under stressful conditions.

Income vs. capital breeding

Reproductive strategies can be characterized along a dichotomy that involves income-breeders on one end and capital breeders on the other (Stephens *et al*. 2009). The reproductive potential of income-breeding herbivores is generally considered a function of adult longevity and the amount of resources acquired during that stage (i.e., adult-acquired resources). Since the current reproductive rate of income breeders depends largely on current food intake, then ecological pressures should have a fairly immediate impact on reproductive rates. Capital breeders, on the other hand, are those

able to stockpile energy reserves, either during immature development or through times of abundant resources (Stearns 1992; Stephens *et al.* 2009). As a result, the reproductive rate of capital breeders can be maintained at a level that is not directly linked to prevailing conditions (Edward & Chapman 2011). Tammaru & Haukioja (1996) proposed that differences in reproductive strategies may explain why the populations of some Lepidopteran species exhibit outbreaks and other do not. Accordingly, selection should favor larger females in capital breeders (those that can store more resources), which should impact important behaviors that result in density-dependence processes such as flight mobility, territoriality, and choosiness of host-plants. Therefore, the size (weight) of adult female capital breeders would be predicted as an important determinant of fecundity.

The reproductive strategy of WFT by most criteria matches that of an income-breeder. First, adult female WFT are relatively long-lived (upwards of 5 weeks), and lay as many as 200 eggs during this time. Furthermore, thrips have two ovaries each comprised of eight ovarioles and oviposit a relatively small number of eggs each day (~upwards of 7), suggesting that under ideal conditions one egg can be produced per ovariole every two days (Reitz *et al* 2009 and sources therein). These results highlight the limited capacity of egg production at any given time and why adult longevity can be a key determinant of lifetime reproductive success of WFT. Second, a positive relationship between feeding and eggs laid was found which tentatively suggests that adult-acquired resources had positive effects on reproductive rates. Third, it was found that imposing certain ecological conditions on thrips for a period of three days resulted in detectable impacts on several aspects of thrips reproduction. However, that the size of the herbivore was an important determinant of reproduction in most cases is more characteristic for capital breeders, rather than income-breeders. Thus, these results highlight that the distinction of income-capital breeding is not a strict dichotomy, but rather a continuum that can vary depending on the environmental conditions.

CHAPTER IV

ECOLOGICAL FACTORS AFFECTING THE FORAGING-DECISIONS OF AN INTRA-CELLULAR FEEDING THRIPS, WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* PERGANDE

Introduction

"All of the interaction with insects occurs initially on the surface of the plant; this is the stage on which evolutionary, and sometimes co-evolutionary, dramas have been played." –(Juniper and Southwood 1986, foreword)

Understanding why herbivores feed where they do is a primary goal in research on HPR. To this end, finer-scale studies involving herbivores feeding on individual leaves can be ideally suited for teasing apart both the proximate and ultimate factors that underlie observed patterns of herbivory. Many examples exist of herbivores foraging non-randomly on individual leaves (eg: Wardle & Simpson 1927; Simmons 1994; Takeuchi et al 2009), but systematic investigations into the ecological mechanisms have mostly focused on chewers (Larrson *et al*. 1997; Korth *et al*. 2006; Schroff *et al*. 2008). For instance, mandibulate herbivores (so-called 'chewers') avoid foraging on major veins in *Medicago truncatula* (Gaertner) due to higher levels of calcium oxalate crystal (Korth *et al*. 2006) and on the periphery and midvein of *Arabidopsis thaliana* (L.) Heynh. leaves due to higher concentrations of allelochemical, like glucosinolates (Schroff *et al*. 2008). However, comparatively less is known about the factors that influence the within-leaf foraging-decisions of haustallate herbivores such as sap-sucking herbivores, which feed on phloem, xylem, and intracellular contents (Whitham 1983; Luft *et al*. 2001).

One noted foraging pattern that deserves additional attention is why some sap-sucking herbivores tend to feed from the undersides (abaxial) of leaves (Wardle & Simple 1927; Fennah 1963; Luft *et al*. 2001). In contrast to mandibulate insects, sap-sucking insects have mouthparts adapted for the consumption of specific plant materials

(xylem, phloem, intracellular contents), which can be differentially accessed from one leaf surface or the other. For example, the silverleaf whitefly, *Bemisia argentifolii* (phloem-feeder) probe for secondary veins predominantly from the abaxial leaf surface (Simmons 1994), and their stylet sheaths left behind after feeding, which represent an exact history of the movement of stylets within leaves, were almost exclusively in the air spaces between the spongy mesophyll (Cohen *et al.* 1998). This raises two possibilities for leaf properties that could lead to differential feeding predominantly from the abaxial surface by sap-sucking herbivores. First, the upper epidermis, which is generally thicker than the lower epidermis (Taiz & Zeiger 2001), could result in the adaxial leaf surface being 'tougher' and requiring more energy for stylet penetration (Peeters *et al.* 2007). Second, once the leaf surface has been fractured, the intercellular movement of stylets, as opposed to moving through cells, could be the path of least resistance while searching for secondary veins (Kingsolver & Daniel 1995). Accordingly, the spongy mesophyll layer which is comprised of irregular-shaped cells and surrounded by large air spaces could provide greater opportunity for intercelullar movement compared to the more densely packed palisade layer (Taiz & Zeiger 2001). Thus, internal and external variation in leaf properties across the dorsi-ventral axis of leaves could differentially affect the feeding of sap-sucking herbivores depending on the leaf surface from which they feed.

Feeding preference from the abaxial leaf surface has been noted previously for other species of thrips, but there is still a limited understanding of which ecological factors influence this preference and how these factors affect the herbivore's fitness (Wardle & Simpson 1927; Fennah 1963). Wardle & Simpson (1927) hypothesized that the preference for abaxial feeding by *Thrips tabaci* was unlikely related to an aversion to light, but rather due to the properties of the leaf itself. Fennah (1963) examined the movement behavior of *Selenothrips rubrocinctus* after manipulating the orientation of individual leaves in field and laboratory conditions and concluded that the preference for the abaxial leaf surfaces was primarily related to avoidance of direct light and only secondarily affected by 'attractiveness' of the abaxial leaf surface.

In this study, I investigated the working hypothesis that an intracellular feeding thrips, WFT, tends to feed on the abaxial leaf surface of cotton cotyledons because: 1) it is less tough than the adaxial leaf surface, and 2) it increases fitness. Thus, my goals were to: 1) quantitatively document the feeding preferences of WFT on individual leaves, 2) assess negative phototaxis and positive geo-taxis as potential factors that influence WFT foraging, 3) evaluate whether properties related to the leaf itself underlie WFT foraging preferences by investigating feeding and fitness consequences of each leaf surface under no choice conditions, 4) characterize the leaf properties based on whether the fitness consequences could be attributed to a reduction in the quantity and/or quality of resource consumed, and 5) investigate the role of leaf 'toughness' as a mechanism that inhibits thrips feeding.

Methods and materials

General Procedures

Cotton plants (*Gossypium hirsutum* L.) were seeded individually in 125 ml pots (Metro-mix 900 [Sun Gro Horticulture, Bellevue, Washington, USA]) and cultivated in a small room (2.25m x 2.75m x 2.25m) (16:8 light:dark cycle, 13.1±5.2 μmol m^{-2} s^{-1}, 34.7±11.1^{o}C). For all experiments, cotton plants were 10-days old (from time of planting) at the time of experimentation. Furthermore, to minimize variation in plant quality between experiments, all experiments were conducted on the same day using plants randomly selected from a source 'batch'. This procedure was repeated for each of two trials. Adult female WFT were obtained from colonies maintained on bean cotyledons under florescent lights (12:12 light:dark, 1.2±0.3 μmol m^{-2} s^{-1}, 25.0±3.3^{o}C). In all experiments, the following two cotton genotypes were used: 07-7-1001 hereafter referred to as "V07" which is an unreleased experimental line developed by Texas AgriLife Research Cotton Improvement Program in Lubbock, TX and All-Tex® Atlas (All-Tex Seed Inc., Levelland, TX; PVP: 9200188; PI 561579). These genotypes were selected based on preliminary experiments that indicated phenotypic variation in the leaf biomechanical properties (see experiment 3 below).

WFT feeding preferences (choice) and effects of leaf orientation

The first objective of this experiment was to document the foraging patterns of WFT on individual cotton cotyledons in terms of feeding on the edge, abaxial, and adaxial surfaces. The second was to evaluate whether the orientation of the cotyledon (i.e., abaxial side-down vs. abaxial side-up) altered their within-leaf feeding preferences, which allowed us to broadly evaluate the roles of negative-phototaxis and positive geotaxis as causal mechanisms.

Individual adult female WFT were sealed in 90-mm-diameter Petri dishes with an excised cotton cotyledon that was placed either abaxial side-down ('normal') or abaxial side-up (up-side down). The petiole 'props-up' when the cotyledon is placed abaxial-side down in the Petri dish ('normal'). Consequently, toothpicks were placed under all cotyledons in both treatment groups to provide access for WFT to the adaxial leaf surface, when the cotyledon was abaxial side-up (i.e., up-side down). After three days, the total area of plant scarring by WFT on the edge, abaxial, and adaxial surface was quantified under a dissecting microscrope, and converted into proportions prior to analysis. If WFT were missing, died, or did not feed during the course of the experiment, the replicate was omitted from the analysis.

The experimental design of the bioassay was a full factorial design with cotton genotype (V07 and Atlas) and leaf orientation (abaxial side-up vs adaxial side-down) (n =10-14 replicates for each treatment combination, each trail). Shapiro-Wilks normality test showed that the proportion of feeding on each of the within-leaf locations was non-normal, so a generalized linear model was used to test for effects of leaf orientation and plant genotype on the proportion of WFT feeding on the abaxial, adaxial, and edge, respectively (R Development Core Team 2009). The model had a quasibinomial error structure and was analyzed using an F test, because the dispersion was estimated by moments.

To establish feeding preferences/non-preferences on the edge, abaxial, and adaxial surfaces, Monte Carlo reshuffling (10,000 times) methods were conducted. A significant preference or aversion was determined if the observed mean proportion of

feeding on a selected leaf surface was significantly larger or smaller, respectively, than the mean proportion of feeding generated from the Monte Carlo simulation. Probability values for obtaining the observed mean proportion of feeding on each leaf surface were generated based on the number of times the re-shuffling resulted in means significantly larger or smaller than the actual means. This analysis was performed independently for each genotype x leaf surface combination, and for each treatment combination the analysis was performed twice, once to determine the upper probability (preference) and once to determine the lower probability (aversion).

No-choice feeding and relative fitness bioassay

To assess the fitness consequences of feeding on each leaf surface, a leaf-disc bioassay was developed that restricted WFT to feeding on either the abaxial or adaxial leaf surface only. Leaf discs (diameter 2.5cm) from cotyledons were placed individually in polyethylene-caps (diameter 2.5cm) of a vial (diameter 2.29cm, height 5.4cm) either abaxial side-up or abaxial side-down. Upon connecting the vial to the cap, a seal was created around the edges of the leaf disc that restricted the access of WFT to only one side of the leaf disc. Measures of feeding and performance of individual thrips was assessed according to the procedure outlined in Chapter 3

If the adult female thrips were missing, died, or did not feed during the course of the experiment, the replicate was omitted from the analysis. The experimental design of the bioassay was a full factorial design with plant genotype (V07 and Atlas) and leaf surface (under-surface vs upper-surface) as treatments (n =11-14 replicates for each treatment combination and each trial). Because the data could not be considered to follow a normal distribution, the number of 1-, 2-, and 3-mm^2 feeding scars was investigated using a generalized linear model (Poisson error structure) with a Chi-square test (R Development Core Team 2009) with initial weight as a covariate. All other variables were analyzed with path analysis.

Path analysis

To investigate the effects of leaf surface on WFT performance, path analysis was applied to the data derived from the aforementioned no-choice bioassay and generated

RAAP for WFT. Two types of RAAPs were generated using path analysis which were identical in all respects except that one included the variable final weight whereas the other included weight gain (Fig. 4.1A and B, respectively). Both measures of weight could not be examined in the model simultaneously because they are perfectly linearly dependent resulting in positive definite matrices. The general procedure used for testing differences between RAAPs was to first test measurement invariance between an unconstrained model for two models combined, followed by a model with the path coefficients constrained, and then both path coefficients and intercepts constrained to be equal. If the chi-square differences statistic was not significant for the original and constrained models then the path coefficients and/or intercepts were invariant between the models. If the chi-square differences statistic was significant for the constrained model implying non-invariance of path coefficients and/or intercepts between models, then post-hoc analysis were conducted to determine which of the specific path strengths and/or means were different. A separate path analyses which included leaf surface as a treatment (coded: abaxial=0, adaxial=1) was also used to determine whether the effects of leaf surface on fitness were due to reductions in the quantity and/or quality of resources.

Do leaf biomechanical properties influence WFT feeding?

No-choice results indicated that feeding was significantly reduced on the adaxial surface (see result section), which led us to investigate the role of leaf biomechanical properties as causal factors that inhibit WFT feeding. To this end, a penetrometer was used to measure the force required to pass a blunt punch (or rod) through either the abaxial or adaxial surface of a cotyledon. The punch, a flat-ended, steel cylinder (diameter=1.5mm), and die (diameter 3.175mm) were installed into a general testing machine (Model TAXT2i, Texture Technologies Corp., Scarsdale, NY, USA). The punch speed was kept constant (0.2 mm s^{-1}) and the machine simultaneously recorded load (N) applied to the sample and displacement (mm) of the punch (every 0.005 s). Because the penetrometer is destructive, I sampled the biomechanical properties from representative cotyledons and correlated these results with WFT feeding data derived

from no-choice conditions. The penetrometer data were collected on the same day that the WFT bioassay was initiated using the same batch of plants. Effort was made to avoid major veins and to sample the biomechanical properties from roughly the same location on the cotyledon as to where the center of each leaf disc would have been.

A penetrometer generates force-displacement curves, which were used to derive two biomechanical properties: punch strength and work to crack initiation (Fig. 4.2, Table. 4.1). Punch strength is the maximum force needed to initiate a crack in the leaf surface (Sanson et al. 2001) (Fig. 4.2A, and Table 4.1), which could be a relevant measure with respect to the feeding of sap-sucking herbivores, as well as for chewers, because each must first initiate a crack in the leaf surface in order to access and consume plant materials beneath. Because work to punch (Fig. 4.2B) would seem to overestimate the energy required during a feeding bout for sap-sucking herbivores (but not for chewers), a new property was derived that represents the work needed to initiate a fracture in the leaf surface, aptly named 'work to crack initiation' (Fig. 4.2C, and Table 4.1). The derivation assumes that work to punch can be viewed as a composite measure of two events, work to crack initiation and work needed to propagate the crack through the leaf (Fig. 4.2C,D and Table 4.1).

The leaf biomechanical assay was a full factorial design with plant genotype (V07 and Atlas) and leaf surface (under-surface vs upper-surface) as treatments (n =12-15 replicates for each treatment combination, each trial). In general, normality could not be achieved and therefore a generalized linear model (gaussian error structure) with an F test to analyze effects of plant genotype, leaf surface, and trial on punch strength and work to crack initiation (R Development Core Team 2009).

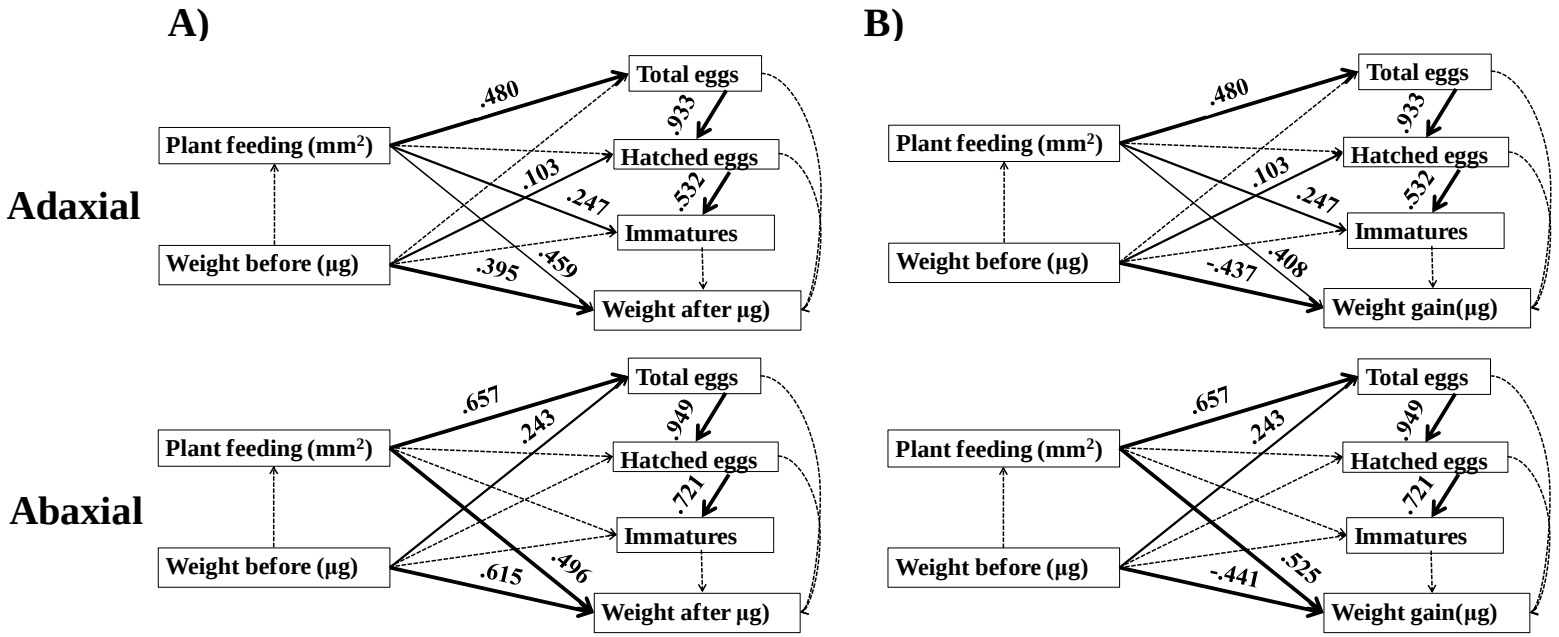

Figure 4.1. Resource acquisition and allocation patterns (RAAP) of Western Flower Thrips (WFT) confined to the abaxial ('under-surface') or adaxial ('upper-surface'). RAAP illustrate the effects of initial weight and plant feeding on four measures of performance: total eggs laid, hatched eggs, alive immature, and either A) final weight or B) weight gain. Matrices for the model were constructed from no-choice bioassays that confined an individual adult female WFT to feeding on either the abaxial or adaxial leaf surface. Standardized path coefficients are shown for significant pathways, and insignificant paths are shown with dotted arrows. Arrows are bolded for significant pathways based on the degree of significance (light bold: $0.01<P<0.05$; heavy bold: $P<0.01$).

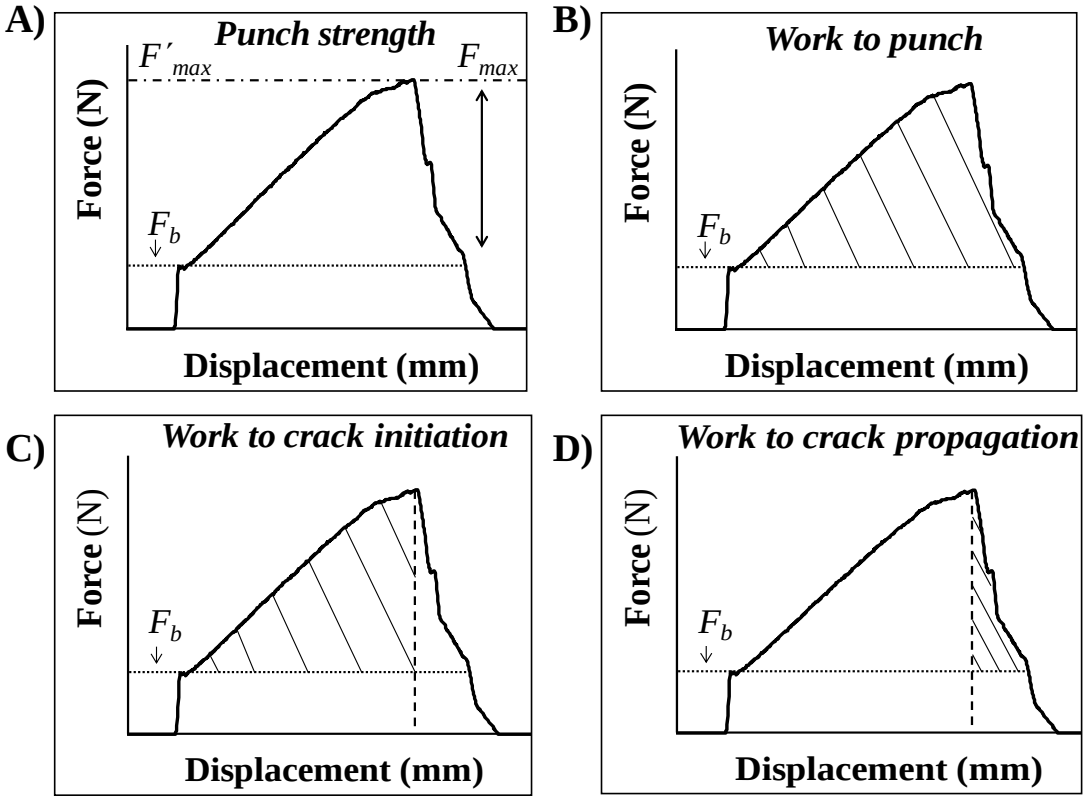

Figure 4.2. A visual representation of four leaf-biomechanical properties plotted on force-displacement curves that were generated from a punch-and-die test of *Gossypium hirsutum*. Most leaves were slightly curved and required force to initially flatten the leaf (base force, F_b) on the die. When the punch started to compress the leaf, a sharp increase in force was observed. The leaf surface is assumed to crack at the maximum force ($F_{\max}$). A) 'Punch strength' is the maximum force (F_{max}) (scaled to the area of the punch) required to initiate a crack in the leaf surface. B) 'Work to punch' is the total amount of work (i.e., area under curve) required to penetrate the entire leaf. Two additional properties were derived called 'Work to crack initiation' (C) and 'work to crack propagation' (D) which represent the total amount of work required to initiate a crack in the leaf surface and the energy needed to propagate a crack through the leaf, respectively.

Table 4.1. Leaf biomechanical properties, their derivation, and the herbivore feeding guild potentially affected.

Leaf biomechanical property	Calculation	Herbivore feeding guild potentially affected
Punch strength (Fig. 4.1A)	$(F_{max}–F_a)/A$	Piercing-sucking and chewing
Work to punch (Fig. 4.1B)	$\int [(F_x–F_b)D_x]/A$	Chewing
Work to crack initiation (Fig. 4.1C)	$\int [(F_y–F_b)D_y]/A$	Piercing-sucking and chewing

F_{max}, maximum force (N); F_b, force needed to flatten the cotyledon against the die; A, area of punch (m^2); F_x, force and D_x, displacement (mm) at any point, *x*, between initiation of leaf compression and complete fracture of cotyledon; F_y, force and D_y, displacement at any point, y, between initiation of leaf compression and the maximum force (F_{max}).

Results

WFT feeding preferences (choice) and effects of leaf orientation

On normally-oriented cotyledons (abaxial side-down), an aversion to adaxial-feeding by WFT was highly significant on both genotypes based on Monte Carlo simulations (P<0.001; Fig. 4.3). Conversely, the preference for feeding on the abaxial (Atlas: P<0.001, V07: P=0.138) and edge (Atlas: P=0.196, V07: P<0.001) varied depending on the plant genotype. By orienting the cotyledon upside down, the proportion of WFT feeding on the adaxial surface increased by ~4% ($F_{1, 110}$=1.559, P=0.014), but the overall aversion to the adaxial surface persisted (P<0.001, each genotype) (Fig. 4.3). Interestingly, when the cotyledon was up-side down, WFT fed 11% more on the abaxial leaf surface ($F_{1, 110}$=1.608, P=0.039) and 19% less on the edge ($F_{1, 110}$=3.440, P=0.005) compared to a normally orientated cotyledon (Fig. 4.3).

No-choice feeding and relative fitness bioassay

WFT were confined to either the abaxial or adaxial surface to investigate how each leaf surface affected feeding (mm^2) or various life history characteristics (LHC) commonly used as indicators of relative fitness. Results showed that RAAPs of thrips on each leaf surface were significantly different when both the intercepts and path coefficients (P=0.001) constrained but not the model with path coefficients (P=0.660)

alone (unconstrained model: χ^2 0.566 (P=0.753) (Table. 4.2). This was interpreted to mean that WFT had the same general resource allocation pattern on each leaf surface (Fig. 4.1A and B), but there were quantitatively differences in the amount of feeding and selected performance. For post-hoc analysis, I used the model with path coefficients constrained, which indicated significant differences in feeding (z=4.53, P<0.001), eggs laid (z=-3.10, P=0.002), and immatures (z=2.26, P=0.024). Accordingly, there was a 32% reduction in total area of feeding on the adaxial leaf surface compared to the abaxial surface (Fig. 4.4A), which could be attributed to 33.5% fewer 2 mm^2 feeding scars ($F_{1, 94}$=6.720, P= 0.009) and 59.1% fewer 3 mm^2 feeding scars ($F_{1, 94}$=11.330, P< 0.001) (Fig. 4.5) on the adaxial surface. While the total number of eggs was no different between each leaf surface (Fig. 4.4B), the path analysis used plant feeding as a covariate for eggs laid which resulted in significant differences in eggs (because the same number of eggs were laid even though there was less feeding on the adaxial surface). The number of hatched eggs on each leaf surface did not vary (Fig. 4.4C), but there was 35% less progeny collected from the adaxial leaf surface (Fig. 4.4D). A separate path analysis, which included leaf surface as a treatment (binary coded: abaxial=0, adaxial=1), indicated leaf surface had both direct (leaf surface → progeny) and indirect (leaf surface → feeding → progeny) effects on immatures (Fig. 4.6; Table 4.3).

Do leaf biomechanical properties inhibit thrips feeding?

In light of the evidence showing WFT fed significantly less on the adaxial surface in no choice condition and at the same time produced fewer 2- and 3-mm^2 feeding scars on the adaxial leaf surface, leaf biomechanical properties were investigated to evaluate their role as a possible causal factor that inhibits WFT feeding. Work to crack initiation was significantly affected by an interaction between plant genotype and leaf surface ($F_{1, 216}$=3.679, P=0.027) (Fig. 4.7B). This was due to the abaxial surface requiring less work to initiate a crack than the adaxial surface for the genotype Atlas, whereas there was no difference between leaf surfaces on genotype V07. While punch strength was not significantly affected by the interaction between genotype and leaf

surface ($F_{1, 216}$=1.783, P=0.183), the general relationship was similar to that observed for work to crack initiation (Fig. 4.7C).

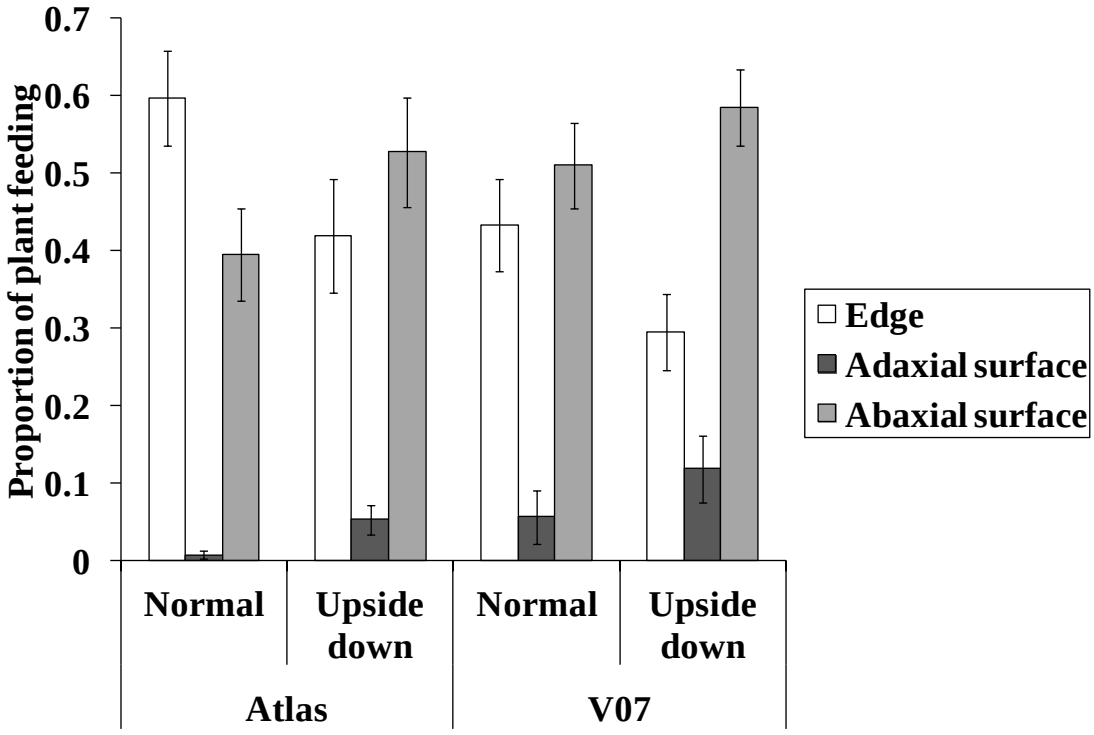

Figure 4.3. Feeding preferences (choice) of individual adult female Western Flower Thrips (WFT) were assessed on excised cotton cotyledons orientated 'normally' (i.e., abaxial-side down) and updside down (abaxial-side down) in Petri dishes. WFT were sealed for 3d with excised cotyledons from either one of two cotton genotypes (Atlas and V07).

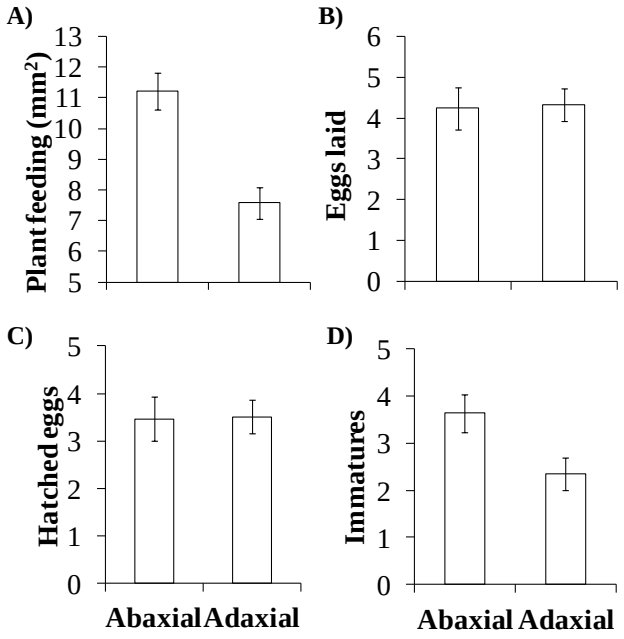

Figure 4.4. The effects of leaf surface on A) plant feeding (mm^2), B) eggs laid, C) hatched eggs, and D) the number of alive immatures produced by an individual adult female Western Flower Thrips during a 3d no-choice bioassay.

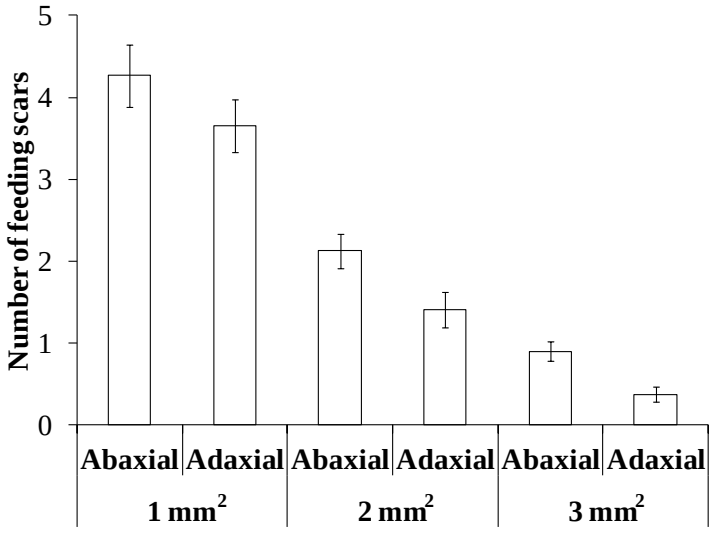

Figure 4.5. The effects of leaf surface (abaxial vs adaxial) on the size (1-, 2-, or $3mm^2$) of feeding scars produced by an individual adult female Western Flower Thrips during a 3d no-choice bioassay.

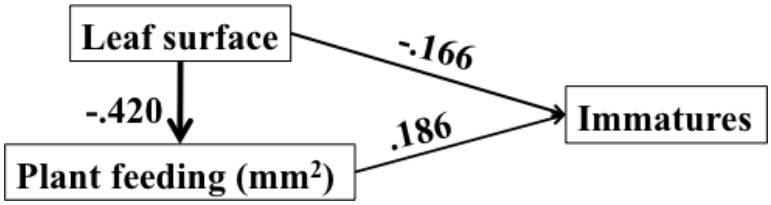

Figure 4.6. Direct and indirect effects (via feeding) of leaf surface (binary coded: abaxial=0, adaxial=1) on the number of alive immatures collected from cotyledons that had been sealed for 3 days with an individual adult female Western Flower Thrips (WFT). Shown above are components of a larger path model (see Table 4.3) that was not presented in full for simplicity. Matrices for the path models were constructed from no-choice bioassays that confined an individual adult female WFT to feeding on either the abaxial or adaxial leaf surface. Standardized path coefficients are shown for significant pathways. Arrows are bolded for significant pathways based on the degree of significance (light bold: $0.01<P< 0.05$; heavy bold: $P<0.01$).

Table 4.2. Results of path analysis examining resource acquisition and allocation patterns of Western Flower Thrips when confined to the abaxial or adaxial leaf surface. Shown is the amount of variance (R^2) in response variables explained in the path analysis, path coefficients and *P* values (in parenthesis) of predictor variables on response variables, and notes for each model (see Fig. 4.1).

		Predictor variable					
Leaf surface	Response variable	Initial weight (μg)	Feeding scars (mm^2)	Eggs laid	Hatched eggs	Immatures	Notes for model
Adaxial	Feeding scars (mm^2)	-0.177 (0.212)	-	-	-	-	Chi-square = 0.566, df=1, *P*=0.452; group normality (c.r.)=0.151
	Eggs laid	0.023 (0.857)	0.480 (<0.001)	-	-	-	
	Hatched eggs	0.103 (0.037)	0.020 (0.716)	0.923 (<0.001)	-	-	
	Immatures	-0.007 (0.945)	0.247 (0.042)	NA	0.532 (<0.001)	-	
	Final weight (μg)	0.395 (0.001)	0.459 (0.001)	0.197 (0.547)	-0.130 (0.716)	0.034 (0.831)	
	Weight gain (μg)	-0.437 (<0.001)	0.408 (0.001)	0.175 (0.547)	-0.116 (0.716)	0.030 (0.831)	
Abaxial	Feeding scars (mm^2)	-0.028 (0.849)	-	-	-	-	Chi-square = 0.001, df=1, *P*=0.981; group normality (c.r.)=1.334
	Eggs laid	0.243 (0.019)	0.657 (<0.001)	-	-	-	
	Hatched eggs	0.022 (0.497)	0.032 (0.422)	0.949 (<0.001)	-	-	
	Immaures	0.105 (0.238)	0.090 (0.430)	NA	0.721 (<0.001)	-	
	Final weight (μg)	0.615 (<0.001)	0.496 (<0.001)	-0.049 (0.917)	0.031 (0.950)	-0.107 (0.536)	

Table 4.3. Results of path analysis examining direct and indirect effects of leaf surface on resource acquisition and allocation patterns of Western Flower Thrips. Specifically shown is the amount of variance (R^2) in response variables explained in the path analysis, beta weights and *P* values (in parenthesis) of predictor variables on response variables, and notes for each model (see Fig. 4.6).

		Predictor variable						
Response variable	(R^2)	Leaf surface	Initial weight (μg)	Feeding scars (mm^2)	Eggs laid	Hatched eggs	Immatures	Notes for model
Feeding scars (mm^2)	0.183	-0.420 (<0.001)	-0.084 (0.362)	-	-	-	-	Chi-square = 0.571, df=2, *P*=0.752; group normality (c.r.)= -0.294
Eggs laid	0.363	0.281 (0.002)	0.162 (0.046)	0.656 (<0.001)	-	-	-	
Hatched eggs	0.927	0.005 (0.878)	0.057 (0.043)	0.032 (0.402)	0.938 (<0.001)	-	-	
Progeny	0.59	-0.166 (0.027)	0.057 (0.396)	0.186 (0.037)	-	0.610 (<0.001)	-	
Final weight (μg)	0.411	0.112 (0.225)	0.484 (<0.001)	0.514 (<0.001)	0.128 (0.656)	-0.124 (0.676)	-0.014 (0.907)	
Weight gain (μg)	-0.441	0.108 (0.225)	-0.453 (0.917)	0.494 (0.950)	0.123 (0.536)	-0.119 (0.676)	-0.014 (0.907)	

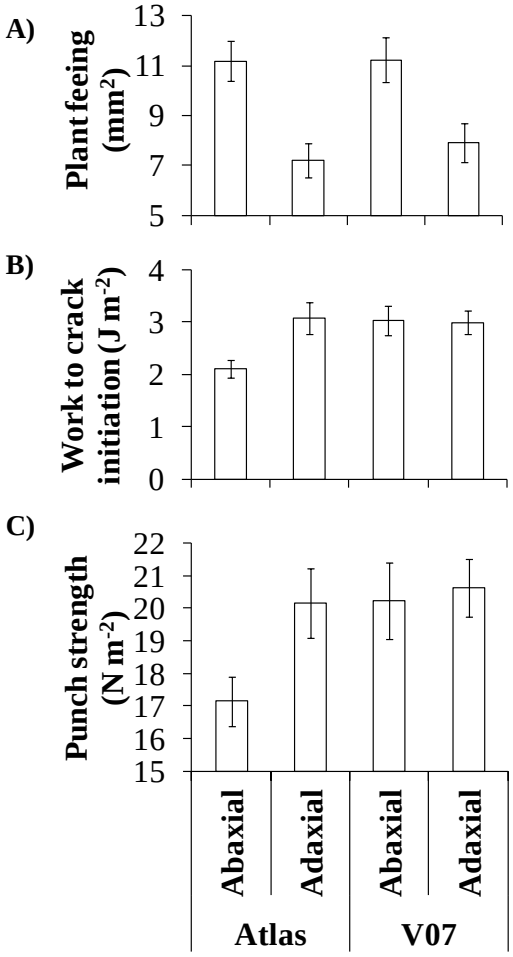

Figure 4.7. Bar graphs illustrating the effects of leaf surface (abaxial vs adaxial) and plant genotype (Atlas and V07) on thrips feeding (A) and two biomechanical properties of cotton (*Gossypium hirsutum* L.) cotyledons: work to crack initiation (B) and punch strength (C). Mean plus SE is shown for each response variable.

Discussion

It has previously been noted for two other species of thrips that feeding on individual leaves tends to be localized on the abaxial surface (Wardle & Simpson 1927; Fennah 1963). The results of this study showed that the foraging of WFT on (normally-orientated) cotyledons was best characterized as an aversion to feeding on the adaxial surface rather than a preference for the abaxial surface. This is based on the evidence showing that the aversion to feeding on the adaxial surface was consistent among plant genotypes, whereas the preferences for abaxial and edge surfaces varied depending on plant genotype.

The first experiment investigated whether thrips aversion to feeding on the adaxial surface was due to an avoidance of light (i.e., negative-phototaxis) and/or an attraction to gravitational forces (positive geotaxis). For this experiment, the orientation of cotyledons was manipulated (normal vs up-side down) and if an aversion to light and/or attraction to gravitational forces influence thirps foraging then I predicted that on up-side down cotyledons thrips would feed predominantly on the adaxial surface. Two lines of evidence did not support these predictions. First, although the proportion of feeding on the adaxial surface increased when the cotyledon was up-side down, feeding on this surface was still characterized as a significant aversion relative to the edge and abaxial surfaces. Second, WFT fed significantly more on the abaxial surface under treatment conditions, which was characterized on both genotypes as a significant preference for the abaxial surface. These results, generated under laboratory conditions, led us to conclude that mechanisms other than negative phototaxis and/or positive geotropism influenced the foraging-decisions of WFT on individual cotton cotyledons.

The next experiment investigated whether an aversion to adaxial-feeding could be attributed to the properties of the leaf itself (Wardle & Simpson 1927). I predicted that if the aversion to adaxial-feeding was due to leaf properties, then under no-choice conditions WFT would have lower performance on the adaxial surface than the abaxial surface. The results of this study supported these predictions. Specifically, WFT produced 35% fewer immatures on the adaxial surface (Fig. 4.4D), which indicates that

the aversion to adaxial-feeding was an adaptive form of foraging. Furthermore, WFT fed 32% less on the adaxial surface compared to the abaxial (Fig. 4.4A), which was associated with fewer larger-sized feeding scars (Fig. 4.5). Thus, variation in leaf properties between the abaxial and adaxial leaf surfaces impacted both the feeding and fitness of an intra-cellular feeding thrips.

To investigate whether the reduction in immatures on the adaxial surface was due to the reduction in the quantity of resources consumed on that surface, a second path analysis was constructed that included leaf surface as a treatment. Support for this hypothesis would be evidenced by an indirect effect of leaf surface on immatures through the following pathway Leaf surface → Feeding scars (mm^2) → Immatures. Direct effects of leaf surface on immatures (Leaf surface → immature) could indicate two possibilities, either reduction in the quality of resources consumed by female thrips resulting in poorer offspring vigor, or reduction in the ability of 1^{st} instar thrips to emerge from and/or establish on the adaxial surface. Results indicated both direct and indirect effects of leaf surface resulted in fewer immatures on the adaxial surface (Fig. 4.6). Thus, a reduction in resources consumed on the adaxial surface could at least in part be attributed to fewer immatures recovered on that surface.

The evidence showing WFT produced fewer feeding scars ≥ 2 mm^2 on the adaxial leaf surface could indicate some sort of mechanical barrier inhibiting the size of thrips feeding scars. A thicker upper epidermis, which is generally common for most plants, could pose a mechanical barrier that requires greater force to fracture. In addition, mechanical resistance could arise from the cellular organization of the palisade layer, which is comprised of columnar-shaped and more densely packed cells compare to the spongy mesophyll (Taiz & Zeiger 2001). Leaf 'toughness' or resistance to mandibular penetration could be a particularly important plant trait for intra-cellular feeding thrips. Thrips mouthparts are characterized as piercing-sucking, and in the sub-order terebrantia, a typical feeding event involves two steps: 1) the mandible which has a pointed tip but no opening is used to punch a hole into the leaf surface, and 2) the maxillary stylets, which interlock and open at the tip to form the feeding tube, enter the

hole created by the mandible and begin to puncture cells and ingest intra-cellular contents (Kirk 1997). The mandible is fused proximally to the exoskeleton and cannot be protracted by direct muscular action, resulting in an indirect force generated from moving the whole head downwards and backwards (Kirk 1997). Thus, the final experiment investigated the hypothesis that leaf biomechanical properties play an important role in thrips foraging-decisions on individual leaves. The approach of this experiment was to examine the relationship between WFT feeding (mm^2) and two leaf biomechanical properties that seemed particularly relevant to an intracellular feeder (Fig. 4.1). Results provided only partial support for the biomechanical-hypothesis as plant feeding and biomechanical properties negatively co-varied on one cotton genotype (Atlas), but not on the other (Fig. 4.7). While the level of precision in this study was fairly unique, the results from this study in general are congruent with the limited research previously conducted. Peeters *et al*. (2007) found the leaf biomechanical properties of 18 co-occuring plant species were not correlated with the densities of shallow-suckers/chewers comprised of Thysanoptera, Diptera larvae, and grubs of unknown order.

This study sought out to identify ecological mechanisms that shape an intra-cellular feeding thrips feeding-decisions. Ultimately, knowledge of the specific factors that influence this class of herbivores will provide a foundation for generating predictions on their distribution and abundance in the field and a focal point to target in plant breeding and advanced biotechnology for development of resistant crop cultivars.

CHAPTER V

EFFECTS OF DROUGHT-STRESS AND RECOVERY ON STOMATAL MORPHOLOGY, LEAF SURFACE TEMPERATURE, AND PHYTOHORMONES IN TRANSGENICALLY-MODIFIED COTTON SEEDLINGS

Introduction

Limited water availability is one of the major abiotic factors limiting crop production worldwide, and is a major issue of concern in future climate change. As a result, significant efforts have been taken to enhance drought tolerance in crop plants through two main approaches: 1) traditional plant breeding at the scale of whole plants (so called 'top-down' approaches), and 2) advanced bioengineering at the scale of individual genes (so called 'bottom-up' approaches) (Sinclair 2011). While advances have been made in the development of drought-tolerant cultivars, an understanding of the metabolic and physiological traits that functionally contribute to the drought-tolerant phenotype remains limited. Such information has both applied and basic value because understanding how genotypes produce a given phenotype will contribute towards closing the gap between the top-down and bottom-up approaches (Miflin 2000), and can be used to develop more efficient protocols for screening candidate drought-tolerant cultivars.

Genomic and genetic analyses primarily in the model plant *Arabidopsis* have elucidated the biosynthetic and signal transduction pathways of abscisic acic (ABA) and its roles in stress response (reviewed in Rock *et al*. 2010; Cutler *et al*. 2011). Altered expression of several different ABA effectors (e.g. *ENHANCED RESPONSE TO ABA1* [*ERA1*/Farnesyl Transferase beta subunit], AtNuclear Factor Y-B1, AtNFY-A5, ABA3/LOW OSMOTIC STRESS RESPONSE5/[LOS5]) has been shown to enhance drought tolerance in canola, corn, and rice (Wang *et al*. 2005; Nelson *et al*. 2007; Xiao *et al*. 2008; Li *et al*. 2008). Based on these results, transgenic cotton (*Gossypium hirsutum* L.) lines were recently generated that over-express the Basic Leucine Zipper Domain transcription factor AtABI5 and Basic3/APETALA2-domain transcription factors

AtRAV1, AtRAV2/TEMPRANILLO2, and AtRAV2L/TEMPRANILLO1. The resultant lines have been characterized in terms of improved drought avoidance, flowering time, and fiber quality (Mittal in prep). However, an understanding of how these transgenic lines physiologically and functionally contribute to drought avoidance has not been elucidated.

This study builds on the aforementioned advances in transgenic cotton and investigates the metabolic and physiological responses to drought-stress and drought-recovery of select cotton isolines over-expressing AtRAV1 and AtABI5 under control of the CaMV 35S promoter. I measured the concentrations of five phytohormones, ABA, Jasmonic acid (JA), Salicylic acid (SA), JA-isoleucine (JA-ILE), and 12-oxo-phytodienoic acid (OPDA). Plant hormones play important roles in regulating growth, development, and signaling networks to a wide range of biotic and abiotic stresses. SA, JA, JA-Ile are known to regulate plant defense pathways related to biotic stressors such as pathogens and insects, but less is known about how they respond to periods of drought and recovery. ABA is an important regulator of plant responses promoting tolerance to drought-stress and is commonly observed at increased levels in drought-stressed plants. One such response governed by ABA is the shrinking and swelling of guard cells (Kim *et al*. 2010), which determines the size of stomata and stomatal apertures. The aperture is the gateway for both CO_2 influxes into plants from the atmosphere and transpiration rates (water loss). Consequently, the areas (μm^2) of stomata and stomatal apertures were measured, and a measure of relative stomatal aperture dominance (SAD) was derived as the scaled relationship between the apertures and stomatal area. SAD has not been previously investigated, but may have important functional consequences on transpiration rates in leaves. Lastly, because stomata behavior affects transpiration rates (i.e., water loss), and transpiration affects the temperature of leaves, I inferred transpiration rates through measurements of leaf surface temperatures (LST). My hypothesis was that the transgenic isolines would exhibit hypersensitive responses to ABA, which would enhance drought-tolerance. Support for this hypothesis would be that the transgenic isolines would have lower levels of ABA

than WT under drought-stress conditions. Since ABA is known to mediate changes in stomatal apertures, I hypothesized that a hypersensitive response to ABA in the transgenics would result in smaller stomata and/or stomatal apertures, which would reduce water loss and thus could be potentially the physiological trait conveying drought-tolerance in the transgenic. Since transpiration (i.e., water loss) can impact LSTs, I predicted that under drought-stressed conditions the transgenic and WT would have similar LSTs but for two different reasons: the WT would have elevated LSTs because of less available water to transpire as a result of being drought-stressed, and the transgenics would have elevated LSTs due to smaller stomata and/or stomatal apertures inhibiting water loss.

Methods and materials

Plant material

Three transgenic cotton lines (collectively referred to as 'isolines') were investigated in this study, all of which were obtained by transformation of the cotton cultivar 'Coker 312'. Thus, the wild-type (WT) refers to untransformed Coker 312 (SeedCo Corporation, Lubbock, TX, PI 528278; PVP 7200100). The specific transgenes with associated event numbers were the following: RAV1-1 (42-1-1-5), ABI5-1 (38-1-1-1), and ABI5-2 (38-13-4-1). All cotton plants (n=21-24 per isoline) were seeded individually in 400 ml pots and cultivated simultaneously in a small room (2.25m x 2.75m x 2.25m) at 34.7±9.9^{o}C under continuous light (13.1±5.2 μmol m^{-2} s^{-1}). From the time of planting, the duration of the experiment was 13 days, and all seedlings emerged 4-5 DAP.

The timeline for the drought-stress and recovery treatment was as follows. Immediately after planting, i.e., 1 day after planting (DAP) and again 5 DAP, 125ml of water was added to each pot. At 9 DAP half of the plants from each isoline were randomly allocated to either a 'well-watered' group which received 125 ml of water on that day, or to a drought-stress (DS) group which did not receive water (n= 10-12 plants per treatment per cultivar). Plants in both treatments were then withheld water for three

days (10-12 DAP) (hereafter referred to as 'drought-stress period'). On 12 DAP, when plants were assumed to be under peak drought conditions with respect to the treatments imposed here (hereafter referred to as 'peak drought'), physiological parameters were assessed (see below) and then DS and WW plants received 125ml of water. On 13 DAP which was 24 h after final watering (hereafter referred to as 'recovery') physiological parameters were assessed and then the experiment was terminated. Summarizing, WW plants received 125ml of water on days 1, 5, 9, 12 DAP, and DS plants on 1, 5, and 12 DAP, with drought recovery measured on 13 DAP.

Leaf surface temperatures

We measured leaf surface temperatures (LST) 9 DAP through 13 DAP using a Fluke 62 IR thermometer. Measurements were taken prior to initiating the drought-treatment on 9 DAP (hereafter referred to as 'pre-drought'). Three subsamples from each cotyledon were collected in rapid succession from the adaxial surface ~2 cm from the petiole. Thus, six subsamples were collected per plant, which were averaged to provide a single data-point per plant. The LSTs of each plant were measured in the same part of the room to minimize variation in measurements. As the drought treatment progressed some of the cotyledons were drooping. In this case, a pen was used to lift the cotyledon so that it was oriented parallel to the ground. The cotyledon was held in place for 30s prior to taking LSTs measurements. Pre-drought (9 DAP) LST did not meet assumptions of normality and a generalized linear model (gaussian error structure) was used with an F test. LSTs collected during the drought-stress period (10-12 DAP) and recovery (13 DAP) met assumptions of normality, and a repeated measure ANOVA used with an error term indicating DAP nested within plant in the former, and an ANOVA was used in the latter (R Development Core Team 2009).

Stomata morphology

Stomatal area parameters and phytohormones were analyzed at peak drought (12 DAP) and drought recovery (13 DAP). On each day, three plants were harvested per treatment with one cotyledon analyzed for stomatal areas and the other analyzed for phytohormones. Stomata measures were collected through a modified technique

reported in Travaglia *et al*. (2010). A layer of clear nail polish (nitrocellulose in ethyl acetate) was brushed onto the abaxial side of a cotyledon, allowed to dry for a few seconds, and then carefully extracted and mounted on a microscope slide. The slide was examined using a standard compound microscope, and digital photomicrographs were taken of individual stomata. The photomicrographs were then imported into Image-J (Rasband 1997-2012), and stomatal areas (μm^2) and stomatal apertures (μm^2) were quantified. Stomata measurements were chosen at random from those that could be clearly focused as to avoid distortion in the measurements. Because of variation in the clarity of stomata, the number of stomata per cotyledon varied ranging from 14-20 stomata per cotyledon. The area of stomata and apertures were summed to produce a single data point for that cotyledon, and then scaled to 14 stomata to correct for the uneven number of stomata collected per cotyledon. Thus, for each cotyledon (n=3 plants per day) the total area of 14 stomata and the total area of their apertures were measured. SAD was investigated by scaling the aperture (μm^2) to the size of the stomata (μm^2). This measure was generated for each cotyledon by dividing the total area of 14 stomata by that of the apertures (and thus avoiding averaging of averages). Stomatal area (μm^2), apertures (μm^2), and the derivative SAD term each met assumptions of normality and were analyzed separately using ANOVA.

LC-MS/MS analysis of acidic plant hormones

The following five phytohormones were analyzed: ABA, JA, SA, JA-ILE, and OPDA. Fresh cotyledon tissue (80-160 mg) was collected from individual cotyledons (n=3) and flash frozen in liquid N_2. The method used for detection and quantitation of acidic plant hormones was developed and performed by the Proteomics & Mass Spectrometry Facility at the Donald Danforth Plant Science Center (St. Louis, MO, USA). The method is similar to (Chen *et al.* 2009), but modified to include additional plant hormone species. Briefly, samples were ground in liquid nitrogen and internal standards (10 mL of 2.5 mM) were added. Samples were extracted with 1.5 mL acetonitrile/methanol (1:1 v:v). After lyophilization, samples were resolubilized in 200 mL of 50% MeOH. For liquid-chromatography (LC) separation, two monolithic C18

columns (Onyx, 4.6 mm x 100 mm, Phenomenex, CA, USA) with a guard cartridge was used flowing at 1 mL min^{-1}. The gradient was from 40% solvent A (0.1% (v/v) acetic acid in MilliQ water), held for 2 min, to 100% solvent B (90% acetonitrile (v/v) with 0.1% acetic acid (v/v) in 5 min. The LC was held at 100% B for 3 min and then ramped back to initial conditions and re-equilibrated for an additional 2 min. To minimize variation from the autosampler, the sample loop was overfilled with 52 mL of sample and the sample storage temperature was set to 8°C. The LC-MS/MS system used is composed of a Shimadzu LC system with LEAP CTC PAL autosampler coupled to an Applied Biosystems 4000 QTRAP mass spectrometer equipped with a TurboIon Spray (TIS) electrospray ion source. Source parameters were set to: CUR: 25, GAS1: 50, GS2: 50 (arbitrary unit), CAD: high, IHE: on, TEM: 550 °C, IS: -4500. Both quadruples (Q1 and Q3) were set to unit resolution. Analyst software (version 1.4.2) was used to control sample acquisition and data analysis. To maximize sensitivity, ABA, SA, OPDA, JA standard solutions were infused into the 4000 QTRAP with a syringe pump (Harvard 22) at 10 mL min^{-1} to select multiple reaction monitoring (MRM) transitions and optimize compound-dependent parameters for MRM detection (Table 5.1).

Table 5.1. Optimized compound-dependent mass spectrometry parameters[a].

Compound	Q1	Q3	DP	EP	CE	CXP
SA	137	93	-49	-22	-5	-5
ABA	263.1	153	-60	-16.7	-9	-9
JA	209	59	-60	-24	-2	-2
H2JA	211	59	-60	-24	-2	-2
D4SA	142	98	-49	-22	-7	-7
OPDA	291.1	165	-75	-30	-5	-5
JA-ILE	322.1	130	-65	-32	-7	-7
D6ABA	269.1	159	-70	-16	-13	-13

[a]D6ABA was used as the internal standard for ABA, D4SA for SA, H2JA for JA and OPDA. Abbreviations of the compound dependent parameters are as follows: Q1, selected m/z of the first quadruple; Q3, selected m/z of the third quadruple; DT, dwell time monitoring each MRM transition (ms); DP, declustering potential of TIS source (V); CE, collision energy (arbitrary unit); CXP, collision cell exit potential (V); EP, collision cell exit potential (V).

Hormone quantitation

For quantitation, a series of standards were prepared containing different concentrations of ABA, SA, OPDA, and JA mixed with the H_2JA and D-labeled ABA, SA, (250 pmol/sample). Correction factors were obtained by adjusting the ratio of standard peak areas to that of internal standards in all samples. The peak areas of endogenous hormones were normalized with the corresponding internal standard and then calculated according to the standard curve. H_2JA was also used for the quantitation of JA-Ile because no D-standard for that compound is commercially available. Abscisic acid concentrations (ηg per mg plant fresh weight) met assumptions of normality and were analyzed using ANOVA, whereas the remaining hormones were non-normal and thus analyzed using a generalized linear model (gaussian error structure) with an F test.

Results

Water availability had the largest effect on LSTs during the drought period (10-12 DAP) ($F_{1, 315}$ =132.316; P<0.0001), and during drought recovery (13 DAP) ($F_{1, 56}$ =5.970; P<0.018) with WW plants having lower LST than drought-stressed plants (Fig. 5.1; Table 5.1). The effect of water availability on LSTs varied significantly among isolines during the drought period ($F_{1, 312}$ =4.562; P=0.003) and drought recovery ($F_{3, 53}$ =2.942; P=0.041), (Table 5.1). Notably, during the drought period there was a general trend in the WT for increased LSTs from one day to the next in both WW and DS conditions, whereas the response of LSTs were considerably more variable each day in the transgenic isolines (Fig. 5.1). At peak drought (12 DAP), LSTs of isolines were significantly different under WW conditions ($F_{3, 53}$ =2.942; P=0.041), but not in DS conditions ($F_{3, 53}$ =2.942; P=0.041). During drought recovery, WT and ABI5-2 had elevated LSTs in DS conditions compared to WW conditions, whereas LSTs for RAV1-1 and ABI5-1 in DS conditions were comparable to those in WW conditions.

Stomatal apertures ($F_{3, 43}$ =3.567; P=0.025) and SAD ($F_{3, 43}$ =4.284; P=0.012) were significantly different among isolines (Fig. 5.2; Table 5.2), and in both cases WT were the largest followed by ABI5-2, ABI5-1, and RAV1-1. The most significant effect

on both stomatal apertures and SAD was due to the drought-stage treatment with apertures and SAD increasing in drought recovery by 14.7% ($F_{1, 45}$ =10.911; P=0.002) and 17.2% ($F_{1, 45}$ =31.605; P<0.0001), respectively (Fig. 5.2; Table 5.2). The effect of water availability on SAD varied significantly among isolines ($F_{3, 43}$ =3.64; P=0.023), with SAD of WT being 7.5% larger in DS conditions compared to WW conditions, whereas SAD for ABI5-1, ABI5-2, and RAV1-1 SAD were 10.5%, 7.7%, and 14.4% smaller in DS conditions compared to WW conditions, respectively.

Drought-stage had the strongest effect on levels of JA ($F_{1, 46}$ =186.557; P<0.001), SA ($F_{1, 46}$ =11.005; P=0.002), JA-ILE ($F_{1, 46}$ =14.268; P<0.001), and OPDA ($F_{1, 46}$ =47.440; P<0.001), which increased by 486%, 72%, 192%, and 137% from the peak drought to drought recovery, respectively (Fig. 5.3; Table 5.3). ABA concentrations were significantly affected by water availability ($F_{1, 46}$ =9.673; P=0.004) and drought-stage ($F_{1, 46}$ =13.337; P=0.001) with concentrations in DS conditions 34.5% higher than in WW conditions, and 24% higher at peak drought than during drought recovery. The strongest effect on ABA was due to an interaction between water availability and drought-stage ($F_{1, 46}$ =35.703; P<0.001), which was seen as to 149% increase in ABA concentrations in WW plants and 14.5% decrease in DS plants from peak drought to drought recovery. SA concentrations were significantly affected by water availability ($F_{1, 46}$ =6.652; P=0.015) with 52% higher concentrations in WW conditions compared to DS. There were significant differences among isolines in OPDA ($F_{3, 44}$ =21.852; P<0.001) and JA ($F_{3, 44}$ =3.973; P=0.016), but the rank order of accessions from highest to lowest concentration was different: ABI5-1, WT, RAVI-1, ABI5-2 in the former, and ABI5-2, WT, ABI5-1, RAVI-1 in the latter.

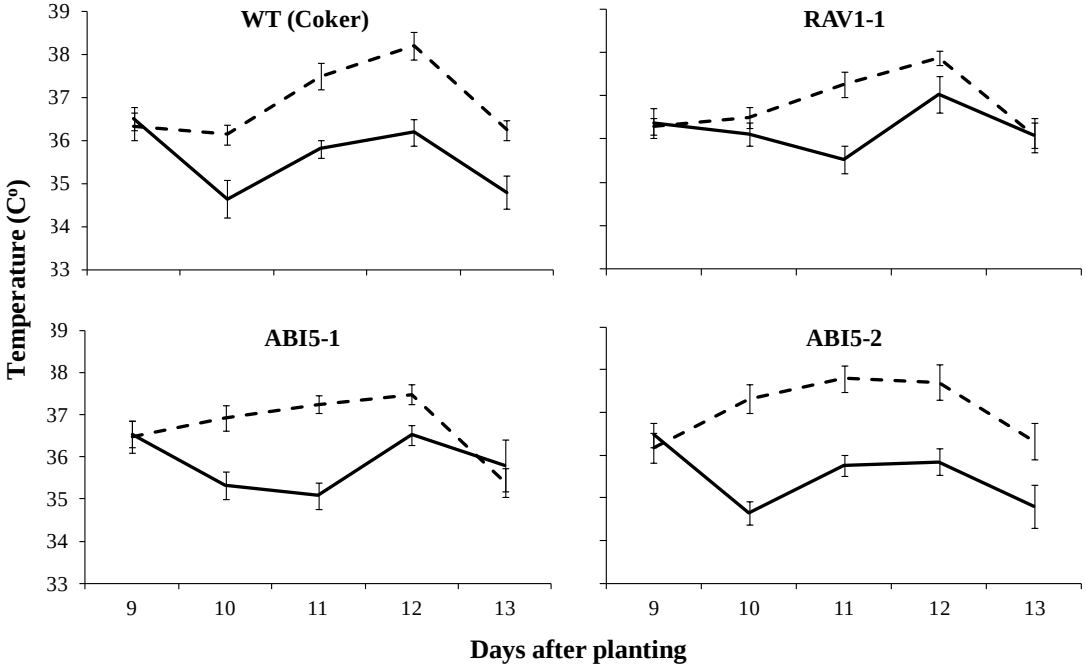

Figure 5.1. The effect of drought-stress and recovery on leaf surface temperatures (LST) of three transgenic cotton lines that overexpress ABA-related transcription factors and wild-type 'Coker 312' (*Gossypium hirsutum L.*). 'Well-watered' (solid lines) plants were given water 5-, 9-, and 12 DAP, whereas periodically drought-stress plants (dashed lines) were given water 5- and 12 DAP. Recovery was measured on 13 DAP. (Note: LSTs were measured prior to watering on 9- and 12 DAP). See Table 5.1. for results.

Table 5.2. Results of the effects of cotton isoline (I), water availability (WA), and days after planting (DAP) on leaf surface temperatures at pre-drought, drought, and recovery.

	Leaf surface temperature					
	Pre-drought (9 DAP)		Drought[2] (10-12 DAP)		Recovery (13 DAP)	
Source of variation	F	P	F	P	F	P
I	0.154	0.927	2.514	0.058	0.827	0.485
WA[1]	0.507	0.479	132.316	<0.0001	5.970	0.018
DAP			1.471	0.226		
I x WA	0.066	0.998	4.562	0.003	2.942	0.041
I x DAP			0.948	0.418		
WA x DAP			5.847	0.016		
DAP x WA x T			1.727	0.161		
N per trt combination	10-12		10-12		7-9	
Shapiro-Wilks	0.034		0.305		0.084	

[1]=Drought-stressed vs. Well watered
[2]=repeated measures ANOVA

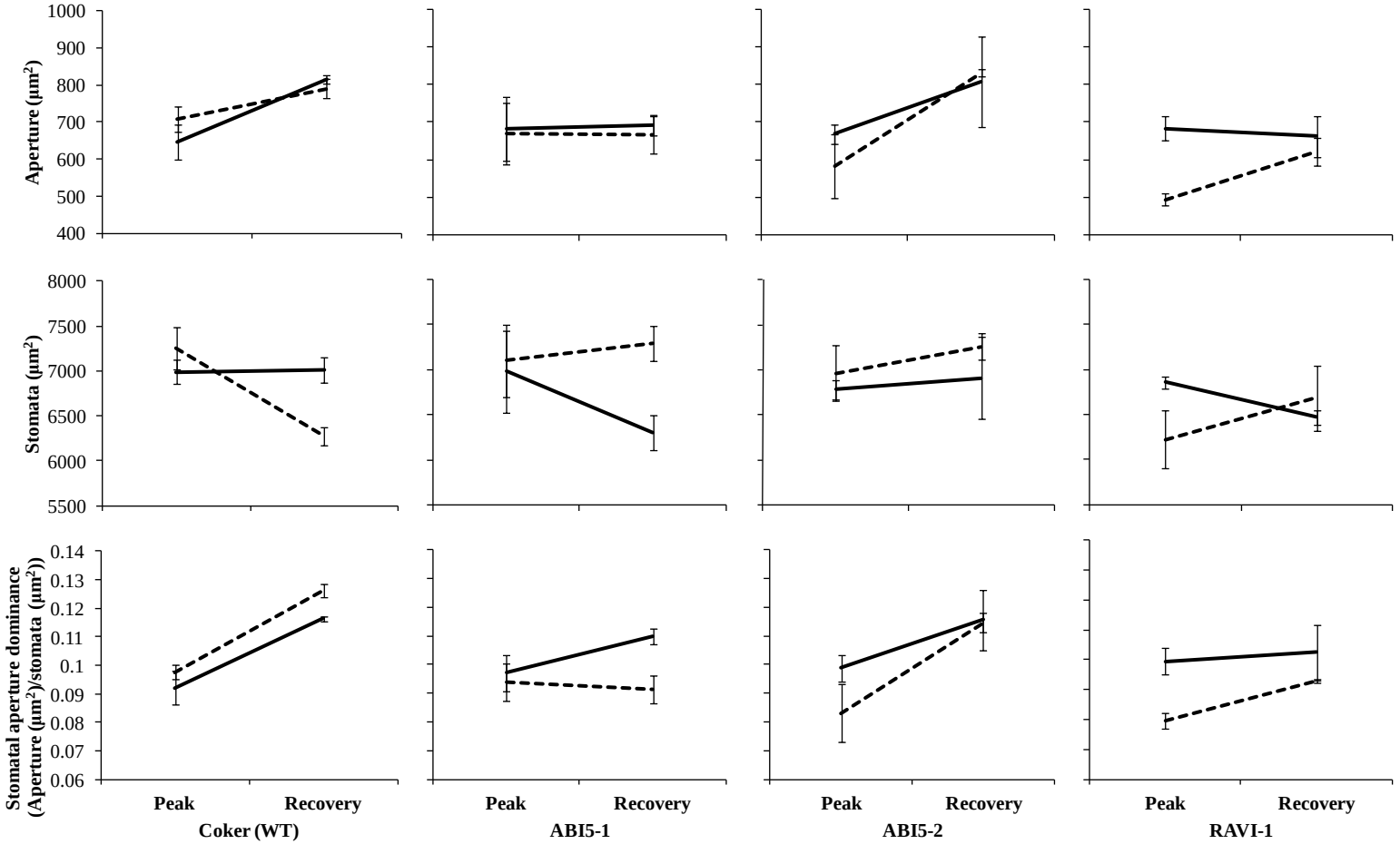

Figure 5.2. Reaction norms illustrating the effects of peak drought and recovery on stomatal aperture (µm^2), stomatal area (µm^2), and relative SAD (aperture (µm^2)/stomata (µm^2)) in the wild-type cotton cultivar 'Coker 312' and three transgenic isolines (ABI5-1, ABI5-2, and RAVI-1) (Table 5.2 for results).

Table 5.3. Results of the effects of cotton isoline, water availability, and drought stage on stomatal aperture (μm^2), stomatal area (μm^2), and relative stomatal aperture.

	Response variable					
	Aperture (μm^2)		Stomata (μm^2)		SAD (Aperture/Stomata (μm^2))	
Source of variation	F	P	F	P	F	P
Isoline (I)	3.567	0.025	1.902	0.15	4.284	0.012
Water availability (WA)[1]	1.419	0.246	0.548	0.465	4.923	0.034
Drought stage (DS)[2]	10.911	0.002	0.918	0.346	31.605	<0.001
I x WA	1.07	0.376	1.958	0.141	3.029	0.044
I x DS	2.191	0.109	1.398	0.262	3.64	0.023
Water x DS	0.571	0.455	0.899	0.35	0.374	0.545
I x WA x DS	0.893	0.456	2.451	0.082	1.368	0.271
Shapiro-Wilks	0.968		0.92		0.793	

Stomatal aperture dominance (SAD) (aperture (μm^2)/stomata (μm^2))
[1]Drought-stressed vs. Well watered
[2]Peak drought vs. Recovery

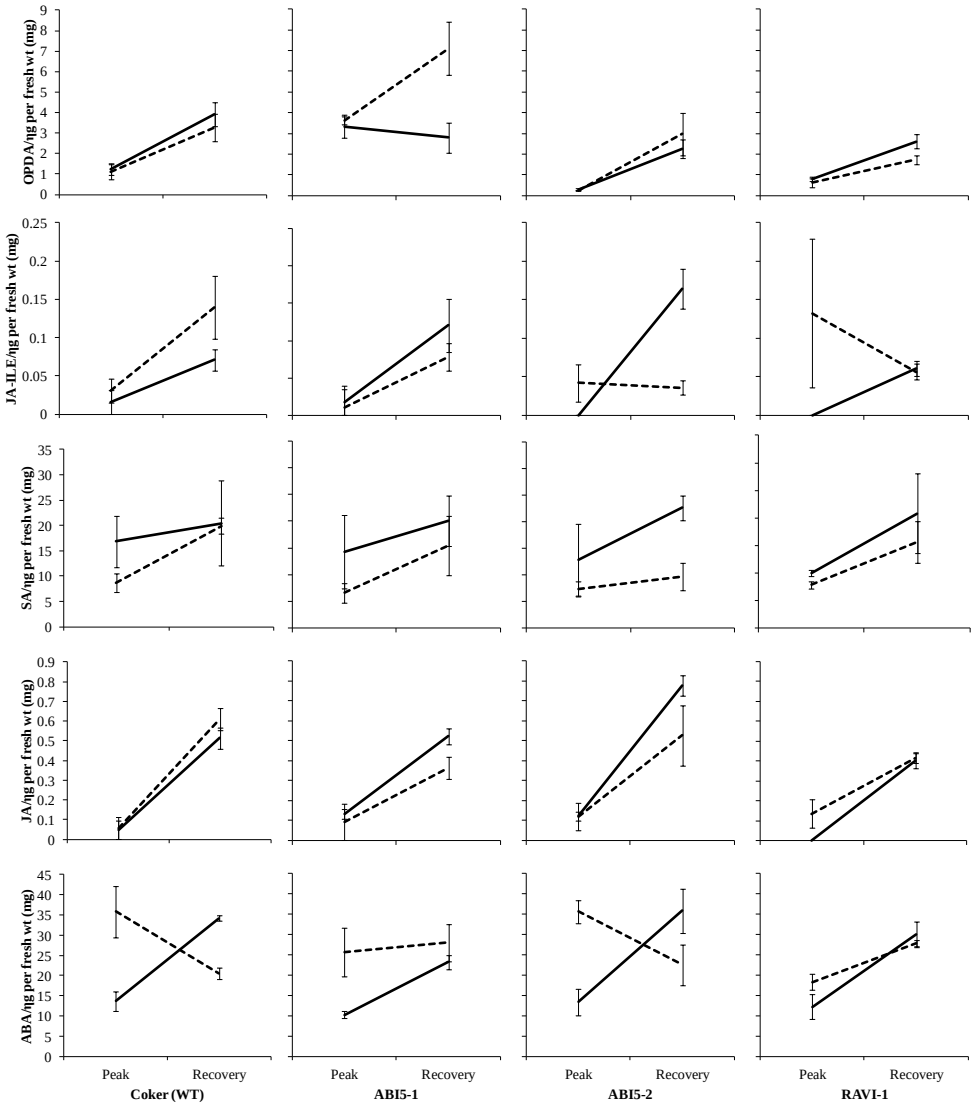

Figure 5.3. Reaction norms illustrating the effects of peak drought and recovery on OPDA, JA-Ile, SA, JA, and ABA in four isolines: wild-type cotton cultivar 'Coker 312' and three transgenic isolines (ABI5-1, ABI5-2, and RAVI-1) (see Table 5.3 for results).

Table 5.4. Results of the effects of cotton isoline, water availability, and drought stage on abundance of five plant hormones.

	Abscisic acid		Jasmonic acid		Salicylic acid		JA-ILE		OPDA	
Source of variation	F	P	F	P	F	P	F	P	F	P
Isoline (I)	1.803	0.167	3.973	0.016	0.434	0.730	0.040	0.989	21.852	<0.001
Water availability (WA)[1]	9.673	0.004	0.610	0.441	6.652	0.015	0.359	0.554	2.420	0.130
Drought stage (DS)[2]	13.337	0.001	186.557	<0.001	11.005	0.002	14.268	<0.001	47.440	<0.001
I x WA	0.893	0.456	2.721	0.061	0.300	0.825	2.659	0.065	5.399	0.004
I x DS	2.244	0.103	2.909	0.050	0.091	0.965	2.013	0.132	0.897	0.453
WA x DS	35.703	<0.001	2.509	0.123	0.001	0.997	5.264	0.028	2.519	0.122
I x WA x DS	4.071	0.015	1.244	0.310	0.543	0.657	2.561	0.072	3.807	0.019
Shapiro-Wilks	0.458		0.003		<0.001		<0.001		<0.001	

[1]Drought-stressed vs. Well watered
[2]Peak drought vs. Recovery

Discussion

This investigated the physiological responses of three transgenic isolines and the WT to drought-stress and recovery. The transgenes AtRAV1 and AtABI5 coded for transcription factors that are either regulated by mechanical or osmotic stress (AtRAV1) (Lee *et al*. 2010; Gutierrez *et al*. 2002) or mediate response to ABA (Finkelstein & Lynch 2000; Finkelstein *et al*. 2005). In particular, ABA has been shown to influence the shrinking and swelling of guard cells (Kim *et al*. 2010), which determines the size of the stomatal aperture and is the primary point of water loss in plants. Therefore, the expectation was that the transgenic isolines would exhibit hypersensitive responses to ABA, which would enhance drought-tolerance through reductions in stomata and/or stomatal apertures. My specific hypothesis was that under drought conditions the transgenic isolines would have lower levels of ABA than WT associated with a reduction in water loss through smaller stomatal apertures.

At peak drought (12 DAP), ABA levels in drought-stressed RAV1-1 were 48% lower than WT. While turgor pressure was not measure, these results suggest that RAV1-1 was less drought-stressed than WT. At the same time, the stomatal apertures of RAV1-1 were 27.7% smaller than WT, which suggests that less water escaped through the stomata in RAVI-1. Leaf surface temperatures were no different between RAVI-1 and WT in DS conditions, which suggest similar transpiration rates (water loss). I hypothesize that two different causes underlie this pattern: in WT elevated leaf surface temperatures under drought-stressed conditions were associated with the plants being drought-stressed and thus having less water was available to transpire, whereas in RAV1-1 plant water potential was higher than WT (inferred based on ABA levels) but transpiration rates were low due to smaller stomatal apertures reducing the rate of water loss. In general, results showed that RAVI-1 was the primary transgenic isoline to support the hypothesis that the transgenes would affect drought-tolerance through altered ABA sensitivity/responsive pathways.

Measuring SAD as a function of the pore apertures and stomatal area provided additional insight into the complexity of stomata behavior and potential plant traits

conferring drought tolerance in RAV1-1. Changes in stomata morphology due to genetic or environmental variation can fall into five categories: 1) smaller or larger aperture without concurrent changes in stomata size, resulting in lower or higher SAD values, respectively, 2) no change in aperture but smaller or larger stomata, resulting in higher or lower SAD values, respectively, 3) smaller or larger aperture accompanied by proportional changes in stomata, resulting in no change in SAD values, 4) smaller or larger aperture accompanied by disproportional changes in stomata, resulting in higher or lower SAD values, and 5) no change in either stomata or aperture, resulting in no change in SAD values. It was found that under drought-stressed conditions RAV1-1 had 27.7% and 16.3% smaller apertures and stomatal areas than WT, respectively, raising the possibility of either category three or four (above) to explain differences among WT and RAV1-1 isolines. Results of SAD indicated stomatal traits fell into category four. Accordingly, the size of the apertures relative to the size of the stomata (i.e., SAD) in RAV1-1was 18.5% smaller than WT, which shows that the basic blue-print of the stomata were considerably different between RAV1-1 and WT under drought-stress conditions.

Pleiotropic effects of individual genes on physiological properties

The transgenic isolines, which were similar except for the addition of one gene coding for ABA-responsive transcription factors, caused a number of pleiotropic affects independent of ABA concentrations. Stomatal apertures and SAD were significantly different among isolines, which show that these genes impacted the morphological of key physiological traits associated with the regulation of water, independent of ABA concentrations. It was also found that the isolines exhibited significant variation in the concentrations of two plant hormones, JA and OPDA. OPDA is precursor to JA (Katsir *et al*. 2008) and so it is interesting to note that the rank order of their concentrations among the accessions was neither positively nor inversely related.

Effects of water availability, drought-recovery, and their interaction on phytohormones

We found water availability, drought recovery, and the interaction between them was each associated with significant effects on phytohormones. Considering the effect of water availability alone, SA concentrations across peak drought and recovery were 52% higher in WW conditions compared to DS. These results from cotton plants in controlled conditions contrast with those of Munné-bosch & Peñuelas (2003) who found that SA increased up to 5-fold during drought in field-grown *Phillyra angustifolia* plants. JA, SA, JA-ILE, and OPDA increased from the peak drought to recovery by 486%, 72%, 192%, and 137%. Overall, these results highlight how the application of water can produce major changes over relatively short periods (24h) in the concentrations of important phytohormones. Notably, JA and SA are important regulators of plant defensive pathways against herbivores, and additional research is needed to determine the ecological consequences of short-term water availability on higher trophic levels. Lastly, the highly significant interaction between water availability and drought recovery on the concentrations of ABA was somewhat unexpected. Across isolines, drought-stressed plants had 14.5% lower ABA concentrations during drought recovery and a 42% lower concentration for WT in particular. These results could be expected assuming ABA levels correlate with plant water availability. However, across all WW isolines, ABA increased 149%, and increased 151% for WT, which is difficult to interpret in light of ABA as a drought-responsive hormone alone. These results highlight the need for additional research to understand the functional consequence of higher ABA concentrations in WW conditions.

CHAPTER VI

THE RELATIONSHIP BETWEEN PHYTOHORMONES, FITNESS, AND STATE-DEPENDENT REPRODUCTION FOR AN INCOME-BREEDER, WESTERN FLOWER THRIPS, *FRANKLINIELLA OCCIDENTALIS* PERGANDE

Introduction

Plants are exposed to numerous types of biotic and abiotic stress. To mitigate the deleterious effects of these stresses, plants have evolved defensive responses that are coordinated and partitioned by various phytohormones. For instance, the primary phytohormone that mediates responses to drought- and salt-stress is Abscisic acid (ABA), to herbivory and necrotrophic pathogens is Jasominc acid (JA), and to biotrophic pathogens is Salicylic acid (SA) (Stout *et al.* 2006; Howe & Jander 2008; Rock & Quatrano 2010; Pieterse *et al.* 2012). Emerging evidence indicates that the response of phytohormones can be linked (so-called 'cross-talk') such that induction of ABA, for example, and the resulting signal cascade is accompanied by alterations in other hormones such as JA and SA (Fujita *et al.* 2006; Pieterse *et al.* 2012). As a result of cross-talk, plant responses to an initial stress such as drought-stress can impact their susceptibility to subsequent, alternative stresses such as herbivory. Thus, one major goal in phytohormonal ecology is to understand how the response of phytohormones to drought-stress affects plants susceptibility to herbivores (Thaler & Bostock 2004).

Previous evidence of the effects of host-plant drought-stress on herbivores have been mixed. As a result, Huberty & Denno (2004) conducted a meta-analysis in an attempt to reconcile the mixed support for the traditionally held view that herbivorous insects respond positively to drought-stressed host-plants. A key conclusion was that the type of drought-stress imposed varied among studies with some investigating the effects of continuous drought-stress on herbivores and others the effects of periodic drought-stress (i.e., a period of drought followed by recovery). Consequently, they proposed the Pulsed-Stress Hypothesis, which posits that herbivores, particularly those in the sap-

sucking functional feeding guild such as aphids, respond positively to periods of drought-stress and recovery (Huberty & Denno 2004). The premise is that drought-stressed plants have elevated levels of nitrogen-containing compounds, e.g., proline (Verslues & Bray 2006), which can be accessed by sap-sucking insects only when the plant regains turgor pressure through drought-recovery. An alternative, but less widely considered, explanation for the mixed evidence supporting the effects of drought-stress on herbivores is the possibility that cross-talk in phytohormone responses associated with drought (i.e., ABA) alters resistance to herbivores (i.e., JA), and that such effects vary depending on the host-plant.

The impact of HPR on an herbivore's fitness can depend on the reproductive strategy and the life-stage of the herbivore being investigated (Awmack & Leather 2002). For example, reproductive strategies can be characterized as a dichotomy that involves income breeders on one end and capital breeders on the other (Stephens *et al.* 2009 for review). The reproductive potential of income breeding herbivores is generally considered a function of adult longevity along with the amount and quality of resources consumed during that stage. As a result, the effects of HPR on adult herbivores would be predicted to have a fairly immediate impact on the reproductive rates of income breeders because reproduction is largely fueled by food intake during that life-stage (Edward & Chapman 2011). On the other hand, reproduction of capital breeders is based on stockpiled energy reserves, acquired during immature development or during times of abundant resources (Stearns 1992; Stephens *et al.* 2009). Consequently, size would be predicted as an important determinant of reproductive success for capital breeders. However, in Chapter 3, it was shown that income versus capital breeding is not a strict dichotomy, but rather a continuum that can vary, even within a given life-stage, depending on the environmental conditions. By most criteria, WFT would be considered an income-breeder because adult-acquired resources, adult-longevity (living up to five weeks in laboratory conditions), and prevailing ecological conditions during the adult-life stage are known to impact life-time reproductive success (Reitz *et al.* 2009 and sources therein). Yet, in most cases examined in Chapter 3, size was a significant

predictor of fecundity, consistent with a capital breeding strategy. Notably, in the one case in which size was not a significant predictor of fecundity, WFT laid relatively more eggs, suggesting that state-dependent fecundity can be associated with poor environmental conditions. The phytohormone JA is known to have strong impacts on the feeding and reproduction of herbivores, including WFT (Abe *et al*. 2009) and thus represents a potential mechanism of HPR. However, the effects of JA on more subtle aspects of reproduction by an income-breeder such as causing shifts to state-dependent becoming an important determinant of reproductive success have not been previously investigated.

This study builds upon the results of Chapter 4 wherein it was found that four cotton (*Gossypium hirsutum* L.) lines consisting of three transgenics and an untransformed WT (Coker 312), showed considerable variation in phytohormone concentrations due to both pleiotropy from the transgenic events, as well as from the effects of drought-stress and recovery. Using plant material from the same experiments in Chapter 4, I then conducted no-choice bioassays on adult female WFT. I tested the working hypothesis that the physiological state of an income-breeder, as inferred based on the weight of WFT which could represent fat stores, immunity, etc., becomes increasingly important for reproductive success under challenging environmental conditions (McNamara & Houston 1996). Thus, the primary objectives of this study were three-fold: 1) to test the hypothesis that as total reproduction decreases, state-dependency will become increasingly important for reproductive success, 2) to explore whether phytohormones negatively correlate with feeding and fitness responses of WFT and positively correlated with state-dependent reproduction, and 3) to evaluate the results in light of the Pulsed-Stress Hypothesis (Huberty & Denno 2004).

Methods and materials

Plant material

No-choice bioassays of WFT feeding and reproductive performance were conducted using the same plant material that was investigated as described in Chapter 5. In brief, four cotton isolines were investigated in total, the untransformed wild-type (WT) variety 'Coker 312', and three transgenic lines with the following event numbers: RAV1-1 (42-1-1-5), ABI5-1 (38-1-1-1), and ABI5-2 (38-13-4-1). Cotton plants were cultivated in either periodically drought-stressed (PDS) or well-watered (WW) conditions. WW plants received 125ml of water on days 1, 5, 9, and 12 days after planting (DAP), whereas PDS plants received water on 1, 5, and 12 DAP. On 13 DAP, 24h after both WW and PDS treatments were irrigated, cotton cotyledons were harvested and used in thrips bioassays. Thus, eight treatment conditions were produced comprised of four cotton lines cultivated in either WW or PDS conditions. For each treatment condition, the following five phytohormones were quantitated as described in Chapter 5: ABA, JA, SA, JA-Ile, and OPDA.

Bioassay

A standardized Petri dish bioassay was used throughout the study to measure feeding and selected measures of WFT performance on plants subjected to the drought stress treatments described above. Accordingly, an individual adult female WFT was first weighed (Mettler-Toledo Ch-8606, Laboratory & Weighing Technologies, Greifensec, Switzerland), and then sealed in a 90-mm-diameter Petri dish with an excised cotton cotyledon for 3d. After three days, thrips were removed, re-weighed and the total area of feeding scars (mm^2) on the cotyledon was quantified. Thrips produce feeding scars characterized as silvery depressions that occur on the leaf surface and along the leaf edges, which can be quantified using a dissecting microscrope and a metric ruler (mm^2). Next, cotyledons were re-sealed in the Petri dish (without adult female WFT) for an additional three days to allow viable thrips larvae to hatch from eggs laid within the cotyledon (thrips in suborder Terebrantia lay eggs in leaf tissue) (Lewis 1997). Three days later, the number of live immature thrips (hereafter referred to

as ‘immatures’) on the cotyledon was quantified. Last, to quantify the number of eggs laid and hatched eggs, the cotyledons were stained with two solutions as per Backus *et al.* (1988). The first solution consisted of 0.2% acid fuchsin (the staining agent) mixed in 95% ethanol and glacial acetic acid (1:1 vol/vol). The second solution enhanced the cotyledons’ transparency and consisted of distilled water, 99% glycerine, and 85% lactic acid (1:1:1 vol/vol/vol). Thrips eggs were visualized with a dissecting microscope and were identified as kidney-shaped with hatched-eggs having a red transparent appearance and unhatched eggs appearing dark-red. If the adult female thrips were missing, died, or did not feed during the course of the experiment, the replicate was omitted from the analysis.

Path analysis

Path analysis was used to produce RAAPs of thrips under each treatment condition. Path analysis is a statistical method used to identify the structure of dependency among variables, and is particularly useful for studying the evolutionary ecology of adult organisms because it can account for the inter-dependent, hierarchical relationship among various life-history traits related to fitness. The method consists of multiple linear regression equations that describe hypothesized relationships among factors, and these equations are solved simultaneously using maximum likelihood methods. The global fit of the model can be evaluated using χ^2 goodness-of-fit. The fit of the models were also assessed with four common indices: NFI <0.95 is acceptable; CFI >0.95 is acceptable; RMSEA <0.05 is acceptable. Analyses were conducted using AMOS software (SPSS Inc.) (Arbuckle 2011).

Two types of RAAP were generated using path analyses which were identical in all respects except that one included the variable final weight whereas the other included weight gain (Fig. 6.1A and B, respectively). Both measures of weight could not be examined in the model simultaneously because they are perfectly linearly dependent resulting in positive definite matrices. For each path coefficient (n =14) and intercept (n = 5) in the RAAP, I used the unstandardized means to investigate for correlations with levels of selected phytohormones. To test for differences between RAAPs, the general

procedure was to first test measurement invariance of an unconstrained model comprised of the two models combined, and then for a model with the path coefficients and intercepts constrained to be equal between the groups. If the chi-square differences statistic was not significant for the original and constrained models then the path coefficients and intercepts were invariant between the models. If the chi-square differences statistic was significant for the constrained model implying non-invariance of path coefficients and intercepts between models, then post-hoc analysis were conducted to determine which of the specific path strengths and/or means were different. A Bonferroni correction was applied to alpha to correct for multiple comparisons between the WT and each of three transgenic isolines in WW and DS conditions, respectively (n=3 comparisons, adjusted $\alpha = 0.0167$).

Phytohormone correlation analyses

We used the phytohormone data to investigate correlations between the concentration of each phytohormone and each of 19 life-history characteristics (LHC) associated with thrips RAAPs. The correlations were based on the means associated with each treatment (n=8). Because each LHC was regressed against five phytohormones, alpha was adjusted using Bonferroni correction (n=5, adjusted $\alpha = 0.01$)

Results

While the fit of the path analysis model associated with all treatments combined was exceptional (NFI=1.000; CFI=1.000; RMSEA=1.000), there was considerable variation in the model fit of each treatment analyzed independently (Table 6.1). Constraining the path coefficients and intercepts resulted in three cases of significantly different RAAPs (Table 6.2). Two of the differences occurred when comparing the WT under PDS conditions to each of the cotton isolines ABI5-1 and ABI5-2 under PDS conditions (Fig. 6.1A and B). Post-hoc analyses of specific differences in path coefficients and means are reported in Table 6.3. The third difference in RAAPs was due to the effects of water availability (i.e. PDS vs WW) on the cotton isoline ABI5-2 (P=0.004) (Fig. 6.1A and B). Based on post-hoc analysis, these differences were associated with a shift from initial thrips weight (μg) being a significant predictor of eggs laid and hatched eggs in WW conditions to plant feeding (mm^2) being a significant predictor of those measures in PDS conditions. At the same time, significantly more eggs were laid and more eggs hatched in PDS conditions. A significant negative correlation was found between path coefficient, Initial weight → Hatched eggs and the intercept of Hatched eggs (r=-0.972, P<0.001; Fig. 6.2A). Similarly, a significant negative correlation was found between path coefficient, Initial weight → eggs laid and the intercept of eggs laid (r=-0.901, P=0.002; Fig. 6.2B). Taken together, these results indicate that as the initial weight of female WFT increasingly predicted the number of hatched eggs or total eggs laid, there were fewer total hatched eggs and eggs laid by that female, respectively.

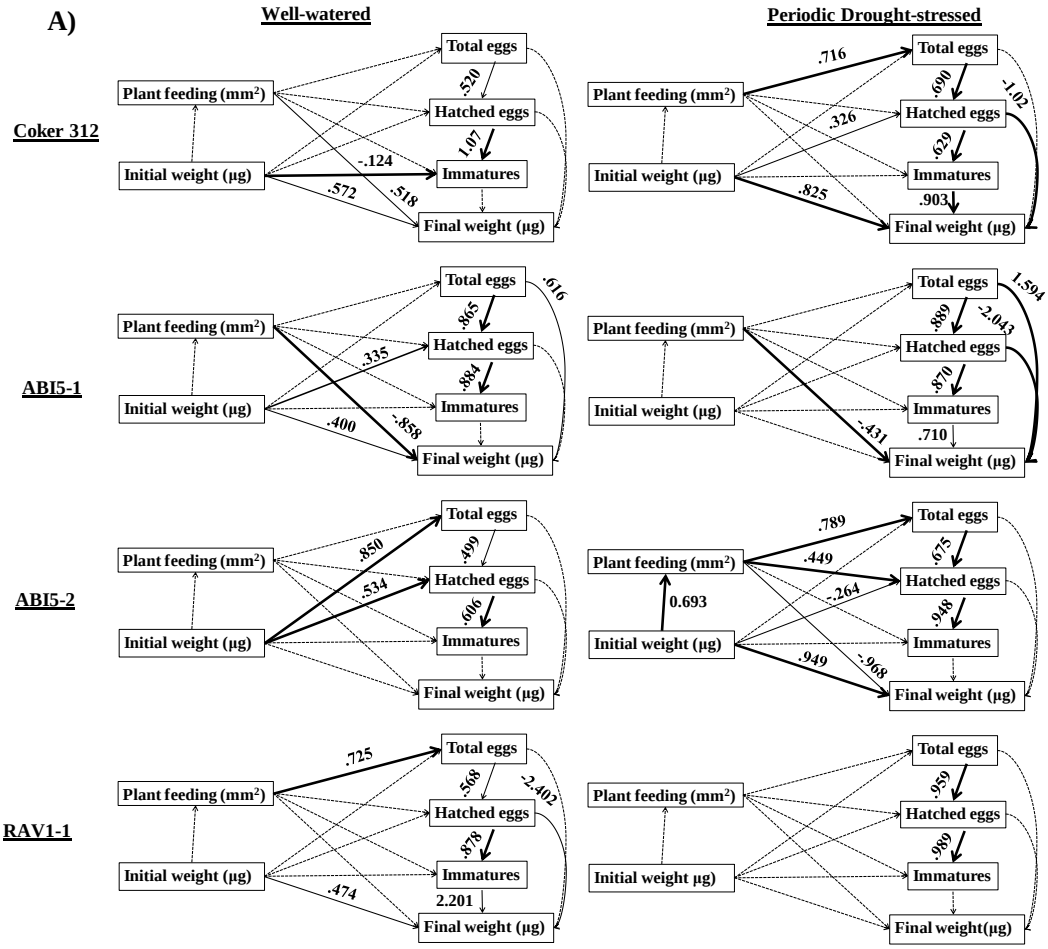

Figure 6.1. Resource acquisition and allocation patterns (RAAP) of Western Flower Thrips were generated for each of eight treatment conditions with path analysis to illustrate the effects of initial weight and plant feeding on four measures of performance: total eggs laid, hatched eggs, alive immatures, and either A) final weight or B) weight gain. Standardized path coefficients are shown for significant pathways, and insignificant paths are show with dotted arrows. Arrows are bolded for significant pathways based on the degree of significance (light bold: $0.01<P<0.05$; heavy bold: $P<0.01$). See Table 6.1 for details on the fit of each model.

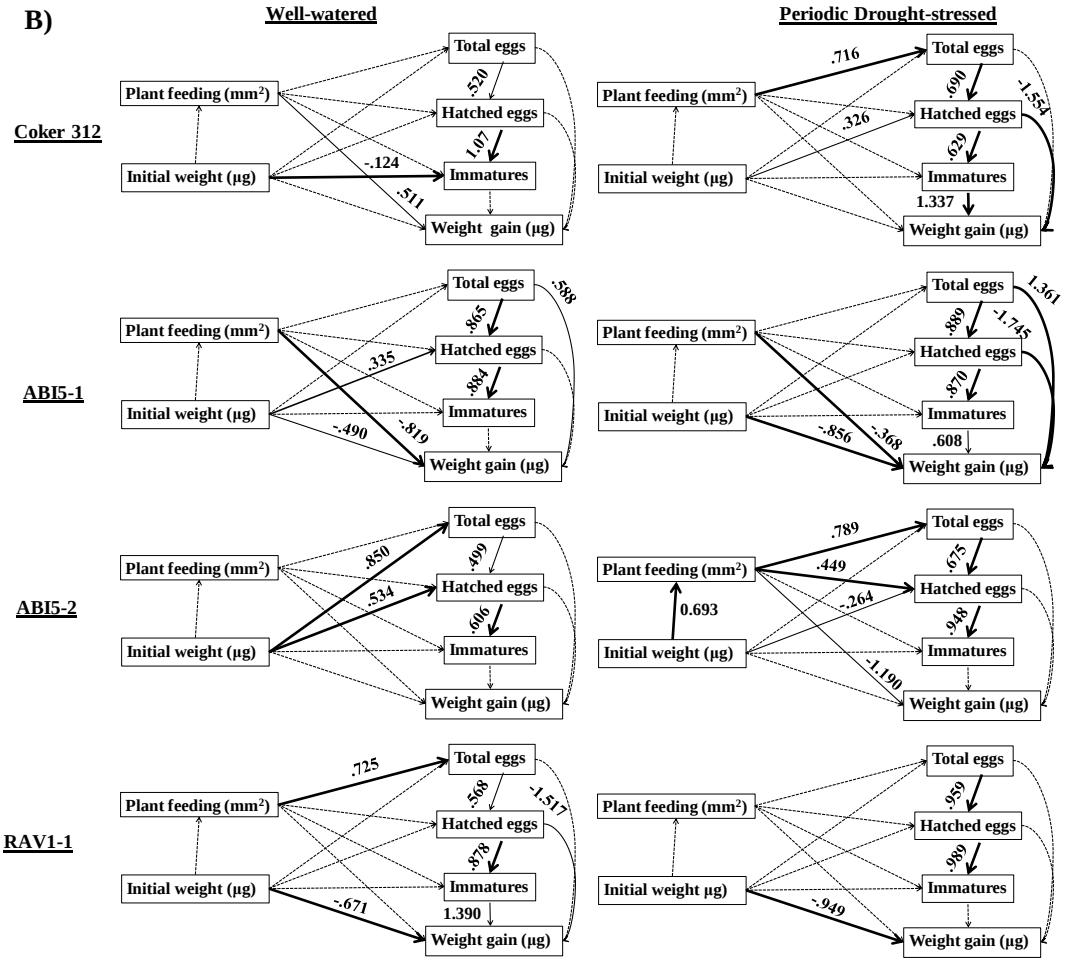

Figure 6.1. Continued.

Table 6.1. Results for each of eight path analyses showing effects of predictor variables on response variables (standardized path coefficient; *P* value) and indices assessing model fit (see Fig. 6.1A and B).

	Cotton Isolines								
	Coker 312 (WT)		**ABI5-1**		**ABI5-2**		**RAV1-1**		
Pathways	**WW**	**PDS**	**WW**	**PDS**	**WW**	**PDS**	**WW**	**PDS**	**Full model**
Initial wt (μg)→ Plant feeding (mm^2)	-0.13; 0.967	0.083; 0.773	-0.111; 0.723	-0.050; 0.869	0.362; 0.244	0.693; 0.002	0.053; 0.867	0.449; 0.112	0.207; 0.045
Plant feeding (mm^2) →Eggs laid	0.482; 0.057	0.716; <0.001	0.316; 0.294	-0.046; 0.879	0.038; 0.834	0.789; 0.005	0.725; <0.001	0.217; 0.518	0.439; <0.001
Initial wt (μg) →Eggs laid	0.256; 0.311	0.221; 0.235	-0.010; 0.975	-0.059; 0.845	0.850; <0.001	-0.022; 0.937	-0.011; 0.960	0.158; 0.637	0.206; 0.025
Plant feeding (mm^2) →Hatched eggs	0.344; 0.130	0.019; 0.993	-0.186; 0.237	-0.028; 0.815	-0.144; 0.181	0.449; 0.002	0.353; 0.123	-0.135; 0.245	-0.006; 0.927
Initial wt (μg) →Hatched eggs	.042; 0.838	0.326; 0.041	0.335; 0.024	-0.152; 0.197	0.534; 0.008	-0.264; 0.027	0.004; 0.982	0.077; 0.503	0.072; 0.215
Eggs laid →Hatched eggs	0.520; 0.027	0.690; 0.003	0.865; <0.001	0.899; <0.001	0.499; 0.012	0.675; <0.001	0.568; 0.015	0.959; <0.001	0.828; <0.001
Plant feeding (mm^2) →Immatures	-0.006; 0.915	0.282; 0.107	0.059; 0.718	0.123; 0.434	-0.032; 0.704	0.010; 0.965	0.126; 0.204	0.001; 0.982	0.042; 0.262
Initial wt (μg) →Immatures	-0.124; 0.004	0.114; 0.506	-0.125; 0.469	0.155; 0.337	0.397; 0.051	0.000; 0.999	-0.099; 0.121	0.003; 0.950	-0.038; 0.295
Hatched eggs →Immatures	1.007; <0.001	0.629; 0.002	0.884; <0.001	0.870; <0.001	0.606; 0.002	0.948; <0.001	0.878; <0.001	0.989; <0.001	0.939; <0.001

Table 6.1. Continued.

Pathways including final weight (µg)	Cotton Isolines: Coker 312 (WT) WW	Coker 312 (WT) PDS	ABI5-1 WW	ABI5-1 PDS	ABI5-2 WW	ABI5-2 PDS	RAV1-1 WW	RAV1-1 PDS	Full model
Plant feeding (mm^2) → Final wt (µg)	0.518; 0.037	-0.288; 0.073	-0.858; <0.001	-0.431; 0.007	-0.190; 0.518	-0.968; 0.017	0.422; 0.270	0.241; 0.387	-0.132; 0.207
Initial wt (µg) → Final wt (µg)	0.572; 0.033	0.825; <0.001	0.400; 0.023	-0.023; 0.896	0.819; 0.302	0.949; <0.001	0.474; 0.055	0.395; 0.144	0.464; <0.001
Eggs laid → Final wt (µg)	-0.476; 0.090	0.372; 0.069	0.616; 0.038	1.594; <0.001	0.870; 0.177	-0.863; 0.058	0.083; 0.838	-0.658; 0.374	0.220; 0.216
Hatched eggs → Final wt (µg)	0.309; 0.830	-1.02; <0.001	-0.064; 0.867	-2.043; <0.001	-1.448; 0.171	1.353; 0.075	-2.402; 0.023	-0.898; 0.621	-0.333; 0.286
Immatures → Final wt (µg)	-0.632; 0.652	0.903; <0.001	-0.297; 0.274	0.710; 0.018	0.337; 0.730	0.513; 0.334	2.201; 0.045	1.715; 0.308	0.300; 0.280

Table 6.1. Continued.

Pathways including final weight (µg)	Cotton Isolines: Coker 312 (WT) WW	Coker 312 (WT) PDS	ABI5-1 WW	ABI5-1 PDS	ABI5-2 WW	ABI5-2 PDS	RAV1-1 WW	RAV1-1 PDS	Full model
Plant feeding (mm^2)	0.511;	-0.439;	-0.819;	-0.368;	-0.164;	-1.190;	0.266;	0.151;	-0.124;
→Wt gain (µg)	0.038	0.074	<0.001	0.008	0.517	0.017	0.269	0.395	0.207
Initial wt (µg→	-0.391;	-0.334;	-0.490;	-0.856;	-0.441;	0.091;	-0.671;	-0.949;	-0.571;
Wt gain (µg)	0.141	0.081	0.003	<0.001	0.518	0.778	<0.001	<0.001	<0.001
Eggs laid→	-0.469;	0.567;	0.588;	1.361;	0.748;	-1.061;	0.052;	-0.413;	0.207;
Wt gain (µg)	0.091	0.070	0.037	<0.001	0.176	0.059	0.837	0.372	0.216
Hatched eggs→	0.304;	-1.554;	-0.061;	-1.745;	-1.246;	1.663;	-1.517;	-0.563;	-0.314;
Wt gain (µg)	0.831	<0.001	0.867	<0.001	0.170	0.076	0.023	0.620	0.286
Immatures→	-0.623;	1.337;	-0.284;	0.607;	0.324;	0.630;	1.390;	1.075;	0.282;
Wt gain (µg)	0.652	<0.001	0.273	0.018	0.729	0.335	0.044	0.306	0.280
Summary of model									
χ^2	0.517	0.432	0.007	0.222	0.941	0.033	0.903	0.406	0.710
NFI	0.994	0.991	0.864	0.970	1.000	0.949	1.000	0.990	1.000
CFI	1.000	1.000	0.838	0.986	1.000	0.949	1.000	1.000	1.000
RMSEA	0.000	0.000	0.796	0.212	0.000	0.595	0.000	0.000	0.000
C.R. for kurtosis	0.070	-0.163	0.407	-0.209	-0.301	-0.235	0.225	0.147	2.665
N (d.f.)	12 (1)	13 (1)	11 (1)	12 (1)	10 (1)	11 (1)	11 (1)	11 (1)	91 (1)

Standardized path coefficients are shown, which represent the change in the response variable given a standard deviation change in the predictor. NFI <0.95 is acceptable; CFI >0.95 is acceptable; RMSEA <0.05 is acceptable.

Table 6.2. Results of constrained and unconstrained models representing resource acquisition and allocation patterns of adult female thrips when confined to cotton isolines (Coker 312 [wild-type]), ABI5-1, ABI5-2, and RAV1-1) that were well-watered (WW) or periodically drought-stressed (PDS).

	χ^2 goodness-of-fit test (*P* value)		
Treatment Comparison	Unconstrained model	Model with constrained path coefficients and means ($\pi_1=\pi_2$)	Specific pathways (P<0.05)
PDS vs WW (α=0.05)			
Coker 312 (WT)	0.596	0.559	NA
ABI5-1	0.012	0.082	NA
ABI5-2	0.104	0.006	Plant feeding →Eggs laid; Plant feeding →Hatched eggs; Plant feeding →Final wt & Wt gain; Initial wt →Eggs laid; Initial wt →Hatched eggs; Eggs laid→Final wt & Wt gain; Hatched eggs →Final wt & Wt gain; Eggs laid (PDS more); Hatched eggs (PDS more)
RAV1-1	0.703	0.479	NA
Effects of transgene (WW) (α=0.0167)			
Coker 312 (WT) vs. ABI5-1	0.020	0.068	NA
Coker 312 (WT) vs. ABI5-2	0.810	0.028	NA
Coker 312 (WT) vs. RAV1-1	0.805	0.831	NA
Effects of transgene (PDS) (α=0.0167)			
Coker 312 (WT) vs. ABI5-1	0.348	0.004	Plant feeding →Eggs laid; Initial wt →Hatched eggs; Initial wt →Final wt & Wt gain; Eggs laid →Final wt; Final wt & Wt gain ABI5-1 more)
Coker 312 (WT) vs. ABI5-2	0.075	0.008	Initial wt →Plant feeding; Initial wt →Hatched eggs; Eggs laid →Final wt & Wt gain; Hatched eggs →Final wt & Wt gain; Plant feeding (WT more)
Coker 312 (WT) vs. RAV1-1	0.520	0.047	NA

Significant positive correlations were found between JA and JA-Ile (R^2=0.589; *P*=0.026), and a marginally significant positive correlation between SA and JA-Ile (R^2=0.489; *P*=0.053) (Figure 6.3A and B; Table 6.3). As such, at unadjusted levels of alpha (α=0.05), the majority of instances of significant correlations between phytohormones and LHCs involved multiple phytohormones correlated to the same LHC (7 out of 9) (Table 6.4). For instance, all three phytohormones were negatively correlated with hatched eggs. Figure 6.4 reports the phytohormones that most strongly correlated with selected LHCs, which were JA and JA-Ile. In these cases, three correlations were below the adjusted alpha levels (*P*<0.01) (Fig. 6.4A,C,D), and two correlations approached significant (*P*=0.011-0.013) (Fig. 6.4B,E). Accordingly, JA was negatively correlated with the path coefficient, Hatched eggs → Immatures (R^2=0.824; *P*=0.002; Fig. 6.4A), positively correlated with the path coefficient, Initial weight → Eggs laid (R^2=0.685; *P*=0.011; Fig. 6.4B), and negatively correlated with the intercept of Eggs laid (R^2=0.802; *P*=0.002; Fig. 6.4C). JA-Ile was positively correlated with the path coefficient, Initial weight → Hatched eggs (R^2=0.780; *P*=0.003; Fig. 6.4D), and negatively correlated with the intercept of Hatched eggs (R^2=0.67; *P*=0.013; Fig. 6.4E).

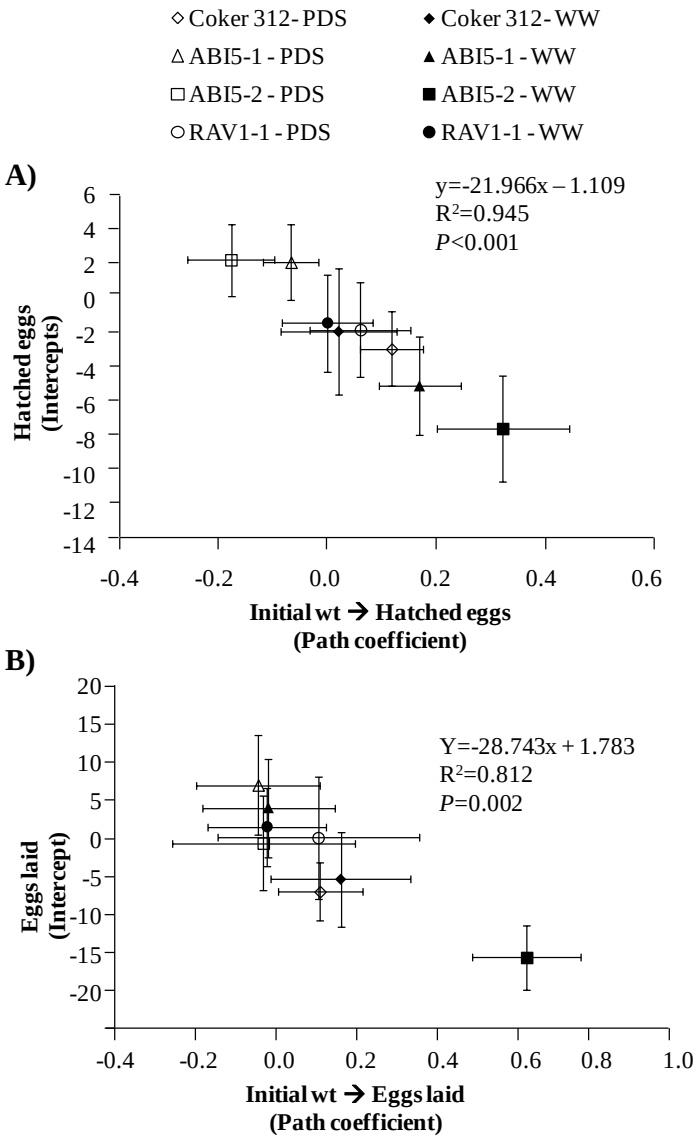

Figure 6.2. Negative correlations between state-dependent reproduction and fecundity and fertility, respectively. A) As the initial weight of female Western Flower Thrips increasingly predicted the number of A) hatched eggs and B) total eggs laid, there were fewer total hatched eggs and eggs laid by that female, respectively.

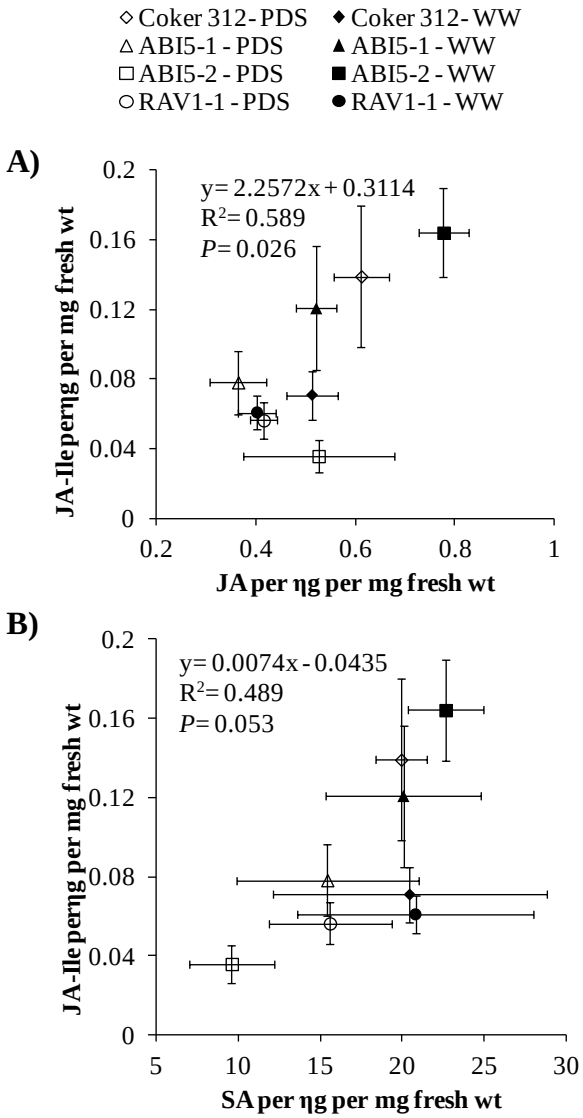

Figure 6.3. Positive correlations between A) Jasonmic acid and Jasmonic acid-isoleucine, and B) Salicylic acid and Jasmonic acid-isoleucine in four transgenic cotton isolines that were cultivated in either well-watered (WW) or periodic drought-stressed (PDS) conditions (see legend).

Table 6.3. Regression results of correlations between each of five phytohormones.

	Abscisic acid		Jasmonic acid		Salicylic acid		Jasmonic acid-isoleucine	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Abscisic acid	-	-						
Jasmonic acid	0.202	0.632	-	-				
Salicylic acid	0.488	0.220	0.412	0.310	-	-		
Jasmonic acid-isoleucine	0.120	0.777	0.768	0.026	0.700	0.053	-	-
OPDA[1]	-0.023	0.957	-0.406	0.318	-0.194	0.645	-0.115	0.786

[1]12-oxo-phytodienoic acid

Table 6.4. Regression results of five phytohormones as predictors of 19 WFT life-history characteristics.

Life-history characteristics	ABA		JA		SA		JA-Ile		OPDA	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Initial wt (μm) → Plant feeding (mm^2)	0.012	0.970	0.437	0.289	-0.393	0.336	-0.158	0.709	-0.656	0.078
Plant feeding (mm^2) →Eggs laid	-0.563	0.146	0.091	0.830	-0.232	0.580	-0.203	0.629	-0.280	0.501
Initial wt (μm) →Eggs laid	0.646	0.084	0.828	0.011*	0.523	0.181	0.667	0.072	-0.343	0.406
Plant feeding (mm^2) →Hatched eggs	-0.060	0.888	-0.087	0.837	-0.410	0.313	-0.552	0.156	0.096	0.819
Initial wt (μm) →Hatched eggs	0.355	0.389	0.696	0.055	0.799	0.017*	0.885	0.003**	-0.407	0.316
Eggs laid →Hatched eggs	-0.302	0.467	-0.528	0.178	-0.405	0.320	-0.286	0.493	0.030	0.943
Plant feeding →Immatures (mm2)	-0.520	0.186	0.043	0.919	0.175	0.679	0.393	0.336	0.309	0.456
Initial wt (μm) →immatures	0.338	0.413	0.618	0.102	0.141	0.739	0.594	0.120	0.083	0.845
Hatched eggs →Immatures	-0.071	0.868	-0.908	0.002**	-0.341	0.408	-0.805	0.016*	0.296	0.477
Plant feeding (mm^2) → Final wt or wt gain (μm)	0.724	0.042*	-0.066	0.877	0.501	0.206	-0.103	0.809	-0.201	0.633
Initial wt (μm) →Final wt or wt gain (μm)	-0.303	0.466	0.598	0.117	-0.213	0.612	0.082	0.847	-0.405	0.320
Eggs laid →Final wt or wt gain (μm)	0.003	0.995	-0.129	0.760	0.219	0.602	0.422	0.298	0.692	0.057
Hatched eggs →Final wt or wt gain (μm)	-0.142	0.738	0.205	0.626	-0.382	0.350	-0.326	0.431	-0.507	0.199
Immatures → Final wt or wt gain (μm)	-0.300	0.470	-0.216	0.607	-0.147	0.729	-0.073	0.864	0.036	0.932
Plant feeding (mm^2)	0.090	0.832	-0.289	0.486	0.604	0.113	0.301	0.468	0.458	0.254
Eggs laid	-0.436	0.281	-0.896	0.003**	-0.463	0.248	-0.602	0.114	0.449	0.264
Hatched eggs	-0.348	0.400	-0.723	0.043*	-0.802	0.017*	-0.819	0.013*	0.546	0.161
Immatures	-0.173	0.682	-0.410	0.313	-0.067	0.875	-0.535	0.172	-0.334	0.418
Final wt or wt gain (μm)	0.071	0.868	-0.67	0.669	0.05	0.907	-0.136	0.747	0.439	0.276
Total significant (P<0.05)		1		4		2		3		0
Total significant (adjusted P<0.01)[1]		0		2		0		1		0

[1]Bonferroni adjusted: α / n=0.050/5= 0.010

*=P<0.05, **=P<0.01

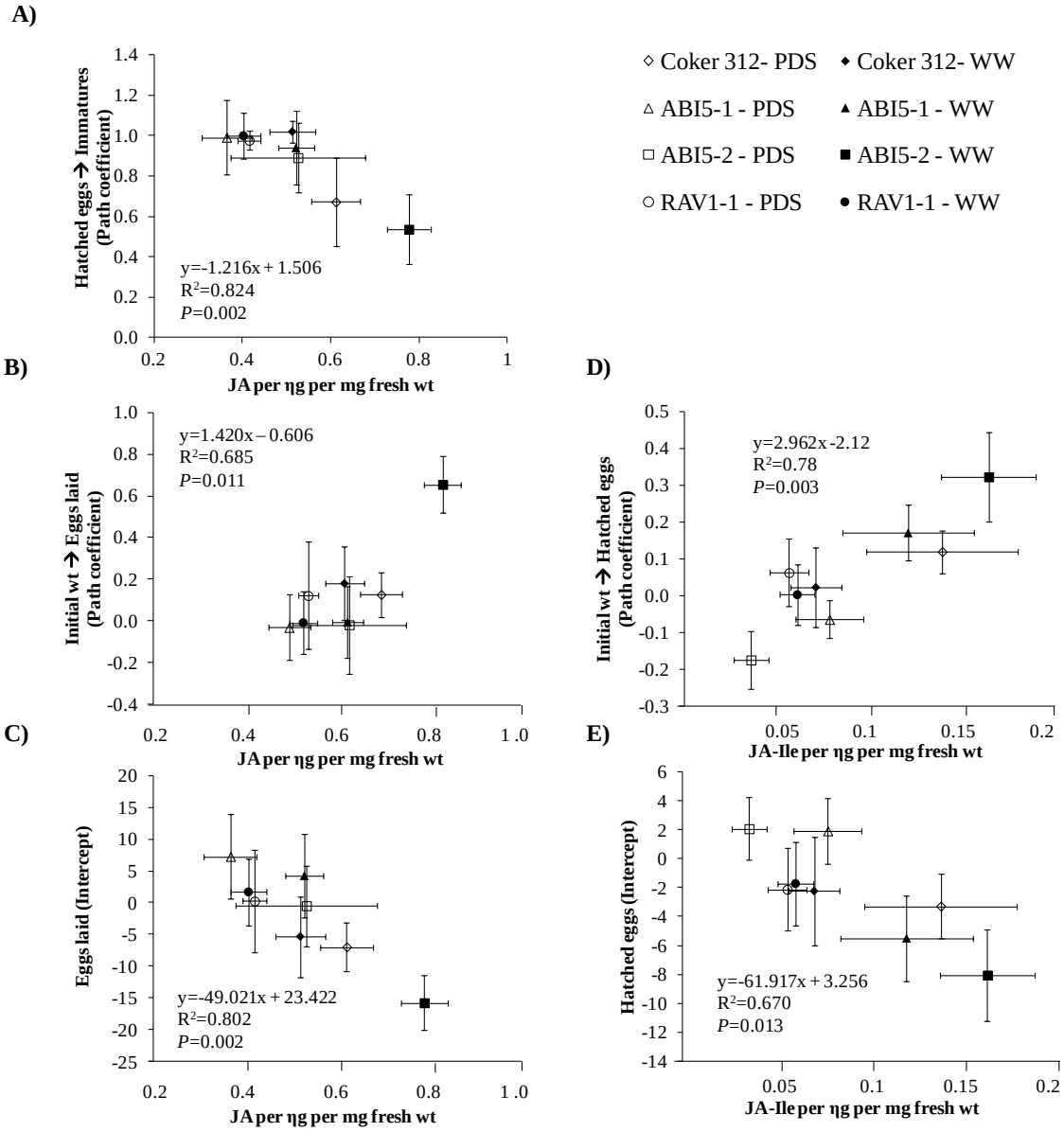

Figure 6.4. Correlations between Jasmonic acid (A-C) or Jasmonic acid-isoleucine (D-E) and select life-history characteristics of Western Flower Thrips. WFT were confined in eight no-choice treatment conditions with cotton cotyledons derived from one of four transgenic isolines that were cultivated in either well-watered (WW) or periodically ('pulsed') drought-stressed (PDS) conditions (see legend). Life-history characteristics shown above were derived from path analyses of each treatment condition.

Discussion

Because adult organisms have many inter-related LHCs that can be affected by environmental variation, the feeding and fitness responses of WFT associated with each of eight treatments were modeled using path analyses to produce RAAPs (Chapter 3). RAAPs were significantly different for three treatment comparisons, indicating that the pattern of resource allocation to, and interactions between, LHCs were altered due to these conditions (Fig. 6.1; Table 6.2). First, RAAPs of thrips were different between WW and PDS conditions on ABI5-2 and was associated with fecundity (eggs laid) and fertility (hatched eggs) (Awmack and Leather 2002) in WW conditions being strongly predicted by the state (weight) of WFT. In contrast, in PDS conditions those measures of reproduction were predicted by resource consumption (i.e., resource-dependent) (Fig. 6.1). At the same time, thrips had higher total fecundity (i.e, eggs laid) and fertility (i.e. hatched eggs) in PDS conditions (Table 6.2). Shifts to state-dependent reproduction with concurrent reductions in reproductive success agree with the results of Chapter 3. Furthermore, there was a significant negative correlation in state-dependent fertility and total fertility (Fig. 6.2A), and state-dependent fecundity and total fecundity (Fig. 6.2B). Notably, the WW and PDS conditions of ABI5-2 were associated with the extreme lower and upper points of the aforementioned correlation, respectively. These results supported the hypothesis that for an income-breeder, state-dependent reproduction is associated with challenging environmental conditions that negatively impact reproductive success.

The other two cases of significantly different RAAPs were associated with PDS conditions only and involved the WT conditions being different than each of ABI5-1 and ABI5-2 (Table 6.2). Accordingly, the final weight of thrips was higher on ABI5-1 than on the WT. Since the weight of thrips was not a significant predictor of the number of eggs laid on WT or ABI5-1, indicating large and small thrips laid similar numbers of eggs, I tentatively interpreted the higher final weight on ABI5-1 as enhanced somatic growth. Notably, egg fertility was state-dependent on WT, but not on ABI5-1, which together with enhanced somatic growth on ABI5-1, supports the hypothesis that state-

dependent reproduction for income-breeders is associated with relatively poorer environmental conditions. Considering WT and ABI5-2, ABI5-2 was a higher quality resource per unit of feeding because WFT fed more on WT, but there were no differences in any of the measures of reproduction or final weight. Again, state-dependent fertility was significantly higher on WT. Based on the state-dependent reproduction hypothesis, I would predict that over a longer experimental period, fitness effects on thrips would become evident in the WT conditions.

The second objective of this study was to explore whether phytohormones negatively correlate with feeding and fitness responses of WFT and positively correlated with state-dependent reproduction. Results showed that the concentrations of JA-Ile and JA were positively correlated with state-dependent fertility (Fig. 6.4D) and fecundity (Fig. 6.4B), respectively, and negatively correlated with total fertility (Fig. 6.4E) and fecundity (Fig. 6.4C), respectively. These results support the hypothesis that JA and JA-Ile underlie the negative effects on WFT reproduction and the associated shift to state-dependent reproductive success. An additional indication of the negative effects of JA on relative fitness of WFT was evidenced by the negative correlation between JA and offspring recruitment, i.e., the path coefficient, Hatched eggs → Immatures (Fig. 6.4A). Negative effects of JA on WFT fitness are in general agreement with those of Abe *et al.* (2009) who showed that JA negatively impacts WFT feeding, reproduction, and population growth using *Arabidopsis* JA-insensitive *coi1-1* mutants and JA exogenous applications.

It is interesting to note that JA and JA-Ile were each associated with impacts on a particular aspect of thrips reproduction (i.e., fecundity and fertility). Because JA and JA-Ile, and JA-Ile and SA exhibited positive cross-talk (Fig. 6.2), it is difficult to rule out the possibility that the combined effects of these phytohormones (either additively or non-additively) may have subtly influenced the observed impacts on thrips fitness. That there was no correlation between JA and SA stands in contrast to a large body of evidence showing indicating JA and SA are antagonistic and exhibit negative cross-talk (Pieterse *et al.* 2012; Thaler *et al.* 2012). Similarly, there was no correlation between

ABA and JA, or ABA and SA whereas previous evidence has shown negative cross-talk between those hormones (Fujita *et al.* 2006). Two possible reasons exist for this discrepancy: 1) the positive correlations were largely attributed to the behavior of the transgenic isolines in WW and PDS, suggesting that the transgenic events may underlie the observed positive responses, and/or 2) to the best of my knowledge, cotton has not been previously investigated for phytohormonal cross-talk, which could indicate that different plant species exhibit unique patterns of cross-talk.

Our results showing that phytohormone concentrations were altered due to periodic-drought stress and that such changes were correlated with relative fitness impacts on WFT has implications on the 'Pulsed-stress hypothesis' (Huberty & Denno 2004). In this study, there was no consistent effect of PDS on WFT, which does not necessarily refute the Pulsed-stress hypothesis because changes in available nitrogen were not measured. Regardless, these results bring to light an additional possible reason for the inconsistent response of herbivores to drought-stress host-plants, which is that drought-stress differentially affects the concentrations of phytohormones depending on the host-plant, or in the present case depending on the isoline, which cascades to differentially affect herbivores. Future research testing this hypothesis may shed light on why the responses of herbivores to drought-stressed host-plants have been mixed and so difficult to predict.

CHAPTER VII

CONCLUSIONS

Herbivory by WFT and drought-stress due to limited water availability are currently two major factors that can severely impact cotton production on the THP and throughout the cotton belt. This dissertation is a contribution to development of biological strategies that reduce the deleterious effects of herbivory and drought-stress on cotton. In particular, the effects of and potential mechanisms underlying HPR and drought-stress in cotton were investigated. An understanding of the mechanisms that functionally contributes to the drought-tolerant/herbivore-resistant phenotype can lead to 'stacking' of mechanisms, more efficient cultivar screening programs and use of seed bank resources, and is key to maintaining a proactive rather than reactive approach in the development of new elite cultivars.

In Chapter 3, a tool was developed that provides a new level of clarity in terms of how environmental variation impacts adult female WFT. Adult organisms have many life history characteristics (LHC) that can be affected by environmental variation. Theory and empirical evidence both suggest that the response of any one adult LHC can be dependent on resource acquisition and allocation to other LHCs, the organism's current physiological state, the response of other LHCs 'up-stream' from the focal LHC (eg: eggs laid, hatched egg), and the type of reproductive strategy (eg: income vs capital breeding). I presented a life-history systems-approach for empirical data that accounts for how interactions among component life-history characteristics can produce emergent effects on adult fitness. The approach was illustrated by investigating the combined effects of plant genotype, plant drought-stress, and prey availability on the LHCs of individual adult female omnivorous thrips using a factorial design. Resulting path models were significantly different in three cases, none of which were predicted based on prevailing ecological-hypotheses. This systems-approach produced three novel insights: (1) LHCs were differentially susceptible to environmental effects, and no

consistent combination of LHCs was affected, (2) physiological trade-offs were evident under stressful conditions, (3) the income versus capital breeding is not a strict dichotomy, but rather a continuum that can vary, even within a given life-stage, depending on the environmental conditions.

Chapter 4 investigated the factors underlying differential feeding on the adaxial ("upper-surface") versus abaxial ("lower-surface") leaf surface of cotton cotyledon by the WFT. WFT showed a significant aversion to adaxial-feeding even when excised-cotyledons were flipped up-side (abaxial-side 'up'), suggesting that mechanisms related to the orientation of the leaf, i.e., negative-phototropism and/or positive-geotaxis, were not a primary cause of thrips foraging patterns. No-choice bioassays showed that adult female WFT produced fewer immature thrips when confined to the adaxial surface, which could be attributed to, in part, a reduction in the quantity of resources consumed from that leaf surface. To test the hypothesis that leaf biomechanical properties inhibited thrips feeding on the adaxial surface, a penetrometer was used to measure two variables related to the 'toughness' of each leaf surface. Neither variable negatively co-varied with feeding.

Chapter 5 compared physiological responses to drought stress and drought recovery of three transgenic cotton isolines relative to the wildtype (*Gossypium hirsutum* L. cv Coker312). The transgenes were the *Arabidopsis thaliana* transcription factors involved in the expression of ABA-Insensitive3/Vivivparous1 (AtRAV1) and ABA-Insensitive5 (AtABI5) over-expressed under control of the CaMV 35S promoter. At peak drought, ABA levels, stomatal area, and stomatal apertures in drought-stressed AtRAV1-1 were 48% lower, 27.7%, and 16.3% smaller than WT. These results suggest that the isoline AtRAV1 was the most drought-tolerant and supports the hypothesis that the transgenes affected drought-tolerance through altered ABA sensitivity/responsive pathways. It was also found that SAD measures of AtRAV1-1 were 18.5% smaller than WT, indicating disproportionate changes in stomatal apertures associated with the AtRAV1-1 transgene. Based on the known role of stomata in mediating water loss in plants, these results support the hypothesis that the changes in stomatal morphology in

the isoline AtRAV1 may be an important phenotype that functionally contributed to drought-tolerance. Results also showed that the amount and timing of water availability altered major phytohormones associated with plant responses to both biotic and abiotic stress.

In Chapter 6 I investigated whether changes in phytohormone concentrations associated with periodic-drought stress in the four cotton isolines studied in Chapter 5 were correlated with feeding, fitness and state-dependent reproductive responses of WFT. Across drought-stress treatments, significant negative correlations were found between state-dependent fertility (i.e., hatched eggs) and total fertility, and state-dependent fecundity (i.e., total eggs laid) and total fecundity, which supported previous observations in Chapter 3 showing that state-dependent reproduction for an income-breeder is associated with relatively poor environmental conditions. Results showed that the concentrations of JA-Ile and JA were positively correlated with state-dependent fertility and fecundity, respectively, and negatively correlated with total fertility and fecundity, respectively. These results support the hypothesis that JA and JA-Ile underlie the negative effects on WFT reproduction and the associated shift to state-dependent reproduction. I proposed an additional hypothesis for the inconsistent response of herbivores to drought-stress host-plants, which is that drought-stress differentially affects the concentrations of phytohormones depending on the host-plant, which cascades to have differential effects on herbivores.

This dissertation has yielded new insights about the effects and potential mechanisms of HPR and drought-stress in cotton. I developed unique protocols to screen for each of HPR and drought-stress, and these short-term outputs represent an additional tool for traditional and molecular plant breeders that may be useful at some point in their own screening programs. Longer-term outputs of this dissertation will require additional research to identify and then introgress candidate traits that serve as mechanisms of host-plant resistant and drought-stress into high yielding, elite cultivars. Moreover, this work has provided new evidence from which hypotheses can be

generated and tested, which hopefully may lead down a path to sustainable food and fiber production in the face of an ever-increasing human population.

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