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Bemisia tabaci B-Arabidopsis Interactions Examined by Electrical Penetration Graphs

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

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in

Cell, Molecular, and Developmental Biology

by

Jaclyn Shuzhen Zhou

June 2014

Dissertation Committee:
Dr. Linda Walling, Chairperson
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Dedication

This dissertation is dedicated to my family and friends who have always been a great source of encouragement, love and support. I want to especially thank my parents and sibling for their constant encouragement and support through this process.

ABSTRACT OF THE DISSERTATION

Bemisia tabaci B-Arabidopsis Interactions Examined by Electrical Penetration Graphs

by

Jaclyn Shuzhen Zhou

Doctor of Philosophy, Graduate Program in Cell, Molecular and Developmental Biology
University of California, Riverside, June 2014
Dr. Linda Walling, Chairperson

In the absence of strong resistance mechanisms to control the world-wide pest *Bemisia tabaci* B, new methods for control must be derived from understanding the plant innate immune response to whiteflies. Using four Arabidopsis defense-signaling mutants, transcriptome and hormone treatment studies, it was shown that *B. tabaci* B activates SA-regulated defenses, suppresses JA-regulated defenses, and the JA-defenses are effective in slowing whitefly nymphal development.

Here we used the Electrical Penetration Graph (EPG) technique to determine the tissue location of resistance traits that influence *B. tabaci* B adult and nymph feeding in wild-type plants and in four defense mutants (*cim10*, *cev1*, *coi1*, and *npr1)*. EPG studies measured 90 variables reflecting adult whitefly feeding behaviors. However, few variables differed significantly among the five genotypes and between pooled data of fast-development genotypes (*cim10* and *coi1*) and slow nymph development genotypes (*npr1* and *cev1*). These data indicated that the suite of variables that are most strongly associated with host plant acceptance/suitability were not consistently associated with how each genotype affected nymphal development. For most of the feeding variables where the ANOVA detected significant differences among the five genotypes, the significant differences were due to *cim10* differing significantly from one or more of the other genotypes.

While evaluating adult whitefly feeding behaviors, EPG classical and three new types of E2 waveforms were identified. All four E2 waveforms were associated with the proxy for ingestion (honeydew production). Interestingly, the second type of E2 waveform, which resembled the E1 (salivation) waveform, was also associated with honeydew production. Finally, whiteflies ingesting phloem sap occasionally entered pathway phase for a brief period, and re-inserted stylets into the same sieve element to reinitiate E2, without an E1 waveform indicating that E1 was not always a prerequisite for E2.

Whitefly nymphs generated two phloem-phase waveforms (H and L) in Arabidopsis. Only waveform H was correlated with honeydew production. When nymph feeding behavior was compared among the four defense mutants or between pooled data of fast- and slow-nymphal development genotypes, no differences were found. Collectively these data indicated that phloem sap quality was the reason for differences in nymphal developmental rate.

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Introduction

Bemisia tabaci B

In the order Hemiptera, the family Aleyrodidae (whiteflies) has over 1500 species, which are divided into two subfamilies: Aleyrodinae and Aleyrodicinae (Mound and Halsey 1978). Aleyrodinae whiteflies are worldwide in distribution and Aleyrodicinae whiteflies occur primarily in Central and South America (Inbar and Gerling 2008). Most whitefly species inhabit climates that are tropical or subtropical. One of the most destructive whiteflies is *Bemisia tabaci B* (also known as *B. tabaci* biotype B, *Bemisia argentifolii*, Middle East-Asia Minor 1), which has caused billions of dollars in crop losses in the United States since its emergence as a pest in the early 1990s (Barinaga 1993). *B. tabaci* B is part of a species complex consisting of at least 24 morphologically indistinguishable species (De Barro et al. 2011). Prior to the 1990s, *B. tabaci A* was the predominant member of the *Bemisia* species complex in the United States, but it was rapidly displaced by *B. tabaci B*.

B. tabaci B feeds on a wider range of plant hosts than *B. tabaci A* and has developed resistance to many insecticides that previously were used for whitefly control (Martinez-Carrillo et al. 2006; Inbar and Gerling 2008). Whitefly damage to plants results from loss of nutrients in the phloem sap which they consume, transmission of plant viruses, and the production of honeydew that supports the growth of sooty mold on the plant surfaces, which reduces the crop's market value (Gelman et al. 2002; Jones 2003). In cotton, honeydew secreted by whiteflies makes the bolls sticky which presents a major challenge in harvesting and milling the fibers (Gamble 2002). Members of the family Aleyrodidae transmit 114 viruses and *B. tabaci* transmits 111 of these, including *Begomovirus*, *Crinivirus*, *Closterovirus*, *Ipomovirus* and *Carlavirus* (Jones 2003). In addition, this more aggressive and dominant *B. tabaci B* induces leaf silvering in squash, irregular ripening in tomatoes and developmental disorders in many other plant hosts (Inbar and Gerling 2008).

Whiteflies have six developmental stages: adult, egg, first instar (crawler), second instar, third instar, and fourth instar. Of these developmental stages, only the adult and crawler have functional legs, which allow them to move; whereas other nymph stages are immobile (Walker et al. 2010). The rate of progression through the nymphal instars is temperature dependent (Yang and Chi 2006). Whiteflies are obligate phloem feeders and crawlers usually travel a short distance (2 to 15 mm) from where they hatched to settle at a feeding site. Once settled, the nymphs continuously feed at their settling site, pausing only to molt, and ultimately emerge as adults (Simmons 2002).

Whitefly Feeding

The whitefly mouthparts consist of the labrum, labium maxillae and mandibles. The maxillae and mandibles are modified as long, thin, flexible stylets that penetrate the plant tissues as a functional unit, the stylet bundle. The maxillary stylets encase the food channel for ingesting plant nutrients and a salivary channel for secretion of saliva into plant tissues (Rosell et al. 1995). The mandibular stylets provide most of the cutting force for the stylet bundle to penetrate the plant. Adult whitefly stylets are approximately 217 µm in length and nymph stylets are range from 113 to 200 µm in length (Freeman et al. 2001).

In adult whiteflies, the labium is a four-segmented tube-like structure which has a deep longitudinal invagination, the labial groove, which extends most of the length of the labium and houses the stylet bundle when the insect is not feeding. The labium of whitefly nymphs is much shorter and, when not feeding, the stylet bundle is housed in an invagination, the crumena, at the base of the labium. At the tip of the adult labium are seven pairs of sensillae which typically are rubbed over the plant surface immediately prior to penetrating the plant with the stylet bundle. Of the seven sensillae, four are mechanosensory and three are either chemosensory or have both chemosensory and mechanosensory abilities (Walker and Gordh 1989). The labrum of adult whiteflies is a small structure at the ventral end of the whitefly's clypeus that forces the styet bundle into the labial groove at the point where the stylets emerge from the head (Walker et al. 2010).

As the whitefly stylet bundle weaves through plant tissue, the stylets take a predominantly intercellular path to reach the phloem, and once it finds a sieve element, the maxillary stylet tips puncture the cell to consume phloem sap (Freeman et al. 2001). Prior to reaching the phloem, the whitefly rarely punctures epidermal and mesophyll cells (Janssen et al. 1989; Walker and Perring 1994; Jiang et al. 1999; Johnson and Walker 1999). Whiteflies prefer to feed from the abaxial side of plant leaves possibly for easier access to the phloem through the spongy mesophyll rather than feeding from the top of the leaf, which requires the whitefly stylets to move through the denser palisade mesophyll. Feeding from the underside of the leaf also allows the sticky honeydew excrement to fall away from the nymphs, shelters the whiteflies from rain, harsh weather, and direct exposure to the sun (Freeman et al. 2001). The movement of whitefly crawlers from the adaxial to abaxial surface of leaf was not due to gravity or light stimuli but possibly due to feeding and texture cues of leaf surface (Simmons 1999).

Whiteflies primarily feed on the sap of phloem sieve elements, but will also occasionally ingest sap from xylem. The xylem and phloem are vascular tissues where photosynthates, nutrients and water are transported throughout the plant (Turgeon and Wolf 2009; Lucas et al. 2013). The xylem transports primarily water, inorganic ions and very little organic nutrients throughout the plant (Raven 1983). The phloem consists of parenchyma cells, sieve element cells and companion cells. The sieve element cells are connected end-to-end creating sieve tubes where organic nutrients including sugars such as sucrose, raffinose, and sugar alcohols are transported throughout the plant (Girousse et al. 1991; Vanhelden et al. 1994; Calatayud et al. 1996). By tapping into the sieve element of the phloem, the whitefly has continuous access to plant nutrients. Whitefly nymphs feed entirely in the phloem, while adults feed primarily in the phloem but also less frequently in the xylem (Lei et al. 1996b; Jiang and Walker 2003; 2007).

Whitefly Saliva

Adult whiteflies have two pairs of salivary glands: principle and accessory glands (Harris et al. 1996; Ghanim et al. 2001). The principle glands consist of at least thirteen cells and the accessory gland consists of four cells (Ghanim et al. 2001). Whiteflies produce two types of saliva as they feed: gelling saliva and watery saliva. The gelling saliva forms a barrier between the whitefly stylets and plant apoplastic defenses (Miles 1999). Gelling saliva is secreted incrementally as the whitefly advances its stylets toward a phloem sieve element. Within seconds after secretion of each droplet of gelling saliva, the saliva gels to become a solid. The stylets then push forward though the gelled droplet and then secrete another droplet which adheres with the prior droplet before the new droplet gels. This incremental secretion of gelling saliva produces a salivary sheath that encases the stylets all the way from the plant surface, where penetration was initiated, to the apex of the stylet bundle. Its incremental secretion gives it the appearance of a string of beads.

Several hypotheses have been proposed for the function of the salivary sheath produced by gelling saliva: it may allow the stylets to advance and retract more easily by acting as a lubricant; it may assist in keeping the stylets in a coherent stylet bundle; it may protect the stylets from plant defensive compounds; or it may make stylet penetration more stealthy thus avoiding eliciting plant defensive responses (van der Westhuizen et al. 1998; Will et al. 2012). In addition, the gelling saliva may aid to seal the phloem element puncture site to prevent the loss of turgor pressure in the sieve element which would trigger a sieve element occlusion response (Tjallingii 2006). Little is known about whitefly saliva composition, whitefly gelling saliva is presumed to be similar to aphid gelling saliva which contains proteins, phospholipids, conjugated carbohydrates, phenoloxidase, and peroxidase (Miles and Peng 1989; Urbanska and Tjallingii 1998; Cherqui and Tjallingii 2000). The recent *B. tabaci* salivary gland transcriptome has identified a set of secreted proteins (Su et al. 2012); some of these proteins may reside in the salivary sheath.

Whiteflies secrete watery saliva into phloem sieve elements (Jiang et al. 2000b) and possibly also secrete watery saliva into the apoplast during stylet penetration as has recently been demonstrated for aphids (Moreno et al 2011). To date, the only enzymes identified in whitefly saliva are polyphenol oxidase, peroxidase, and alkaline phosphatase (Funk 2001; Peng et al. 2013). A greater understanding of phloem-feeder saliva content is primarily gleaned from aphid studies that have detected phenol oxidases, phenolic glycosides, peroxidases, pectinases, amylases, alkaline and acidic phosphatases, amino acids, and cellulose in aphid saliva (Miles 1959; 1972; 1999).

Watery saliva also contains a number of proteins and molecules that may trigger or suppress defense signaling (Peng et al. 2013). Watery saliva also has been hypothesized to be important in countering plant defenses (Will et al. 2007; Mutti et al. 2008). For example, the polyphenol oxidase and peroxidase in whitefly saliva may serve to detoxify plant phenolics that could be detrimental to the whiteflies (Peng et al. 2013). Also, it is hypothesized that alkaline phosphatase may facilitate absorption and metabolism of nutrients in phloem sap (Yan et al. 2011).

Innate immunity and Effector-Triggered Immunity

For insects to feed successfully on a host plant, the insect has to overcome many plant defense mechanisms. Unlike chewing insects that destroy plant tissues, piercing-sucking insects cause much less mechanical damage to the plant (Walling 2000; Puthoff et al. 2010). Phloem-feeding insects (whiteflies and aphids) alert plants of their presences by mechanical movements of their stylets and the secretion of sheath and watery saliva. It is presumed that plant receptors perceive signals in insect saliva. The molecules recognized by plants are categorized as herbivore-associated elicitors (HAEs) or herbivore-associated molecular patterns (HAMPs) (Walling 2009; Bonaventure 2012). Elicitors induce plant immune responses, whereas effectors induce or suppress the activation plant defense mechanisms.

Since mechanism used by plants to perceive insect HAEs and HAMPs are likely similar to plant perception of pathogens elicitors, the mechanism of plant innate immune responses to insects is described in the context with what is known about pathogen perception and immune responses (Bonaventure 2012). Upon attack by pathogens, plants innate immunity is induced. During the innate immune response, plant pattern recognition receptors (PRRs) detect the presence of pathogen-secreted pathogen-associated molecular patterns (PAMPs) (Monaghan and Zipfel 2012). PAMPs are highly conserved molecules and provide an critical function for the pathogen. PAMPs are readily distinguished from host plant because they are normally not present in the plant. The interaction of plant PRR with pathogen PAMP triggers signal-transduction cascades that lead to the activation of defense responses resulting in PAMP- triggered immunity (PTI); this is a powerful resistance mechanism and confers non-host immunity.

While numerous PAMPs are known for microbial pathogens (Muthamilarasan and Prasad 2013), far less is known about HAEs and HAMPs (Bonaventure et al. 2011; Bonaventure 2012). Prior to the functional genomic approaches, the identification of insect elicitors was more challenging and relied on the collection of insect saliva in artificial diet and characterizing the salivary components (Rodriguez and Bos 2013). With the availability of genome and transcriptome sequences along with proteomic tools, rapid advances have been made in identifying insect elicitors or effectors particularly in aphid saliva. For example, by deducing the *Myzus persicae* (green peach aphid) secretome and screening proteins that had no matches in other aphid species, candidate elicitors were identified (Bos et al. 2010). Two of these proteins (Mp10 and Mp42) are to be elicitors, since their expression in *Nicotiana benthamiana* reduces aphid fecundity (Bos et al. 2010). In another study, it was shown *M. persicae* saliva induced plant defenses in *A. thaliana* that decreased aphid performance (De Vos and Jander 2009). Although the identity of this elicitor was not known, the size of the peptide was 3 to 10 kDa.

Over evolutionary time, pathogens/pests have evolved many mechanisms to suppress and overcome the plant innate immune response. Many successful pathogens/pests produce effectors to inhibit PAMP-triggered immunity (Walling 2009; Dou and Zhou 2012). For example, pathogen effectors can inhibit cell surface PRRs and prevent plant recognition of PAMPs. Pathogen effectors can also inactivate the MAPK signaling cascade by dephosphorylating MAPKs and subsequently preventing the activation of defense response genes essential for innate immunity. Downstream plant defense signaling such as RNA metabolism, vesicle trafficking, hormone accumulation, and programmed cell death (HR responses) are all possible targets of pathogen effectors (Chisholm et al. 2006; Zhang and Zhou 2010). Similarly, there may be hundreds of hemipteran effectors that target distinct steps in the plant innate immune responses. Through the stylets, the effectors can be released into plant cells, the phloem and apopastic space (Rodriguez and Bos 2013).

The function and targets of these insect effectors may be similar or distinct from pathogen effectors (Hogenhout and Bos 2011). In *Acyrthosiphon pisum* (pea aphid), the salivary gland *C002* gene was identified and encodes for a protein that is secreted into host plant tissues (Mutti et al. 2008). The overexpression of *M. persicae C002* (MpC002) in *N. benthamiana* results in enhanced aphid performance (Bos et al. 2010). In *Macrosiphum euphorbiae* (potato aphid), two effectors (Me10 and Me23) were identified and when expressed in *Nicotiana benthamiana* increase aphid fecundity (Atamian et al. 2012a). In addition, the expression of Me10 in tomato also increases aphid fecundity (Atamian et al. 2012a).

To defend against pathogen/insect effectors, plants respond with effector-triggered immunity (ETI) (also known as gene-for-gene resistance) (Flor 1971; Jones and Dangl 2006; Thomma et al. 2011; Gassmann and Bhattacharya 2012). During effector-triggered immunity, the plant specific resistance (R) protein directly or indirectly recognizes the pathogen/pest effector leading to plant resistance. Many *R* genes encode leucine-rich repeat (LRR) proteins (Belkhadir et al. 2004; Eitas and Dangl 2010). If either the *R* gene is absent in the plant or the cognate effector gene is absent from the pathogen, the host plant is unable to rapidly recognize the pathogen resulting in disease susceptibility (Glazebrook 2005). The decoy model and guard hypothesis are common models to explain the interactions between effectors and host plant proteins (de Wit 2002; van der Hoorn and Kamoun 2008).

Effector-triggered immunity (ETI) or *R*-gene mediated resistance leads to the activation of many plant defense mechanisms. During ETI, the MAPK signaling cascade used in PTI is activated and there is considerably overlap in the transcriptional responses to PTI and ETI (Thomma et al. 2011) . However, the ETI response is more rapid and robust after pathogen/insect perception and may also induced ETI-specific responses (Bhattacharjee et al. 2013; Gao and He 2013). During ETI, there is a strong oxidative burst and reactive oxygen species (ROS) are generated (Glazebrook 2005; Muthamilarasan and Prasad 2013). ROS generation is often associated with hypersensitive cell death responses (HR), which is a plant programmed cell death that prevents pathogen access to water and nutrients (Glazebrook 2005). ETI induces salicylic acid (SA) synthesis and, ultimately, systemic-acquired resistance (SAR), which is a broad spectrum defense beyond the site of local infection (Fu and Dong 2013).

Plant Resistant (R) Genes to Hemiptera

The specific mechanisms that mediate ETI through *R* gene protein recognition of insect effectors remain to still be elucidated. However, current data suggest there are strong parallels in plant-pathogen and plant-insect *R* gene protein and effector interactions (Bonaventure 2012). First, the three plant *R* genes (*Mi1.2*, *Bph14*, and *Vat*) that have been isolated and confer resistance to hemipteran insects encode LRR proteins; these motifs are found in most *R* genes that recognize pathogen effectors (Hogenhout and Bos 2011). Second, similar to pathogen effectors, there are numerous putative effectors that have been identified based on the proteomics of hemipteran saliva (Carolan et al. 2011; Nicholson et al. 2012) and predicted by the salivary gland secretome (Bos et al. 2010; Rao et al. 2013).

While many plant resistance genes against hemipterans have been identified genetically (Walling and Thompson 2012), for most the mechanism of resistance has not been elucidated. However, a few plant resistance genes have been well characterized and the mechanisms of resistance have been examined. Three plant resistance genes that are cloned and characterized are *Vat* in melon, the rice *Bph14* gene and the tomato *Mi1.2* gene. The *Mi1.2* gene was the first insect resistance gene cloned and it is part of the *R* gene family of leucine zipper, nucleotide binding (NB), LRR proteins (Milligan et al. 1998). In tomato, the *R* gene *Mi-1.2* confers resistance to potato aphid (*Macrosiphum euphorbiae*), whiteflies (*B. tabaci B* and *Q*), a psyllid (*Bactericera cockerelli*), and four species of root knot nematodes (*Meloidogyne spp*.) (Roberts and Thomason 1986; Rossi et al. 1998; Nombela et al. 2003; Casteel et al. 2006). Since *Mi1.2* confers resistance to four types of animals, it is uncertain if these nematodes and insects provide the same or different effectors that are recognized by the Mi1.2 protein. It is important to note that the mechanism of resistance to these animals appears to be different. Resistance to *M. euphorbiae* is phloem mediated. In contrast, resistance to whiteflies is not phloem-mediate and resistance appeared to be along the path to the phloem (Jiang et al. 2000b). If a whitefly reaches a phloem sieve element on Mi1.2 plants, feeding was not hindered.

Resistance of aphids conferred by *Mi-1.2* is dependent on HSP90, SGT1, and SA (Bhattarai et al. 2007a; Bhattarai et al. 2007b). In tomato, the plasma membrane-localized somatic embryogenesis receptor kinase 1 (SERK1) is required for *Mi-1* mediated resistance to potato aphids but not to root knot nematode (Mantelin et al. 2011). In addition, tomato *RME1* (for resistance to Meloidogyne) and *SlWRKY70* gene has been implicated in *Mi-1* mediated resistance to both potato aphid and root knot nematode (de llarduya et al. 2001; Atamian et al. 2012b). When transgenic eggplant express the *Mi1.2* gene, only the resistance to the root-knot nematode was conferred (Goggin et al. 2006). The aphid susceptibility of these transgenic plants suggests that additional factors present in tomato are needed for expression resistance to *M. euphorbiae* (Goggin et al. 2006)

In rice, 27 resistance genes have been identified that confer resistance to *Nilaparvata lugens* (brown planthopper) biotypes (Jena and Kim 2010; Cheng and Zhu 2013; He et al. 2013). At the present time, only the *Bph14* gene has been cloned and confers resistance to both the brown planthopper and white-backed planthopper (*Nilaparvata lugens* Stal) (Tan et al. 2003; Du et al. 2009). The *Bph14* gene encodes a coiled-coiled NB–LRR protein. Transgenic planthopper-susceptible rice expressing *Bph14* reduced brown planthopper population growth and nymph survival. However, neither insect settling nor oviposition were affected (Du et al. 2009). Moreover, electrical penetration graph studies that monitored planthopper feeding behaviors showed that *Bph14* resistance is mediated by resistance factors in both pathway and phloem (Du et al. 2009). Although the mechanism of *Bph14*-mediated resistance to brown planthopper remains to be elucidated, it most likely involved the core plant defense hormone (SA, JA, ET) in ETI response (Cheng and Zhu 2013).

Relatively few plants have resistance to whiteflies. As described in the next section the glandular trichome-mediated resistances from wild tomatoes are effective against whiteflies and other insects (Liedi et al. 1995; Snyder et al. 1998; Glas et al. 2012), and the tomato *Mi1.2* gene provides a weak resistance to whiteflies (Nombela et al. 2003). Unlike aphid and planthopper (Nombela et al. 2003; Klingler et al. 2009; Zhang et al. 2009a; 2010; Cheng and Zhu 2013), there are no known gene-for-gene mediated resistance mechanisms that protect plants from whiteflies. Genetically uncharacterized resistance to whiteflies has been reported in cultivated and wild cotton and soybeans (Wilson et al. 1993; Walker and Natwick 2006) (Arioglu 1987; Gulluoglu et al. 2012; Miyazaki et al. 2013). Cassava and alfalfa cultivars also display resistance to whiteflies that is non-trichome mediated. Significant resistance to the Latin American whitefly *Aleurotrachelus socialis* was observed in cassava (*Manihot esculentum*) cultivars. Whitefly resistant cassava display both nymph mortality and repellency; these genotypes have lower rates of oviposition and nymph developmental times are increased, and nymph mortality is increased (Bellotti and Arias 2001; Carabalí et al. 2010). A similar type of resistance to whitefly was discovered in alfafa (*Medicago sativa* L.). On whitefly-resistant alfalfa genotypes, whiteflies either do not emerge from eggs or rarely proceed beyond their first instar (Teuber et al. 1996; Jiang and Walker 2003). This resistance is primarily mediated by phloem determinants as nymphs ingested more phloem sap on susceptible genotypes relative to resistant genotypes (Jiang and Walker 2007). At the present time, the mechanism of resistance to whitefly is unknown.

Constitutive Defenses

Unlike induced defenses (eg., PTI, effector-induced susceptibility, and ETI) that require re-directing of resources from growth and development to plant defense, constitutive defenses are present in both healthy and infested plants. Constitutive defenses includes the protection mediated by cell walls, cuticles, trichomes, thorns, and stored secondary metabolites (Walling 2000; Mithöfer and Boland 2012). Trichomes contribute to the physical appearance and texture of leaves. Trichomes are either non-glandular or glandular with the latter containing round "head" structures at the tip of the trichomes (Walling 2000; Tissier 2012). Glandular trichomes may act as sensors which alert the plant of insect attack . Trichomes can either physically deter insects from feeding or harm insects with stored toxic chemicals decreasing insect survival on the plant.

Wild tomato plants have glandular trichomes that store secondary metabolites that provide a broad spectrum resistance to insects, including whiteflies; these resistance traits are multigenic. The *Lycopersicum pennnellii* type IV trichomes store acylsugars that irritate, trap, and reduce whitefly oviposition (Liedi et al. 1995; Snyder et al. 1998). The resistance from *S. habrochaites* is associated with volatile sesquiterpenes that repel whiteflies and deter oviposition (Bleeker et al. 2009b; Bleeker et al. 2011). Recently, cultivated tomatoes were engineered to produce the sesquiterpene (7-epizingiberene) in its glandular trichomes resulting in an increase in whitefly adult mortality and a decrease in fecundity; resistance to other herbivores was also noted (Bleeker et al. 2012). Finally, the type IV trichomes of *S. habrochaites* var *glabratum* contain high levels of methyl ketones that confer repellency and mortality of whitefly adults (Eigenbrode and Trumble 1993; Maliepaard et al. 1995)

Secondary metabolites are produced and stored in plants during growth and development and some provide an immediate defense against herbivores (Mithöfer and Boland 2012; Kliebenstein 2013). Secondary metabolites attract or repel an insect and they can slow insect growth and development. Phenylpropanoids, terpenoids, alkaloids, hydrozamic acids, and glucosinolates are examples of secondary metabolites with roles in plant defense against phloem-feeding insects (Wink 2003). Some secondary metabolites are volatiles, which have various roles in plant-insect interactions (Rodriguez-Saona and Frost 2010; War et al. 2011). Insects are able to distinguish between the volatiles released by host verses non-host plants and this would affect insect settling and oviposition (Baldwin 2010; Rodriguez-Saona and Frost 2010) . Volatiles released by healthy and infested plants are different, and insects are able to assess the density of insects feeding by the different volatiles released (Rodriguez-Saona and Frost 2010). Volatiles released from infested plants also may activate plant defense or alert neighboring plants (War et al. 2011). The most important is the role of volatiles in the attraction of the herbivore's natural enemies (parasitoids and predators) to the infested plant (Walling 2000; McCormick et al. 2012). In tomato, the presence of terpene volatiles (ie., zingiberene and curcumene), and monoterpenes (ie., *p*-cymene, α-terpinene, and α-phellandrene) make plants less attractive to whiteflies (Bleeker et al. 2009a; Bleeker et al. 2010).

In Brassicaceae plants, glucosinolates play an important role in insect defenses (Winde and Wittstock 2011). The biosynthesis of glucosionolates is dependent on primary and secondary metabolism. In wild-type Arabidopsis plants, the amino acid side chains of glucosinolates are derived from Met, homo-Phe and Trp (Halkier and Gershenzon 2006). Glucosinolates are anionic thioglucosides and are stored as non-toxic glucose conjugates that are stored in cells adjacent to the vasculature. Myrosinase releases the toxic nitriles, isothiocyanates, epithionitriles, and thiocyanates that are harmful to the insects by removing the glucose moiety. Myrosinase in stored in the idioblasts in the phloem (Andreasson et al. 2001; Winde and Wittstock 2011). This distinct storage of glucosinolates and myrosinases allows this potent defense to only be deployed upon tissue and cellular damage that occurs with wounding or herbivory.

The effects of aliphatic and indolic glucosinolates on *B. tabaci* B and Q life history parameters was investigated using transgenic Arabidopsis lines that over-express these glucosinolates (Elbaz et al. 2012). Species specific responses were observed. For *B. tabaci* B, high levels of aliphatic glucosinolates decreased oviposition, while high levels of indolic glucosinolates decreased oviposition, nymph survival and developmental rate. In contrast, for *B. tabaci* Q, high levels of aliphatic glucosinolates decreased oviposition, nymph survival and developmental rate, and high levels of indolic glucosinolates decreased developmental rate. While these experiments show that aliphatic and indolic glucosinolates are potent resistance factors for whiteflies and aphids, it is interesting to note that both whiteflies and aphids suppress the expression of sulfur metabolism and gluocinsolate biosynthetic gene expression during infestation of Arabidopsis (Kempema et al. 2007; Kim and Jander 2007; Kim et al. 2008; Kusnierczyk et al. 2008)

Salicylic Acid (SA)

While many hormones have been implicated in plant defense responses, this project focuses on the roles of salicylic acid (SA) and jasmonic acid (JA) in innate immunity (Bari and Jones 2009; Erb et al. 2012). In response to pathogen attack, chorismate is used to synthesize SA by the isochorismate synthase 1(ICS1) and isochorismate pyruvate lyase (IPL) in the chloroplasts (Wildermuth et al. 2001). SA is also synthesized from cinnamate by phenylalanine ammonia lyase (PAL) when the plant is under abiotic or biotic stress (Lee et al. 1995). SA-regulated defense is important in PTI and ETI and influences defenses in the infected leaves, as well systemically. Systemic acquired resistance (SAR) is a sustained defense response protecting non-infected tissues against bacterial, fungal and viral attack (Fu and Dong 2013). The SA-defense signaling pathway activates the transcription of a complex array of genes including *Pathogenesis-related protein* (*PR)* genes (Glazebrook 2005; Boatwright and Pajerowska-Mukhtar 2013).

There are many positive and negative regulatory components that control SA-regulated defenses. NONEXPRESSOR OF PR GENES 1 (NPR1) is an key component in the this pathway (An and Mou 2011). In the absence of SA, NPR1 protein is mainly an oligomer in the cytoplasm. As the level of SA increases, NPR1 oligomers dissociate into monomers by reduction of disulfide bonds (Mou et al. 2003; Tada et al. 2008). The NPR1 monomers enter the nucleus (Tada et al. 2008). Through its ankyrin repeat domain, NPR1 binds to TGA transcription factors (TGA 2,TGA 5, and TGA6) to activate the expression of defense genes such as *PR1* gene (Zhang et al. 1999). The full expression of *PR1* gene requires transcription factors TGA 2,TGA 5, TGA6, WRKY70, and AtWhy1 (Zhou et al. 2000; Johnson et al. 2003).

The level of NPR1 is controlled by the NPR1 paralogues, NPR3 and NPR4, which are the SA receptors (Fu et al. 2012). Of the two SA receptors, NPR4 has a greater affinity for SA than NPR3. In the presence of high level SA, NPR3 mediates NPR1 degradation through the 26S proteasome, and at low level of SA, NPR4 mediates the degradation of NPR1 (Fu et al. 2012). Fu et al. (2012) proposed that NPR3 and NPR4 sense the level of SA and thereby controls NPR1 homeostasis in the cell. At low level of SA, NPR4 binds to NPR1, and NPR1 is degraded. As a consequence, the plant is highly susceptible. As the level of SA increases at the site of infection (an intermediate level of SA), SA binds to NPR4, and the “free” NPR1 activates plant defense genes. At the highest level of SA, NPR3 binds SA and NPR1, which leads to its degradation and ETI (Moreau et al. 2012). At sites distal from the local infection, the level of SA is intermediate, which allow for NPR1 accumulation and degradation, which is optimal for SAR (Fu et al. 2012; Gust and Nurnberger 2012; Fu and Dong 2013)

To study plant SA-regulated defenses in Arabidopsis, a larger number of mutants that impair or enhance SA defenses have been identified (An and Mou 2011; Fu and Dong 2013). This project uses a transgenic line (NahG) and two mutants [*npr1* and *constitutive immunity 10* (*cim10*)] that impact SA signaling. NahG transgenic lines express a bacterial salicylate hydroxylase protein (NahG) that catabolizes SA into catechol (Van Wees and Glazebrook 2003). In NahG plants, SA levels are low, *PR1* genes are not expressed and there is no SAR (Shah 2003). *npr1* mutants are insensitive to defense inducers such as SA, 2,6-dichloroisonicotinic acid (INA), and pathogens (Cao et al. 1994) and have an impaired innate immune response and enhanced susceptibility to virulent pathogens (Dong 2004). It should be noted that although not well studied, some SA-regulated defenses are independent on NPR1 (Glazebrook 2005; Moreau et al. 2012).

The *cim10* mutant has high levels of SA and constitutively activate *PR* genes (Maleck et al. 2002). Little is known about this mutant. The *cim10* mutant maps between the dihydroflavonol 4-reductase (DFR*)* (At5g42800.1) and length of the floral internodes *LEAFY3/LFY3* (At5g61850.1) on chromosome 5. This region includes the MRC-J region, which intriguingly is rich in *R* gene homologs. *cim10* is an optimal *cim* mutant for whitefly studies since it has a robust rosette and does not have spontaneous lesions, which are common in the other *cim* mutants (Maleck et al. 2002).

Jasmonic acid (JA)

JA-signaling pathways are involved in plant development, defense and wound responses (Koo and Howe 2009). Necrotrophs and tissue-damaging insects (eg., caterpillars and beetles) activate JA-dependent and -independent wound-response genes (Howe and Jander 2008). Damage to plant tissues activates the octadecanoid pathway, resulting in the production of the 12-carbon fatty acid JA from the 18-carbon linoleic acid. Galactoplipids of chloroplast membranes releases linoleic acids and through multiple biosynthetic steps to generate JA (Creelman and Mullet 1997; Ryan 2000; Howe et al. 2006). JA is conjugated to the amino acid isoleucine by the JA-amino acid synthetase (JAR1). JA-Ile is the bioactive form of JA (Staswick and Tiryaki 2004; Guranowski et al. 2007; Fonseca et al. 2009). JA-Ile regulates transcription of many plant defense genes (Wasternack and Hause 2013), including synthesis of secondary metabolites, volatile blends and, in the Brassicaceae, glucosinolates (Krumm et al. 1995; Mikkelsen et al. 2003; Mewis et al. 2006).

In Arabidopsis the JA-defense signaling pathway involves the functions of the CORONATINE-INSENSITIVE PROTEIN 1 (COI1), JASMONATE-RESISTANT 1 (JAR1), JASMONATE-INSENSITIVE 1/MYC2 (JIN1/ MYC2), and JASMONATE ZIM-DOMAIN (JAZ) proteins (Wasternack and Hause 2013). COI1 is an F-box protein involved in the degradation of proteins via the proteasome. COI1 interacts with CULLIN 1, S-phase kinase-associated protein 1 (Skp1)-like proteins, and RING-box protein 1 (AtRbx1) to form an E3 ubiquitin ligase (Devoto et al. 2002; Xu et al. 2002; Ren et al. 2005). This SCF complex helps stabilize the COI1 protein (Wasternack and Hause 2013) and is crucial for degrading Jasmonate ZIM domain (JAZ) proteins that repress JA-signaling (Chini et al. 2007; Thines et al. 2007). By binding and sequestering the MYC2 (JASMONATE INSENSTIVE1) transcription factor, JAZ proteins repress JA signaling (Lorenzo et al. 2004; Chico et al. 2008). The repression of MYC2 also involves co-repressors TOPLESS (TPL), TPL-related proteins and adaptor protein Novel Interactor of JAZ (NINJA) (Pauwels et al. 2010).

When levels of JA-Ile rise, COI1 binds JA-Ile (Wasternack and Hause 2013) and promotes the binding of JAZ proteins COI1 in the SCF complex. This leads to the ubiquitination and degradation of JAZ proteins (Thines et al. 2007). In the absence of JAZ proteins, the MYC2 transcription factor activate JA-responsive genes (Chini et al. 2007). Finally, the JAZ proteins interact with several other transcription factors involved in other defense signaling pathways (Pauwels and Goossens 2011).

Two JA defense-signaling mutants were used in this project including: *coronatine-insensitive protein 1* (*coi1*) and *constitutive expression of VSP1* (*cev1*). The

Arabidopsis *coi1* mutant was discovered as insensitive to a bacterial compound similar to JA, coronatine (Feys et al. 1994). As described above, COI1 is the JA receptor and plays a crucial role in JA-regulated defenses (Yan et al. 2009). In the absence of COI1, JA-regulated defenses are impaired. In the *cev1* mutant, JA- and ethylene (ET)-regulated defense-signaling pathways are activated (Ellis and Turner 2001). CEV1 encodes a cellulose synthase (CeSA3). *cev1* roots contains less cellulose compared to WT (Ellis et al. 2002). Reduced levels of cellulose synthesis impacts lignin in cell wall, and causes activation of JA, ET, and other defense- signaling pathways (Ellis et al. 2002; Caño-Delgado et al. 2003).

Defense-Signaling Crosstalk

Plants derive the most effective defense against pathogen and pest attack by successful crosstalk between signaling pathways (Derksen et al. 2013). This dissertation has focused on the roles of the SA and JA defense-signaling pathways and their roles in influencing whitefly nymph development rates (Zarate et al. 2007) and their impacts on adult and nymph feeding behaviors. While SA and JA are at the core the plant immune response to pest and pathogens, the plant JA and SA defense networks not only communicate with each other (cross-talk) but they are intimately intertwined with additional phytohormone pathways including ethylene, abscisic acid, auxin, gibberellic acid, cytokinin, brassinosteroids, and bioactive peptides (Erb et al. 2012; Pieterse et al. 2012). For example, in many cases, the JA- and ET defense-signaling pathways act cooperatively and synergistically, whereas the SA signaling pathway antagonizes the JA/ET-regulated defenses (Bari and Jones 2009; Derksen et al. 2013).

Unraveling the defense signaling networks is a difficult task, and microarrays and RNAseq are additional approaches to look at large scale gene expression profiles among/between multiple signaling pathways (Tsai et al. 2011; van Verk et al. 2011; Tamaoki et al. 2013). Many regulatory nodes (often transcription factors) are involved in of the coordination of multiple intersecting signaling pathways (van Verk et al. 2011). So far, few large scale transcriptome analysis with plant infested with whiteflies or whiteflies and the SA mimic (benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester, BTH were used to identify a cohort of genes induced by the insect and BTH (Kempema et al. 2007; Park and Ryu 2013). However, more microarray studies have been completed using plant defense mutants, transgenic lines overexpressing key defense regulators, and hormone treatments (ie., SA and JA) to identify a cohort of genes up-regulated or down-regulated, and genes shared between pathways (Wang et al. 2006; Sato et al. 2007; Moffat et al. 2011; Tsai et al. 2011; Tamaoki et al. 2013). Evident in all these studies is that defense signaling is complex, and many of the results vary depending on the treatment used; to date no transcriptome studies have focused on the complete set of defense mutants used in Zarate et al. (2007) and this Dissertation. Such studies would shed light on the fluxes in the defense pathways that influence pathogen/pest interactions.

However, the regulatory nodes between defense signaling pathways and mechanisms of crosstalk between the JA- and SA-signaling pathways are beginning to emerge (Van der Does et al. 2013). For example, NPR1 has a dual function in that it activates transcription of SA-responsive defense genes in the nucleus, while there appears to be a cytosolic mechanism that represses JA-regulated defenses (Spoel et al. 2003). In addition, other regulatory genes that have shown opposing effects on JA- and SA-regulated signaling pathways include: ENHANCED DISEASE SUSCEPTIBILITY 1 (EDS1), EDS4, PHYTOALEXIN DEFICIENT 4 (PAD4), WRKY transcription factors, and the glutaredoxin GRX480 (Zhou et al. 1998; Falk et al. 1999; Gupta et al. 2000; Li et al. 2004; Ndamukong et al. 2007). Most recently, it was shown that SA suppresses JA-regulated defenses through modulation of the transcription factor ORA59 (Van der Does et al. 2013).

A lot still remains to be understood in crosstalk between different hormone-signaling pathways but some components of crosstalk have been elucidated between JA and other hormone signaling pathways. For example, the TPL co-repressor involved with repression of JA signaling by sequestering MYC2 (Pauwels and Goossens 2011), is also co-repressor for auxin mediated gene repression (Szemenyei et al. 2008). Similarly, GA signaling and JA signaling reciprocally regulate JAZ protein levels. In the absence of GA, DELLA proteins bind JAZ1, preventing JAZ1 binding of MYC2, thereby activating JA-regulated gene expression (Hou et al. 2010). In the presence of gibberellic acid, DELLA protein is degraded, JAZ1 binds MYC2, and JA signaling is repressed. A similar example links, ethylene (ET) and JA signaling. JAZ1, JAZ3 and JAZ9 bind and repress transcription factors, EIN3 and EIL1. In the presence of JA-Ile, JAZ proteins are degraded allowing the ethylene dependent activation of EIN3 and EIL1. Thus, in this case, JA and ET- pathways act in synergy (Zhu et al. 2011).

Whiteflies and Plant Immunity

To date, there are few mechanisms that confer resistance to whiteflies. Therefore, studies have primarily focused on the role of plant’s innate immune responses in the whitefly-plant interactions. Studies of several whitefly species (*B. tabaci* B, *B. tabaci* A, *B. tabaci* Q, and *Trialeurodes vaporariorum*) on different host plants (eg., squash, tomato, Lima bean, and Arabidopsis) have given some insight into the complexities of plant-whitefly interactions. For example in squash, *B. tabaci* B nymph feeding induced *SLW1* (M20b peptidase-like gene) and *SLW3* (leaf-specific β-glucosidase-like gene) RNAs to accumulate locally and systemically (van de Ven et al. 2000). While, *B. tabaci* A induced *SLW3* RNAs locally and did not induce *SLW1*. While *SLW1* gene is regulated by the JA/E pathways, *SLW3* appears to be regulated by a novel signaling pathway (van de Ven et al. 2000). The different induction of *SLW1* and *SLW3* by *B. tabaci* B and A may be due to different quality or quantity of elicitors secreted by whiteflies into the plant.

Whitefly feeding causes little mechanical damage (Kempema et al. 2007; Puthoff et al. 2010). Thus, it is not surprising that in tomato plants the transcript level of wound response genes *LapA* and *pin2* were not observed after adult whitefly feeding (Puthoff et al. 2010). In tomato, both *T. vaporariorum* and *B. tabaci* B nymph feeding induces the accumulation of pathogenesis-related (PR) proteins and RNAs (Mayer et al. 1996; Puthoff et al. 2010). In tomato, the most strongly induced *PR* genes were regulated by JA and ET and a more modest induction of SA-regulated *PR* genes was seen (Puthoff et al. 2010). Finally whitefly nymphs induce transcripts for the JA- and ET-regulated

Whitefly-induced 1 (*Wfi1)* gene, which encoded an NADPH oxidase. *Wfi1* RNAs accumulate locally and systemically in tomato leaves during nymph feeding, but this was not observed after adult whitefly feeding (Puthoff et al. 2010).

The importance of tomato's JA-regulated defenses in defense against *B. tabaci* B was examined using the mutant *suppressor of prosystemin-mediated responses2* (*spr2*) and in the transgenic line that over-expresses Prosystemin (*35S:ProSys*) (Sanchez-Hernandez et al. 2006; Cui et al. 2012). The *spr2* mutant accumulates low levels of JA and JA-regulated volatiles and while *35S:ProSys* plants have JA-regulated defenses constitutively activated. Surprisingly, the data in the two studies were not consistent. While whiteflies had increased oviposition on the *spr2* mutant plants and decreased nymph survival on *35S:ProSys* plants in the studies reported by Sanchez Hernandez et al. (2006), surprisingly, *B. tabaci* B fitness parameters were similar in WT, *spr2* and *35S:ProSys* plants in a study by (Cui et al. 2012).

Cui et al. (2012) also examined the impact of whitefly prior infestation of tomato plants, which they called preconditioning, on *B. tabaci* B fitness (Cui et al. 2012). Whiteflies preferred to feed and oviposit on non-infested plants rather preconditioned plants. Nymph development was also longer on preconditioned plants. Relative to non-infested plants, the preconditioned plants had similar JA levels, lower concentrations of soluble sugars and free amino acids, while increased levels of SA and total phenolics were detected. The changes in tomato biochemistry and whitefly fitness after preconditioning were similar in WT, *spr2* and *35S:ProSys* plants (Cui et al. 2012). In contrast to the (Sanchez Hernandez et al. 2006), Cui et al. (2012) concluded that SA regulates the deterrents to whiteflies.

In contrast to the studies in tomato, the importance of JA-regulated defenses in interfering with whitefly nymph development was clearly shown in *Arabidopsis thaliana-B tabaci* B interaction by Kempema et al. (2007); Zarate et al. (2007). Transcriptome analyses showed that SA-biosynthesis and SA-regulated RNAs increase locally and systemically after *B. tabaci* B nymph feeding; concurrently, there was no change or a decrease in transcript levels of JA- and ET-regulated gene RNAs (Kempema et al. 2007). To investigate the importance of SA- and JA-regulated defenses in whitefly success, they determined the rate of nymph development on five defense mutants (*cim10, coi1, cev1, npr1,* and NahG) (Kempema et al. 2007; Zarate et al. 2007). Nymph development is accelerated in *cim10,* which has high levels of SA and constitutively activates SA-regulated defenses, and *coi1,* which cannot perceive JA. Reciprocally, *B. tabaci* B nymphal development is delayed in *npr1, NahG* and *cev1*. While *npr1* and the transgenic line expressing salicylate hydrolase (*NahG*) block SA signal transduction and accumulation of SA, respectively, the *cev1* expressed both JA- and ET-dependent defenses constitutively. These data suggested that JA-regulated defenses deter nymph development and this was confirmed by the fact that methyl jasmonate (MeJA) treatments of Arabidopsis plants cause severe delays in nymph development (Zarate et al 2007). Using a different set of Arabidopsis defense mutants and assessing defense gene expression and whitefly nymphal development, Zhang et al. (2013) provided further evidence for the ability of *B. tabaci* to suppress effective JA-regulated defenses (Zhang et al. 2013).

Given the increases in SA levels and reciprocal regulation of SA- and JA-regulated defenses after whitefly infestation Kempema et al. (2007); Zarate et al. (2007); (Zhang et al. 2013) (Zarate et al, unpublished results), it was proposed that whiteflies manipulate Arabidopsis defense signaling to suppress the most effective JA-regulated defenses that deter the development of whitefly nymphs. One likely mechanism of regulation is JA-SA crosstalk. Recent studies have shown that SA suppresses JA-regulated defenses through modulation of the transcription factor ORA59 (Van der Does et al. 2013); not surprisingly, ORA59 transcripts are down regulated after *B. tabaci* B feeding (Kempema 2007; Kempema et al. 2007).

Whitefly co-infestations with other herbivores have also been used to elucidate the defense pathways that are induced or suppressed by whitefly feeding and to test the idea that whiteflies suppress JA-regulated defense via SA-JA crosstalk (Zhang et al. 2009b; Zhang et al. 2012). When Lima bean (*Phaseolus lunatus*) plants are co-infested with whiteflies and spider mites (*Tetranychus urticae*), the volatiles emitted are less attractive to predatory mites (*Phytoselulus persimilis*) than the volatiles from spider mite infested plants (Zhang et al. 2009b). This was correlated with reduced the emission of the JA-regulated volatile (E)-β-ocimene. In addition, co-infested plants had lower levels of JA-regulated gene RNAs (eg., *Lipoxygenase* and *Ocimene Synthase*), as well as reduced levels of both JA and SA. The mechanisms of JA-defense suppression is not known, but is not correlated with SA-JA crosstalk (Zhang et al. 2009b).

Similar to Lima bean, the dual infestations of *B. tabaci* and the caterpillar (*Plutella xylostella*) caused changes in emitted volatile blends and the attraction of the parasitoid wasp (*Diadegma semiclausum*) relative to caterpillar infested plants (Zhang et al. 2012). They used WT and four Arabidopsis mutants that are JA deficient (*delayed-dehiscence2-2; dde2-2), ET insensitive (ein2-1*), cannot accumulate SA (*NahG*) or constitutively express SA-regulated defenses (*constitutive expressor of PR gene-6* ; *cpr-6*) to genetically dissect this response. They found that relative to caterpillar infested plants, the whitefly-caterpillar volatile blend was deficient in volatiles that were up-regulated by JA and ET and suppressed by SA (Zhang et al. 2012). Zhang et al. (2012) also showed that whiteflies repressed many genes that were induced by caterpillar feeding, including three glucosinolate biosynthesis genes.

Collectively the data from *B. tabaci*-Arabidopsis interactions and *B. tabaci* interactions with other herbivores in Lima bean and Arabidopsis suggests that *B. tabaci* suppresses JA-regulated defenses and thereby alters both direct and indirect defenses. In the future it will be important to understand the nature and the mechanisms of action of the defense traits that actively interfere with whitefly growth and development.

Whitefly Electrical Penetration Graphs

JA regulates the defenses that interfere with *B. tabaci* nymph development in Arabidopsis (Zarate et al 2007), and since nymphs primarily feed on the phloem, the traits that deter nymph development are likely to be phloem-localized. However, since JA causes large changes in plant metabolism (including alteration of volatile blends and secondary metabolites) (Wasternack and Hause 2013), it is possible that JA regulates defenses in other locations (eg., leaf surface and apoplast) that will alter whitefly behavior. This Dissertation sought to determine if JA- and SA-regulated defenses altered *B. tabaci* B adult and nymph feeding behavior. To this end, feeding behaviors of adult and second-instar nymphs was studied on Arabidopsis WT and four plant defense mutants (*coi1*, *npr1*, *cev1*, *cim10*).

Unlike chewing insects whose feeding behavior can be observed visually, the feeding behavior of phloem feeders requires the aid of electrical penetration graphs (EPGs) (Tjallingii 1978; 1988; Walker 2000). EPGs measure changes in electrical resistance and electromotive forces (emf) in the plant-insect interface during stylet penetration. To record an EPG, a voltage is applied through an output wire into the soil of a potted plant and the insect is glued to a platinum or gold wire using an electrically conductive adhesive. When the insect inserts its stylets into the plant, a circuit is completed and current flows from the voltage source to the plant, through the insect, and into an input resistor. Depending on the feeding behavior of the insect, different voltage fluctuations occur across the input resistor and are recorded (Tjallingii 1978; 1988; Walker 2000). Different feeding behaviors produce distinct patterns of voltage fluctuation that are referred to as waveforms and therefore EPGs allow the researcher to indirectly observe a multitude of feeding behaviors in real time (Tjallingii 1988; Walker 2000). Two general EPG systems have been used, DC and AC systems, which differ in the electrical phenomena that they record (Walker 2000) most whitefly EPG studies have used the DC system. Numerous studies have used EPGs to study hemipteran feeding behaviors. There is a vast literature in aphids and more limited studies with leafhoppers, planthoppers and whiteflies. These studies have been useful in establishing the relative acceptability of hosts, the locations of resistance mechanisms within plant tissues, and mechanisms of virus transmission. An overview of EPG studies with whiteflies in virus-free hosts is provided below.

The first whitefly EPG study was with adult *T. vaporariorum* feeding on cucumber (*Cucumis sativus*)(Janssen et al. 1989) and used a DC system. Six waveforms were defined: waveform pattern C (whitefly stylets penetrating intercellularly through plant tissue) which in later studies is referred to as pathway phase; potential drops (intracellular punctures with their stylet tips into plant cells), waveform pattern G (xylem sap ingestion), waveform pattern E(pd) (activities with stylet tips in a phloem sieve element) which in later studies is referred to as phloem phase; waveform pattern E(c) (unknown behavior); and waveform pattern F (unknown behavior) (Janssen et al. 1989). Later, two distinct DC EPG waveforms were found to comprise phloem phase: E(pd)1 which has been correlated with salivation into a sieve element and E(pd)2 which is produced during phloem sap ingestion (Lei et al. 1997; Jiang et al. 2000a).

The second whitefly EPG study used an AC system to study feeding behavior of *B. tabaci B* on lima bean leaves and the whitefly *Parabemisia myricae* on lemon leaves (Walker and Perring 1994). The AC EPG waveforms were called sawtooth (equivalent to waveform pattern C of the DC study), transition (initiation of phloem phase), high flat (phloem phase), low flat (in some cases, but not all, produced during xylem sap ingestion), and pseudotransition (brief intracellular punctures with the stylet tips) (Walker and Perring 1994; Johnson and Walker 1999).

The advantage of the DC system for studying whitefly feeding behavior is that sieve element salivation and phloem sap ingestion can be distinguished during phloem phase while these two behaviors were indistinguishable in the AC studies. Also xylem sap ingestion can be unambiguously distinguished with the DC system but not in the AC system. On the other hand during pathway phase, several features of the sawtooth waveform in AC EPG systems can be used to detect partial stylet withdrawal and provide estimates of the speed of stylet advancement in the plant tissue (Jiang and Walker 2001); currently the DC system cannot provide this information. Regardless of the system, pathway-phase is very complex and many of the feeding behaviors associated with the variations seen in pathway phase are not yet know (Jiang and Walker 2001).

Feeding behavior of *T. vaporariorum* and *B. tabaci* B nymphs have been studied using the DC EPG system. The first studies were with *T. vaporariorum* nymphs on tomato, cucumber, gerbera, and pepper plants (Lei et al. 1996a; Lei et al. 1996b). Three waveforms produced by *T. vaporariorum* nymphs were distinguished: pathway (waveform C), high frequency (waveform H - phloem sap ingestion) and low frequency (waveform L - noningestion behavior with stylet tips in a phloem sieve element; possibly salivation) (Lei et al. 1996a; Lei et al. 1996b). In a later DC-EPG study of *B. tabaci* B nymphs on alfalfa waveforms produced were similar to those produced by *T.vaporariorum* nymphs (Jiang and Walker 2003). *B. tabaci* B nymphs produced the C, H and L waveforms but it also produced a J waveform, which was not reported from *T. vaporariorum* on tomato, cucumber, gerbera or pepper (Jiang and Walker 2003). The *B. tabaci* B nymph waveform J was observed immediately after penetration of a sieve element and was brief in duration; the behavioral correlation of waveform J is unknown (Jiang and Walker 2003).

The EPG technique has been used in many studies to understand whitefly plant host acceptability. *Trialeurodes vaporariorum* adults produced fewer, but longer bouts of phloem phase on a highly preferred host, cucumber, compared to a less preferred host, tomato (Lei et al. 1998), although the total time in phloem phase was similar for the two plants. The data indicate that phloem sap ingestion is more continuous (fewer interruptions) on cucumber than on tomato. The feeding behavior of *T. vaporariorum* on cucumber, tomato, gerbera and sweet pepper was compared by (Lei et al. 2001). Cucumber was the most acceptable for the whitefly feeding, sweet pepper was the least acceptable and tomato and gerbera had an intermediate acceptability (Lei et al. 2001). On sweet pepper, whiteflies make many short probes, had a long duration of xylem phases, had short phloem phases, and had the shortest duration of first probes. In contrast on cucumbers, whiteflies made longer probes, shorter duration of xylem phases, longer duration of phloem phases, and longer first probes.

The EPG technique has also been used to assess feeding behaviors of *B. tabaci* B on susceptible and resistant genotypes of tomato and alfalfa (Jiang et al. 2000b; Jiang and Walker 2007). The ability of *B. tabaci* B to feed on plants expressing the *Mi-1.2* gene in tomato was compared to an untransformed near-isogentic line. On the *Mi-1.2* transformed plants, whiteflies made more probes before reaching phloem phase, had longer duration of non-probing time, longer time before their first intracellular puncture, and fewer whiteflies reached phloem phase. However, once the whitefly reached the phloem feeding was not hindered. Thus, resistant factors of *Mi-1.2*-transformed plants appear to be located in the epidermis and/or mesophyll tissue and not in the phloem (Jiang et al. 2000b). In Jiang and Walker (2007) first instar *B. tabaci* B feeding behavior was examined on resistant and susceptible alfalfa genotypes. Nymphs were able to locate and penetrate a sieve element with equal success on susceptible and resistant genotypes. However, phloem sap ingestion was reduced on resistant genotypes suggesting phloem localized resistance.

Goals of Dissertation: Whitefly behavior on plant defense mutants

Using defense mutants and MeJA treatments, Kempema et al (2007) and Zarate et al (2007) showed that JA-regulated defenses slow whitefly nymph development. The goal of this Dissertation is to determine of the location of these resistance traits and if alterations in defense signaling pathways by the *cim10*, *cev1*, *coi1*, and *npr1* mutants alter *B. tabaci* B adult and second instar feeding .

In Chapter 1, EPGs were used to determine if adult *B. tabaci* B displayed altered feeding behaviors on the defense signaling mutants that accelerate (*coi1, cim10)* or slow (*npr1, cev1*) nymphal development as described in Zarate et al. (2007). Since nymph development was accelerated on *cim10* and *coi1* mutants, we hypothesized that there would be fewer feeding deterrents at the surface, in the apoplast and in the phloem of these plants. With fewer deterrents, adult whitefly would more readily access the phloem and engage in longer bouts of phloem sap ingestion on *cim10* and *coi1* mutants relative to *cevl* and *npr1* mutants, which had delayed nymph development.

In Chapter 2, *B. tabaci* B second instar feeding on WT and the defense mutants (*coi1*, *npr1*, *cev1*, *cim10)* were examined using EPGs. Since nymphal development was accelerated on *cim10* and *coi1* mutants, we hypothesized that nymphs would have longer duration of phloem sap ingestion on these mutants relative to the mutants *cevl* and *npr1* that delayed nymphal development.

In Chapter 3, we report the new phloem-phase waveforms that were discovered during EPGs that were recorded for chapter 1. We correlate these waveforms and the nymphal H and L waveforms with phloem-consumption by making high magnification video recordings on the whiteflies while simultaneous recording EPGs. The simultaneous video/EPG recordings were used to identify EPG waveforms that were correlated with honeydew production which was used as a proxy for phloem sap ingestion.

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Chapter 1

Adult Whitefly (*Bemisia tabaci* B) Feeding Behavior on Arabidopsis Salicylic Acid- and Jasmonic Acid-Signaling Mutants

Abstract

The feeding behavior of adult *Bemisia tabaci* B on wild-type *Arabidopsis thaliana* and four salicylic acid (SA)- and jasmonic acid (JA)-signaling mutants were examined by electrical penetration graphs (EPG). Prior studies in Zarate et al. (2007) demonstrated that whitefly nymphal development was accelerated on mutants where JA-regulated defenses are impaired (*coi1*) or where SA-regulated defenses are constitutively active (*cim10*). Conversely, nymphal development was delayed on mutants where SA-regulated defenses are impaired (*npr1*) or where JA-regulated defenses are constitutively active (*cev1*). To understand the feeding behaviors of adult whiteflies on wild-type (WT) and defense mutants (*coi1*, *cim10*, *npr1*, and *cev1*), the EPG technique was used. The EPG results showed that with 90 variables measured, only 12 variables differed significantly among the five genotypes. Data from the fast-development ("FastDev") genotypes (*cim10* and *coi1*) from nymph study were pooled and compared to data from slow nymph development ("SlowDev") genotypes (*npr1* and *cev1*) from nymph study. A comparison of FastDev versus SlowDev genotypes yielded only four variables that differed significantly out of 90 variables. In general, the differences in nymph developmental rate observed among the genotypes in Zarate et al. (2007) did not translate into alterations of adult whitefly feeding behavior. However, among the genotypes, *cim10* (FastDev) had

the most number of variables that differed significantly. Since genotypes that were grouped together as either “FastDev” or “SlowDev” did not appear to have the same effect on whitefly behavior, the data suggest that the underlining SA- and JA- signaling defense mechanism is complex.

Introduction

The whitefly *Bemisia tabaci* B (also known as *B. tabaci* biotype B, *Bemisia argentifolii*, and *B. tabaci* Middle East-Asia Minor 1) has cost billions of dollars in crop losses in the United States since its introduction in the early 1990s (Barinaga 1993). *B. tabaci* B is part of the *Bemisia* species complex consisting of at least 24 morphologically indistinguishable species (De Barro et al. 2011). Prior to the 1990s, another member of the *Bemisia* species complex, *B. tabaci* A was the only known member of the complex to occur in the United States, but it was rapidly displaced by *B. tabaci* B.

B. tabaci B differs from *B. tabaci* A in that it has the ability to acquire insecticide resistance more rapidly and feed on a wider range of plant hosts (Inbar and Gerling 2008; Martinez-Carrillo et al. 2006). *B. tabaci* B also induces developmental disorders in a wide variety of plants; these disorders include leaf silvering in squash and irregular ripening in tomatoes (Inbar and Gerling 2008). Whitefly damage to plants results from loss of nutrients by whitefly ingestion of phloem sap, transmission of plant viruses, and production of honeydew that supports the growth of fungi on the plant (Gelman et al. 2002; Jones 2003). Whitefly adults and nymphs feed on phloem sap using highly flexible mouthparts called stylets (Pollard 1955). During penetration through the plant tissue, whitefly stylets weave between and around cells causing minimal mechanical damage, and finally penetrate a phloem sieve element where they begin ingesting phloem sap (Freeman et al. 2001).

Biotic stresses, such as pathogen and pest attack, alter phytohormone levels and/or perception to regulate plant defenses. Abscisic acid, auxin, gibberellic acid, cytokinin, and brassinosteroid, salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are all known to influence plant defense and the networks that interlink the different hormone-signaling pathways are complex (Erb et al. 2012). These defense-signaling pathways may act antagonistically, cooperatively, or synergistically with each other to induce the transcription of a complex array of plant defense genes (Derksen et al. 2013; Mur et al. 2006; Spoel and Dong 2008).

The SA- and JA-defense signaling pathways are at the core of defense responses. SA-regulated defenses are usually most effective against biotrophic pathogens, whereas JA-regulated defenses are most effective against insects and necrotrophic pathogens (Thaler et al. 2012). However, given the complexity of defense networks in plants, inactivation or constitutive activation of either the SA- or JA-defense pathways are likely to have ramifications on each other (cross-talk), as well as on the signaling pathways controlled by abscisic acid, auxin, ethylene, gibberellic acid, cytokinins, brassinosteroids, and/or peptide hormones (Pieterse et al. 2009; Pieterse et al. 2012; Robert-Seilaniantz et al. 2011).

Upon infestation, plants recognize phloem-feeding insects by herbivore-associated elicitors (HAEs), herbivore-associated molecular patterns (HAMPs) and effectors present in the saliva that is secreted into plant tissue via stylets (Bonaventure 2012). Recognition of HAEs and HAMPs activate signal-transduction cascades to trigger the transcription of a suite of defense genes that provide protection against insects. Insects counter these induced defenses by secreting effectors that inactivate or slow plant immune responses leading to plant susceptibility (Rodriguez and Bos 2013).

While the chemical identities of whitefly HAMPs, HAEs, and effectors of whiteflies are currently unknown, it is clear that general and species-specific HAEs are secreted by whiteflies. For example, gene discovery studies with *B. tabaci* infestations of squash (*Curcubita pepo*) showed that *B. tabaci* B secretes a different complement HAEs than *B. tabaci* A and thereby specifically activates two *B. tabaci* B-specific genes (*Silverleaf whitefly-induced 1* and *SLW2*) (van de Ven et al. 2000). In contrast, *B. tabaci* B and greenhouse whitefly, *Trialeurodes vaporariorum,* cause similar changes in tomato (*Solanum lycopersicum*) gene expression suggesting that whiteflies also have a set of shared elicitors (Puthoff et al. 2010). Both whiteflies species strongly induce several tomato JA- and ET-regulated *Pathogenesis-Related (PR)* RNAs, proteins and activities (Mayer et al. 1996; Puthoff et al. 2010; Sanchez Hernandez et al. 2006). In addition, consistent with minimal cellular disruption and the intercellular route of whitefly stylets (Freeman et al. 2001), neither *B. tabaci* B nor *T. vaporariorum* activate wound-response genes in tomato. The importance of tomato's JA-regulated defenses in defense against whiteflies was demonstrated using the mutant *suppressor of prosystemin-mediated* *responses2* (*spr2*) that accumulates low levels of JA and JA-regulated volatiles and in the transgenic line that over-expresses Prosystemin (*35S:ProSys*) and therefore has JA-regulated defenses constitutively activated (Sanchez-Hernandez et al. 2006).

Relative to tomato, Arabidopsis responds in a distinct manner after whitefly feeding (Kempema et al. 2007; Puthoff et al. 2010; Zarate et al. 2007; Zhang et al. 2013b). Both SA-biosynthesis and SA-regulated RNAs increase locally and systemically after *B. tabaci* B nymph feeding. Concurrently, there was no change or a decrease in transcript levels of JA- and ET-regulated defense gene RNAs (Kempema et al. 2007; Zarate et al. 2007). Furthermore, Zarate et al. (2007) showed that nymphal development is accelerated in the defense mutant *constitutive immunity 10* (*cim10),* which has high levels of SA and constitutively activates SA-regulated defenses (Maleck et al. 2002), and *coronatine insensitive 1 (coi1),* which cannot perceive JA (Feys et al. 1994). Reciprocally, *B. tabaci* B nymphal development was delayed in the three defense mutant lines (*npr1*, *NahG* and *cev1*). While *non-expressor of PR genes1* (*npr1)* and the transgenic line expressing salicylate hydrolase (*NahG*) block SA signal transduction and accumulation of SA, respectively, the *cev1* (*constitutive expression of VSP1*) expressed both JA- and ET-dependent defenses constitutively (Ellis and Turner 2001). Consistent with these findings Zarate et al. (2007) showed that activation of JA-regulated defenses by methyl jasmonate (MeJA) treatments causes severe delays in nymphal development.

Since SA levels increase after *B. tabaci* B infestation of Arabidopsis (Zarate et al., unpublished results) (Zhang et al. 2013b), these data suggest that crosstalk between SA-and JA-signaling pathways suppresses the effective JA-regulated defenses to promote nymphal development. Using a different set of Arabidopsis defense mutants and assessing defense gene expression and whitefly nymphal development, Zhang et al. (2013b) provided further evidence for the ability of *B. tabaci* to suppress effective defenses and the potential role of SA-JA cross talk in mediating the suppression of JA-defense response genes (Zhang et al. 2013b). Recent studies have shown that SA suppresses JA-regulated defenses through modulation of the transcription factor ORA59 (Van der Does et al. 2013); not surprisingly, ORA59 transcripts are down regulated after *B. tabaci* B feeding (Kempema 2007; Kempema et al. 2007)

While *B. tabaci* effectively reduces direct defenses to promote nymph success, it is not as successful in reducing indirect defenses (volatiles). While there are small changes in volatile emissions after whitefly feeding, the rise in SA induces the release of β-myrcene that attracts parasitoid wasps (Zhang et al. 2013a). Finally, the ability of *B. tabaci* to induce SA and suppress JA-regulated defenses is not a unique characteristic of Arabidopsis-whitefly interactions. *B. tabaci* infestations of Lima bean also showed that pre-infestation with whiteflies reduce the levels of volatiles emitted by spider mite- or caterpillar-infested plants and this influences natural enemy attraction (Zhang et al. 2009).

The ability of *B. tabaci* to manipulate plant defense signaling to enhance the rate of nymphal development by suppressing JA-regulated defenses suggested that there were changes in phloem quality in WT, JA-signaling mutants and SA-signaling mutants. In the mutants (*npr1, cev1*) that slowed nymphal development (SlowDev), these changes in plant biochemistry could include increases in compounds that are detrimental to nymphal development or interfere with nymph feeding. Reciprocally, in the mutants (*coi1*, cim10) that displayed accelerated nymphal development (the fast development genotypes, FastDev) reduced levels of detrimental phytochemicals that influence nymphal development or feeding or enhanced levels of growth-promoting chemicals in the phloem could be present. While there was no difference in host choice when whiteflies were provided all five Arabidopsis genotypes (WT, *cim10, coi1, cev1, npr1*, and *NahG*) (Kempema 2007), it is currently unknown if the interactions and feeding behaviors of adult *B. tabaci* B will be similar in all genotypes or if there will be a correlation with the SlowDev and FastDev genotypes. In this Chapter, we used DC Electrical Penetration Graph (EPG) (Tjallingii 1988) to determine if adult *B. tabaci* B displayed altered feeding behaviors on the defense signaling mutants that accelerate or slow nymphal development.

Materials and Methods

Plant Growth and Insect Rearing

Five Arabidopsis (*Arabidopsis thaliana*) plant genotypes were used in the experiments: wild type ecotype Columbia-0 (WT), and the mutants *coil*, *npr1*, *cev1*, and *cim10* (Kempema et al. 2007; Zarate et al. 2007). Seeds were pipetted onto Murashige & Skoog agar plates (1% sucrose and 0.8% [w/v] agarose) and were allowed to germinate and grow for one week. Due to their slow growth rate, *cev1* plants were plated one week prior to all other genotypes (Zarate et al. 2007). For *coi1*, seeds were supplemented 30 µM methyl jasmonate (MeJA) in 0.01% ethanol to select for homozygous seedlings; after one week, MeJA-insensitive homozygous *coi1* seedlings were selected based on elongated root and green cotyledons (Feys et al. 1994).

Whiteflies primarily feed on the abaxial side of the leaf. Leaves of plants grown in conventional pots grew too close to the soil to allow access to the abaxial sides of the leaves for EPG recording of whitefly feeding. Therefore, seedlings were transplanted into 5.5-cm tall by 2.5-cm diameter plastic bottles (Fisher, Cat# 03402B) with six 3-mm diameter holes for watering and EPG electrode entry. Plants were grown in Sunshine Mix Number 1 soil (JM McConkey Company, Inc, Cat# SUSMLC1), which was supplemented with fertilizer (Osmocote 14–14–14; Scott Horticulture Solutions). When plants matured, the leaves extended away from the relatively narrow bottles; the bottles were inverted for EPG recording allowing access to the abaxial sides of leaves. Plants in bottles were kept in 28 x 28-cm flats and were sub-irrigated. Plants were grown under fluorescent and incandescent lights (180 µE m^{-2} s^{-1}) at 22℃ under short-day (8-hr light and 16-hr dark) conditions. Plants were 4-weeks old (or 5-weeks old for *cev1*) when used in the EPG experiments.

The whitefly (*Bemisia tabaci* B) colony was maintained on *Brassica napus* var ‘Florida Broad Leaf’ (W. Atlee Burpee & Co.) in a growth room at 27℃, 55% relative humidity, and long day (16-hr light and 8-hr dark) conditions. *B. napus* plants were grown in 15-cm pots containing UC Soil Mix Number 3 (Matkin & Chandler 1957) and fertilized with Miracle-Gro (Scotts, Marysville, Ohio) under 24℃, 150 to 250 µE m^{-2} s^{-1}

lights with 12-hr light and 12-hr dark conditions. Only adult female whiteflies were used in the EPG experiments.

Electrical Penetration Graph (EPG)

All EPG experiments were performed on intact plants using Giga-4 DC-EPG monitors with 1 Giga-ohm input resistance (EPG systems, Wageningen, The Netherlands). The substrate voltage was applied to the soil of the plant via a copper wire inserted through one of the bottle's watering holes. At the beginning of each recording, the substrate voltage for each channel was adjusted to fit the +5 to -5 volt vertical axis in each channel's display window in the WINDAQ recording software. Output was analogue-to-digitally converted at 100 samples per second per channel with a DI 720-P analog-to-digital converter, and recorded using WINDAQ software (hardware and software from DATAQ Instruments, Akron, Ohio, USA).

Wiring Adult Whiteflies

Adult female whiteflies were glued to the ends of 2.5-µm diameter platinum wires (Wollaston process wire, Sigmund Cohn Corp., Mt. Vernon, New York) using electrically conductive silver glue (Electrodag 503, Ladd Research Industries, Williston, Vermont). Wollaston process platinum wire is encased in a silver sheath that was removed prior to use. First, one end of a 2-cm piece of the silver-encased platinum wire was attached to the head of a 3-mm diameter nail using silver glue. A very small drop of silver glue was then brushed onto tip of the other end of the wire. When the glue was dry, the free end of the wire was submerged in 40% nitric acid to dissolve the silver casing and expose approximately 1 cm of the 2.5-µm diameter platinum core. The exposed core was gently submerged twice in water to remove residual nitric acid and then attached to the whitefly as described below.

Whiteflies were collected in 15-ml tubes and placed at -20°C for 2 minutes. After 2 minutes, the immobilized whiteflies were placed in a Petri dish that was chilled on a cold plate (Sigma-Aldrich Techware, Milwaukee, WI). The cold plate and Petri dish were placed under a stereomicroscope to view attachment of wires to the whiteflies. A cardboard windscreen was placed around the Petri dish to shield the wire from air currents during attachment of the wires to the whiteflies. A 1 x 1-cm plastic sheet containing a fresh drop of silver glue was placed inside the Petri dish in the same field of view as the whiteflies. Using forceps to hold the nail with the platinum wire, the tip of the wire was dipped into the glue and quickly placed onto the thoracic dorsum of a female adult whitefly. The previously applied dried silver glue at the apex of the wire facilitated this process by serving as a nucleus for the fresh wet glue.

Experimental Procedure

For recording EPGs, plants were inverted so the abaxial sides of the leaves faced up. The nail with the platinum wire-tethered whitefly was inserted into the input of the head-stage amplifier of the EPG, and the head-stage amplifier was positioned so that the whitefly came in contact with the abaxial leaf surface. EPGs were recorded for 7 hours. There were five treatments: the Arabidopsis genotypes WT, *coi1, cev1, npr1*, and *cim10*. The order of genotypes was randomized, each recording used a different whitefly and plant, and recordings were made until there were 20 replications of each genotype. In a given recording session, six to eight whiteflies on randomly selected genotypes were recorded simultaneously.

Waveform Annotation and Statistical Analysis

For each recording, the beginning of each waveform was annotated using WinDaq Waveform browser. The waveforms that were annotated were: non-probing (np, whitefly stylets not in the plant), pathway phase (path, stylets penetrating through plant tissue, mostly intercellularly), potential drop (pd, intracellular punctures during pathway), sieve element salivation (waveform E1, injection of watery saliva into sieve element), sieve element ingestion (waveform E2, ingestion of phloem sap), and xylem ingestion (waveform G, ingestion of xylem sap) (Janssen et al. 1989; Jiang et al. 2000; Jiang et al. 1999; Johnson and Walker 1999). The annotated files were imported into the EPG analysis software of Sarria et al. (2009), which calculated 119 feeding behavior variables for each whitefly.

Eighty-five of the most relevant variables (Table 1.1) were analyzed using JMP software (SAS Institute, Cary, North Carolina). Means and standard deviations were calculated for each variable. Each variable was compared among the five Arabidopsis genotypes using one-way ANOVA for a completely randomized design. Variables that did not meet the ANOVA requirements of normality and homogeneity of variances were log 10- or square root-transformed and re-analyzed by one-way ANOVA. If the transformed data still did not meet the assumptions of ANOVA, data were re-analyzed using the non-parametric Wilcoxon test. Following significant ($P < 0.05$) ANOVA or Wilcoxon tests, genotype means were compared using Tukey-Kramer's All Pairs Comparison or the Dunn All Pairs for Joint Ranks Mean Comparison for ANOVA or Wilcoxon, respectively. In five variables, the AVOVA or Wilcoxon tests were significant ($P < 0.05$), but means separation tests did not detect any significant differences among the five genotypes at the $\alpha = 0.05$ level. Since ANOVA and Wilcoxon are more powerful than their respective means separation methods, in these variables the highest mean was designated as significantly different from the lowest mean, and all other means intermediate.

Additionally, the two genotypes (*cev1* and *npr1*) that delay nymphal development and the two genotypes (*cim10* and *coi1*) that accelerate nymphal development in Zarate et al. (2007) were designated SlowDev (slow nymphal development) and FastDev (fast nymphal development). The SlowDev and FastDev genotypes were pooled. For each variable, the SlowDev and FastDev groups were compared using a t-test, and if neither the untransformed data, log 10-transformed, nor square root-transformed data met the parametric assumptions, then the nonparametric Kruskal-Wallis test was used. Finally, the proportion of whiteflies that reached phloem phase during the 7-hour recording was compared among the five genotypes and between the pooled SlowDev and pooled FastDev genotypes using the Chi-squared statistic.

Eighteen of the variables were selected for principle component analysis (PCA). Variables selected included the most relevant variables relating to resistance mechanisms encounter during either pathway or phloem phase, while minimizing the total number of variables (Table 1.2). PCA was performed with the Factor procedure in PC/SAS application software (SAS Institute, Cary, North Carolina). The first four PCA factors, which accounted for approximately 73% of the total variance, were compared among the five genotypes and compared between the pooled SlowDev and pooled FastDev genotypes as described previously for the other variables.

Results

Adult Whitefly EPG Recordings and Waveforms

EPG recording success rate was 54%. Many of the unsuccessful recordings were caused by whiteflies detaching from the platinum wire, whiteflies abandoning the leaf, or electrical noise, which made the recordings uninterpretable. One hundred usable EPG recordings (twenty replicates for each Arabidopsis genotype) with seven hours of recording and minimal background noise were analyzed. Adult whitefly feeding on all five Arabidopsis genotypes produced the major waveform patterns seen in other whitefly studies: pathway (path), potential drops (pd), xylem sap ingestion (G), phloem salivation (E1), and phloem sap ingestion (E2) (Janssen et al. 1989; Jiang et al. 2000; Jiang et al. 1999; Johnson and Walker 1999; Lei et al. 1997).

Stylet penetration (a "probe") always begins with pathway phase as the whitefly stylets penetrate between epidermal and mesophyll cells, and advance deeper into the leaf tissue (Fig. 1.1-1.2). Pathway phase waveform patterns vary greatly in frequency, amplitude and duration, and the behavioral correlations of all the variations are not completely known. Whitefly stylets penetrate predominantly in an intercellular manner during pathway phase, but occasionally make brief intracellular punctures with their stylet tips into plant cells, and these are indicated by potential drops (pds) in the EPG recordings (Fig. 1.3). Penetration of the cell membrane at the beginning of a pd always causes an abrupt voltage (potential) drop due to the cell membrane potential of living cells: negative intracellular and positive extracellular. Conversely, pds always end with an abrupt increase in voltage as the stylets withdraw from a negative intracellular position to a positive extracellular position. In this study and in a prior study, whiteflies generally make intracellular punctures only after their stylets have penetrated deep into the plant during long probes (Johnson and Walker 1999). The occurrence of intracellular punctures during a probe was usually indicative that the whitefly will reach phloem phase (penetration of a phloem sieve element) later in the probe. Phloem phase is usually preceded by a few intracellular punctures.

Phloem phase (Fig. 1.4) takes place immediately after the stylet tips penetrate a sieve element and always begins with an abrupt drop in voltage in the recordings due to the membrane potential of the sieve element. The voltage level usually stays low throughout phloem phase. Phloem phase always begins with salivation into the sieve element (waveform E1) prior to ingestion of phloem sap (waveform E2) (Fig. 1.4). Waveform E1 is characterized by consistent upward peaks (Fig. 1.4, inset). Waveform E1 is usually followed by waveform E2, ingestion of phloem sap. The “classic” E2 waveform is very distinctive with a regular frequency of low-amplitude plateaus alternating with sharp downward spikes (Fig. 1.5). The amplitude and frequency of classic E2 is usually very consistent and does not vary within a phloem phase. However, there were many variations of the whitefly E2 waveform on Arabidopsis plants among the recordings; these are described in Chapter 3.

Whiteflies are presumed to ingest xylem sap when they are unable to ingest phloem sap or dehydrated, as has been demonstrated for aphids (Pompon et al. 2010; Spiller et al. 1990). The xylem consists of mainly dead cells transporting water and limited nutrients. Xylem phase (waveform G) is always preceded by pathway phase (Fig. 1.6). Waveform G is characterized by downward peaks with very steady frequency and amplitude. No voltage level drop occurred when whiteflies transition from pathway into xylem phase; this is reflective of the absence of change in membrane potential (Fig. 1.6). The transition from pathway to xylem phase is usually associated with an increase in waveform amplitude. Xylem phase usually proceeds uninterrupted for long periods (>2203 sec) (Table 1.1, variable 14), but in some cases, whiteflies may exit xylem phase, return to pathway, and then re-enter xylem phase again. Usually, the probe is terminated shortly after the end of a xylem phase.

Principal Component Analysis

PCA was completed using eighteen variables. These analyses identified four PCA factors that combined accounted for 73% of the total variance with factors 1-4 accounting for 44%, 14%, 8% and 7% of the variance, respectively (Table 1.2). The loadings of the 18 variables analyzed are given in Table 1.2. There were seven variables with high positive loading (> 40%) for PCA factor 1. Five of these variables were unique to Factor

1 including total duration of nonprobe periods before the first phloem phase (variable 7), time from first probe to first phloem phase (variable 11), time from first probe to first sustained E2 (phloem sap ingestion > 10 min) (variable 48), time from first probe to first E2 (variable 51), and time from the beginning of the first probe to first pd (variable 77). Variable 2 (number of probes before the first E1) had high positive loading with both PCA Factor 1 and Factor 2. Although the variables are not unique to PCA factor 1, variables with high negative loading (< -40%) for PCA factor 1 include average number of pds per probe (variable 9), number of E2 (variable 20), number of sustained E2 (variable 21), total duration of E2 (variable 32), and number of pd (variable 39).

Variables with high positive loading (> 40%) for PCA factor 2 were number of probes before the first E1 (variable 2), number of probes (variable 35) and number of short probes (< 3 min) (variable 37). Number of probes (variable 35) and number of short probes (< 3 min) (variable 37) are unique for PCA factor 2. Although none of the variables with high negative loading (< -40%) were unique for PCA factor 2, they include the average number of pds per probe (variable 9), total duration of E2 (variable 32), and mean duration of non-probing (variable 45). Variable 45 had high negative loading on PCA2 in contrast to its high positive loading on PCA1.

Variables with high positive loading (> 40%) for PCA factor 3 were average number of pds per probe (variable 9), number of E2 (variable 20), number of sustained E2 (variable 21), and number of pd (variable 39), but none of the variables were unique to PCA factor 3. Mean duration of G (variable 14) is the only variable with high negative loading (< -40%) and unique for PCA factor 3. Lastly, two variables with high positive loading (> 40%) and unique for PCA factor 4 were duration of first probe (variable 3) and duration of the second nonprobe period (variable 6). Number of single E1 (variable 19) had a high loading (< -40%) and was unique for PCA factor 4.

Comparison of Behaviors among Arabidopsis Genotypes

Ninety variables, 86 calculated directly from the EPG recordings plus 4 PCA factors, were used to compare whitefly feeding behaviors among the five Arabidopsis defense mutant lines *coi1*, *npr1*, *cev1*, *cim10* and WT plants, and between FastDev (combined *cim10* and *coi1*) and SlowDev (combined *cev1* and *npr1*) genotypes (Table 1.1). Very few of the variables differed significantly among the genotypes. Out of the 90 variables, 12 differed significantly among the five genotypes and four differed significantly between the pooled FastDev and SlowDev genotypes (Table 1.1).

Of the 12 variables that differed significantly among the five genotypes, the number of whiteflies that reached phloem phase during the 7-hour recordings (variable 90) is the most relevant to potential resistant factors that may interfere with locating and recognizing a phloem sieve element. The number of whiteflies that reached phloem phase was significantly higher for *cim10* (FastDev) than for *coi1* (FastDev) and *cev1* (SlowDev). However, two other variables that also reflect potential resistant factors that may interfere with locating and recognizing a phloem sieve element, time to reach phloem phase from first probe or from beginning of recording (variables 10 and 11, respectively), did not differ significantly among the five genotypes.

Immediately after penetrating and recognizing a sieve element, whiteflies inject saliva into the sieve element (E1) before initiating sap ingestion (E2). Three variables related to whitefly salivation into sieve elements differed significantly among the five genotypes (variables 24, 25, and 33). The duration of E1 followed by the first E2 (variable 25) was significantly shorter on *cim10* (FastDev) than on *cev1* (SlowDev). The duration of E1 followed by first sustained E2 (variable 24) was significantly shorter on *cim10* (FastDev) than on WT. And, the mean duration of E1 (variable 33) was significantly shorter on *cim10* (FastDev) than on *coi1* (FastDev). These three variables indicate that whiteflies salivate a shorter duration after penetration of a sieve element on *cim10* (FastDev). However, the total duration of E1 (variable 28), the total duration of E1 followed by E2 (variable 30), and the total duration of E1 followed by sustained E2 (variable 29) did not differ significantly among the five genotypes, although the latter (variable 29) was almost significantly different (P = 0.0647), with *cim10* again having the lowest mean (Table 1.1).

Two variables related to phloem sap ingestion, the mean duration of E2 (variable 34) and the duration of longest E2 (variable 79) differed significantly among the five genotypes (Table 1.1). In both variables, the values for *cev1* (SlowDev) was significantly greater that the values for *coi1* (FastDev). Two other variables related to phloem sap ingestion, the potential E2 index (variable 26), which calculates the percentage of time the whitefly spends in E2 after reaching the first E2, and the total duration of E2 (variable 32) showed the same trend (*cev1* was highest and *coi1* lowest), but the difference was not significant.

Two variables relating duration of probing and non-probing differed significantly among the genotypes. Duration of the second probe (variable 4) was significantly longer on *cim10* (FastDev) compared to *npr1* (SlowDev). In contrast, duration of first probe (variable 3) did not differ significantly among the five genotypes. Total duration of non-probing time (variable 42) was significantly higher for *coi1* (FastDev) than for *cim10* (FastDev) and *cev1* (SlowDev).

The four remaining variables that differed significantly among the five genotypes (variables 62, 68, 69, 75) measured behaviors during a specific hour of the EPG recording: the number or the average duration of potential drops or the number of probes during fourth or fifth hour of recording. Number of probes during the fifth hour (variable 75) was significantly greater on *coi1* (FastDev) than on *cim10* (FastDev). Number of pds during the fourth hour (variable 62) was significantly greater on *cim10* (FastDev) than on *coi1* (FastDev). Average duration of pds during the fourth hour (variable 68) was significantly greater on *cev1* (SlowDev) than on *cim10* (FastDev) or *npr1* (SlowDev). Average duration of pds during the fifth hour (variable 69) was significantly greater on *cev1* (SlowDev) than on *coi1* (FastDev). However, number of probes (variable 35), number of pds (variable 39), and mean duration of pds (variable 8) over the entire 7-hour recording did not differ significantly among the five genotypes.

In comparisons between the pooled FastDev and pooled SlowDev genotypes, four variables differed significantly (variables 4, 9, 29, and 30). The duration of the second probe (variable 4) was significantly longer on the pooled FastDev genotypes. In contrast, duration of first probe (variable 3) did not differ significantly between the pooled SlowDev and pooled FastDev genotypes. Total duration of E1 followed by E2 (variable 30) and total duration of E1 followed by sustained E2 (variable 29) were significantly lower on the pooled FastDev genotypes than on the pooled SlowDev genotypes. In contrast, there was no significant difference between the pooled FastDev and pooled SlowDev genotypes in the total duration of E1 (variable 28) and mean duration of E1 (variable 33). Finally, the average number of potential drops per probe (variable 9) was significantly higher on the pooled SlowDev genotypes than on the pooled FastDev genotypes.

Discussion

Previous studies that monitored defense gene expression and nymphal development on JA- and SA-defense mutants and after MeJA treatment indicated that elevated JA-regulated defenses are important in constraining *B. tabaci* B nymphal development on Arabidopsis plants (Kempema et al. 2007; Zarate et al. 2007). Nymphal development is accelerated on *cim10* and *coi1* plants but delayed on *npr1,* NahG and *cev1* mutants relative to WT plants (Zarate et al. 2007). Defense mechanisms that slow insect development are important resistance strategies that can negatively impact insect population expansion, as well as providing a larger window of opportunity for natural enemies to attack their insect host (Arimura et al. 2010; Holopainen 2004). Based on nymphal development rates and the fact that SA- and JA-response genes were reciprocally regulated in the FastDev and SlowDev genotypes in Zarate et al. (2007), we hypothesized that resistance factors would be elevated in the SlowDev genotypes (*cev1* and *npr1*) relative to WT plants and therefore adult whitefly feeding would be negatively impacted. Reciprocally, the FastDev genotypes (*cim10* and *coi1)* should express fewer deterrents to adult whitefly feeding. The EPG studies presented in this Chapter do not support this simple hypothesis.

Principal Component Analysis

Principal component analysis performed on eighteen variables yielded eighteen PCA factors. Four criteria are generally used to determine the number of PCA factors to retain (Lehman et al. 2005). The first criterion is that the PCA factor accounts for at least 10% of the proportion of variance. With this rule, only PCA factors 1 and 2 would be retained (Table 1.2). The second criterion is that PCA factors with an eigenvalue greater than 1 are retained, and PCA factors 1 to 5 met this rule. The third criterion uses a scree plot, which plots the PCA factors on the x-axis and the eigenvalues on the y-axis, and only PCA factors before the point of sudden drop on the graph should be retained. A scree plot indicated that only the first two PCA factors in this study should be retained. Lastly, the fourth criterion is that the cumulative variance of the PCA factors combined is greater than 70%. Using this criterion the first four PCA factors should be retained in this study for a cumulative variance of 73% (Table 1.2).

Variables with high positive loading (> 40%) for PCA factor 1 were: number of probes before the first E1 (variable 2), total duration of non-probe periods before the first E (variable 7), time from first probe to first E (variable 11), means duration of non-probing (variable 45), time from first probe to first sustained E2 (variable 48), time from first probe to first E2 (variable 51), and time from the beginning of the first probe to first pd (variable 77) (Table 1.2). Variables with high negative loading (< -40%) for PCA factor 1 were: average number of pds per probe (variable 9), number of E2 (variable 20), number of sustained E2 (variable 21), total duration of E2 (variable 32), and number of pd (variable 39). The variables with high positive correlation with PCA factor 1 reflects long duration of non-probe periods, higher number of probes and longer time to reach phloem phase and potential drops. Whiteflies make potential drops (pds) only when they have penetrated deep into the plant tissue (Johnson and Walker 1999). High negative variables for PCA factor 1 reflects low numbers of pds, low number of phloem sap-ingestion and sustained ingestion phases, and short duration of phloem sap-ingestion. Consequently, high positive loadings for all of these variables indicate PCA factor 1 was associated with whiteflies having difficulty reaching and locating a sieve element. PCA factor 1 did not differ significantly between the pooled SlowDev and pooled FastDev genotypes, and among the five individual genotypes.

Variables with high positive loading (> 40%) for PCA factor 2 were: number of probes before the first E1 (variable 2), number of probes (variable 35) and number of short probes (variable 37). Variables with high negative loading (< -40%) were: average number of pds per probe (variable 9), total duration of E2 (variable 32), and mean duration of non-probing (variable 45). For phloem sap-feeding insects, there is generally an inverse relationship between number of probes and probe duration. On favorable hosts, the probes are generally longer and consequently, there are fewer probes during a given period of time (Sauge et al. 1998). In contrast, on unfavorable plants, insects generally withdraw their stylets without having penetrated far, and then try probing again over and over, resulting in a greater number of short-duration probes. Consequently, it is not surprising that both of these variables are associated with the same PCA factor. Since whiteflies rarely puncture epidermal and mesophyll cells (Freeman et al. 2001), greater numbers of shallow probes (ie., short-duration probes) could be interpreted as the whiteflies detecting something in the apoplast of epidermis or mesophyll cells that causes them to terminate their probes. Furthermore, high number of probes before the first E1 and low total duration of E2 are variables indicative of poor whitefly feeding behavior. PCA factor 2 did not differ significantly between the pooled SlowDev and pooled FastDev genotypes, nor among the five individual genotypes.

Variables with high positive loading (> 40%) on PCA factor 3 were: average number of pds per probe (variable 9), number of E2 (variable 20), number of sustained E2 (variable 21), and number of pd (variable 39). These variables all had a high negative loading for PCA factor 1. Mean duration of G (variable 14) was the only variable that had a high negative loading (< -40%) and was unique to PCA factor 3. The negative loading of variable 14 indicated that high values of PCA factor 3 were associated with short mean duration of xylem ingestion. When phloem sap-feeding insects have difficulty ingesting phloem sap, they often increase their ingestion of xylem sap. Reciprocally, if the insects are successfully ingesting phloem sap, they tend to spend less time ingesting xylem sap (Powell and Hardie 2002; Spiller et al. 1990). Accordingly, PCA factor 3 factor reflected high number pds, high number of E2s and sustained E2s. Consequently, PCA factor 3 should be associated with whiteflies feeding favorable. PCA factor 3 did not differ significantly between the pooled SlowDev and pooled FastDev genotypes, nor among the five individual genotypes.

Lastly, variables with high positive loading (> 40%) for PCA factor 4 were: duration of first probe (variable 3) and duration of the second nonprobe period (variable 6). Number of single E1 (variable 19) was the only variable with high negative loading (> -40%) and was unique for PCA factor 4. The significance of the two variables that had high positive loading for PCA factor 4 was difficult to interpret. The high negative loading of number of single E1s (phloem phases that failed to make the transition from salivation to ingestion) indicated that whiteflies with high values of PCA factor 4 tended to have difficulty initiating ingestion after penetrating a sieve element. PCA factor 4 did not differ significantly between the pooled SlowDev and pooled FastDev genotypes, and among the five individual genotypes.

Pooled FastDev Verses Pooled SlowDev Genotypes

Data from two FastDev (*cim10* and *coi1*) genotypes were combined and compared to the two SlowDev (*cev1* and *npr1*) genotypes. This comparison used ninety variables, of which four are PCA factors and provided little support for the hypothesis that adult whiteflies feed better on the SlowDev than on the FastDev genotypes. Out of ninety variables, only four variables differed significantly between the pooled FastDev and pooled SlowDev genotypes: duration of the second probe (variable 4), average number of potential drops per probe (variable 9), total duration of E1 followed by E2 (variable 30), and total duration of E1 followed by sustained E2 (>10 min) (variable 29).

The duration of second probe (variable 4) was significantly shorter on both SlowDev genotypes. The duration of the first probe (variable 3) was also much shorter on the SlowDev genotypes, but due to the high variance, this difference was not significant. This analysis of pooled SlowDev and pooled FastDev genotypes indicated that whiteflies detected a difference between SlowDev and FastDev genotypes in the earliest probes and responded by terminating the probes sooner on the SlowDev genotypes.

Average number of potential drops per probe (variable 9) was significantly greater on the SlowDev genotypes. Whiteflies make cell punctures (pds) after they have penetrated deeply into leaf tissue (Johnson & Walker 1999), so a significantly greater number of potential drops per probe in the SlowDev genotypes may suggest that the whiteflies spend more time with their stylets deep in the leaf tissue. However, there was no significant difference between the pooled SlowDev and pooled FastDev genotypes in number of whiteflies that reached phloem phase (variable 90) or in the time it took them to reach phloem phase (variables 10 and 11). Consequently, the relevance, if any, of number of potential drops per probe to resistance/susceptibility is unclear.

Long duration of salivation (E1) into sieve elements preceding sap ingestion (E2) has sometimes been interpreted as an indication of difficulty initiating sap ingestion, which may contribute to resistance (Tjallingii 2006). On the SlowDev genotypes, the total duration of E1 followed by E2 (variable 30) and the total duration of E1 followed by sustained E2 (variable 29) were both significantly greater than the FastDev genotypes. In contrast, there was no significant difference in the total duration or mean duration of phloem sap ingestion (E2) (variables 32 and 34) when the pooled SlowDev and pooled FastDev genotype data was compared.

It is important to note that with 90 variables and a significance level of $\alpha = 0.05$, four to five variables would be expected to be declared significant by random chance. Consequently, with only four variables differing significantly between the pooled SlowDev and pooled FastDev genotypes, the difference in feeding behavior between the SlowDev and FastDev genotypes should be viewed cautiously. Furthermore, the main feeding behavior variables that would reflect changes in plant resistance or susceptibility factors that are shared in SlowDev and FastDev genotypes would be the number of whiteflies that reached phloem phase (variable 90), time it took them to reach phloem phase (variables 10 and 11), and time spent ingesting phloem sap (variables 32 and 34). None of these differed significantly between the pooled SlowDev and pooled FastDev genotypes.

Comparison of Behaviors among Arabidopsis Genotypes

Comparisons among the five individual genotypes yielded more significant differences in adult feeding behaviors than did the comparisons between the pooled SlowDev and pooled FastDev genotypes. Collectively these data indicate that while the *coi1* and *cim10* mutants promote accelerate nymphal development and *npr1* and *cev1* slow nymphal development (Zarate et al, 2007), the resistance traits that alter nymphal development did not clearly translate to adult behaviors that are correlated with both FastDev or both SlowDev genotypes. Each of the defense mutants used in this study alter adult whitefly feeding behaviors distinctly as described below.

The comparisons among the five genotypes identified 12 variables that differed significantly among the five genotypes. However, again, with a significance level of $\alpha = 0.05$ and comparisons of 90 variables, it is expected that four to five variables would be significant by chance alone. Consistent with this fact, four of the 12 significant variables had no clear relation to whitefly feeding more or less favorably. These variables included the number of potential drops during the fourth hour (variable 62), average duration of potential drops during the fourth and fifth hours (variables 68 and 69), and number of probes during the fifth hour (variable 75).

Duration of second probe (variable 4) was significantly longer on *cim10* (FastDev) than on *npr1* (SlowDev) Duration of the first probe (variable 3) was also much longer on *cim10* (FastDev) than on all the other genotypes except for WT, but due to the high variance, this difference was not significant. The biological interpretation of longer early probes is unclear. It could mean that the whiteflies detected something favorable during initial contact with the plant or it could mean it took them longer to reach the depth at which they make a decision whether or not to withdraw stylets. Nonetheless, the significantly greater duration of the second probe on *cim10* (FastDev) versus *npr1* (SlowDev) is consistent with the significantly faster nymph developmental rate on *cim10* compared to *npr1* (Zarate et al. 2007). However, this correlation was not seen in the other FastDev (*coi1*) and SlowDev (*cev1*) genotypes.

While the remaining seven variables that differed significantly among the five genotypes may reflect differences in resistance or susceptibility factors, there were no adult feeding behaviors among the five genotypes that strictly correlated with the nymph FastDev or SlowDev phenotypes. The number of whiteflies that reached phloem phase during the 7-hour recordings (variable 90) was significantly higher on *cim10* (FastDev) than on *coi1* (FastDev) and *cev1* (SlowDev). This observation concurred with accelerated nymph growth on *cim10* plants compared to *cev1*. However, based solely on nymphal development rates, it was counter-intuitive to discover that the FastDev genotype *coi1* had the lowest number of whiteflies reaching phloem phase. Furthermore, the number of whiteflies reaching phloem phase was not significantly greater on *cim10* (FastDev) compared to *npr1* (SlowDev) or WT plants. These data suggest that there was no strict correlation of nymphal development rates with the ability of adult whiteflies to reach the phloem. While it appears that whiteflies may have easier access to the phloem on *cim10* plants compared to *coi1* and *cev1*, other variables that reflect ease or difficulty reaching the phloem did not differ significantly among the five genotypes. These additional variables included the time from start of EPG to first E (variable 10), time from first probe to first E (variable 11), and time from the beginning of that probe to first E (variable 12).

Long durations of non-probing are generally considered an indication of low host acceptability. While there were genotypes differences in this behavior they did not correlate with the ability of nymphs to develop on the FastDev and SlowDev genotypes. For example, the total duration of nonprobing time (variable 42) was significantly longer on *coi1* (FastDev) than on *cim10* (FastDev) and *cev1* (SlowDev). If the resistance factors that delay nymphal development were co-regulated with resistance traits perceived by whitefly adults, we would have expected longer periods of non-probing on the SlowDev *cev1* and *npr1* genotypes.

At the start of each phloem phase, whiteflies salivate into the sieve element (waveform E1) before ingesting sap (waveform E2). Long durations of E1 are usually associated with difficulty initiating sap ingestion after a sieve element is penetrated (Tjallingii 1994; 2006). While several of the variables related to the duration of E1 differed significantly among the five genotypes, adult whiteflies did not behave similarly on FastDev (*cim10* and *coi1*) versus SlowDev (*cev1* and *npr1*) plants. However, it is noteworthy that in each of these statistically significant behaviors (variables 24, 25 and 33), *cim10* had the shortest duration of E1. For example, the duration of E1 followed by the first E2 (variable 25) was significantly shorter on *cim10* than on *cev1*, which corresponded to the slower nymphal developmental rate on *cev1* compared to *cim10.* In addition the duration of E1 followed by first sustained E2 (variable 24) was significantly shorter on *cim10* plants than on WT, which corresponds to the accelerated nymph developmental rate on *cim10*. Finally, the mean duration of E1 (variable 33) was significantly shorter on *cim10* than on *coi1;* surprisingly both these genotypes had accelerated nymphal development. Collectively, these sets of pairwise comparisons with *cim10* suggest that *cim10* plants are more acceptable than other genotypes.

The duration of E2 is often associated with host plant suitability. Longer E2s are reflective of a better quality phloem sap indicating better nutrition or reduced feeding deterrents or toxins. Here we showed that the mean duration of E2 (variable 34) and the duration of longest E2 (variable 79) were both significantly longer on *cev1* (SlowDev) than on *coi1* (FastDev) mutant. This observation about adults is counter-intuitive relative to nymphal development rates. However, this finding is interesting as that *cev1* and *coi1* have opposite effects on JA-regulated defenses. While *cev1* expresses JA- and ET-regulated defenses constitutively, *coi1* cannot perceive JA-Ile and therefore has an impaired JA-defense response (Ellis and Turner 2001; Feys et al. 1994). While variables 34 and 79 suggest that the adult whitefly was able to ingest phloem-sap longer on *cev1* (SlowDev) compared to *coi1* (FastDev), two other variables related to phloem sap suitability are not consistent with this conclusion. For example, the potential E2 index (variable 26) and the total duration of E2 (variable 32) were not significant among the five genotypes.

Conclusion and Future Directions

Zarate et al. (2007) found significantly faster nymphal developmental rates in many paired comparisons of the JA-signaling and SA-signaling mutants and WT plants (eg., *cim10* >WT, *cev1, npr1; coi1* > *cev1, npr1;* and WT > *cev1, npr1*). In this Chapter, we tested the hypothesis that genotypes with fast (*cim10, coi1*) versus slow (*cev1, npr1*) nymphal development rates would display distinct adult feeding behaviors. We found that adult whitefly feeding behaviors were not strictly correlated with these genotype-dependent changes in nymphal development. In fact, there were few adult feeding variables that differed significantly among the five genotypes (12 out of 90 variables) and even fewer that differed significantly between the pooled data analysis (four out of 90 variables).

Each of the adult feeding behaviors that differed significantly among genotypes had some relevance to host plant acceptance/suitability (variables 4, 24, 25, 33, 34, 42, 79, 90). However in most cases, there was only one pair of genotypes where this comparison was significant. In addition, the genotype pairs that were significantly different were not the same for each of these variables. Despite these limitations, adult whiteflies on *cim10* plants repeatedly displayed different behaviors (variables 24, 25, 33, 90). However, it is difficult to interpret meaning of the differences in variables 24, 25, 33, 90, since other major variables that indicate a favorable feeding were not significantly different in *cim10* or other genotypes.

Collectively these data indicated that the compelling differences in nymphal development rates that were observed when *cim10* and *coi1* were compared with WT, *cev1*, and *npr1* did not translate to consistent changes adult feeding behaviors. These data suggest that the changes in phloem biochemistry in the JA- and SA-defense mutants used in this study that accelerate or slow nymphal development are: (1) in insufficient quantities to influence adult behaviors, (2) are not perceived by adults, or (3) are influence nymphs and adults feeding behaviors in distinct manners. In Chapter 2, we test hypothesis 3 and determine whether nymph feeding behaviors are altered in FastDev (*cim10*, *coi)* and SlowDev (*npr1*, *cev)* mutants relative to WT plants.

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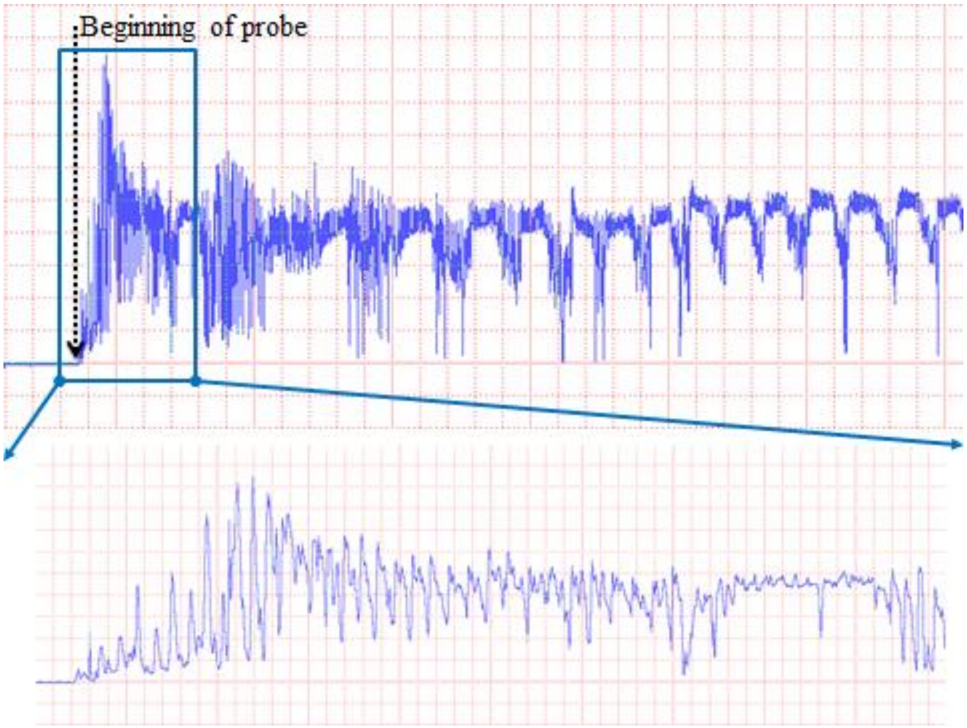

Figure 1.1. Adult whitefly (*B. tabaci B*) pathway phase (stylet penetration) waveform. Vertical axis is the voltage. Horizontal axis is time. Divisions on the horizontal axis: 2 sec per division. Inset: detailed view at 0.2 sec per division. Beginning of a long probe with a long pathway phase phase.

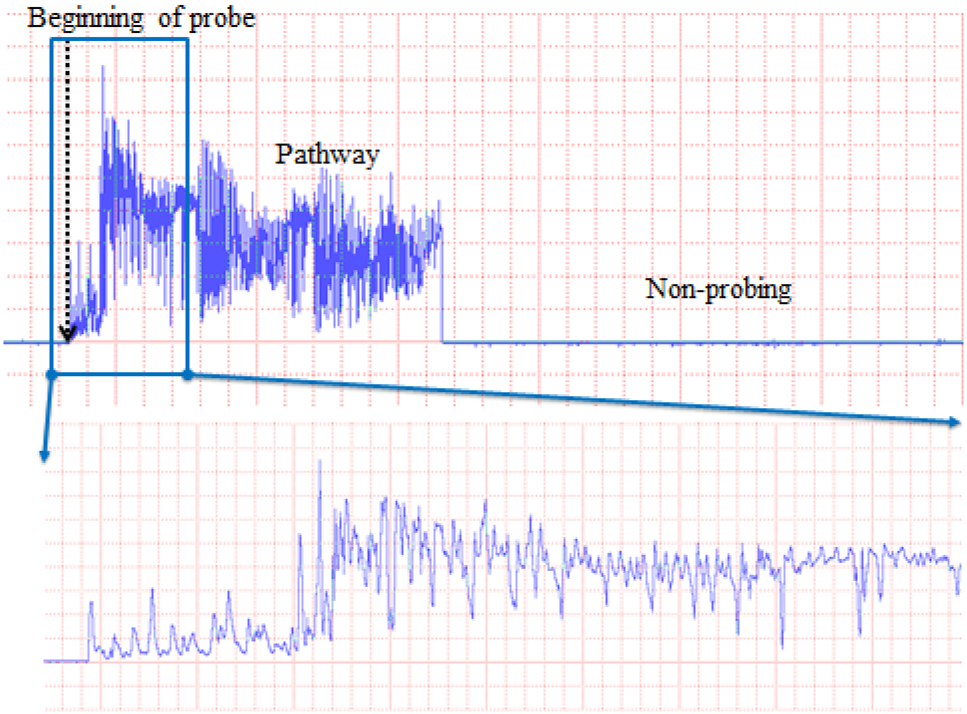

Figure 1.2. Adult whitefly (*B. tabaci B*) pathway phase (stylet penetration) waveform. Vertical axis is the voltage. Horizontal axis is time. Divisions on the horizontal axis: 2 sec per division. Inset: detailed view at 0.2 sec per division. Short probe consisting entirely of pathway phase.

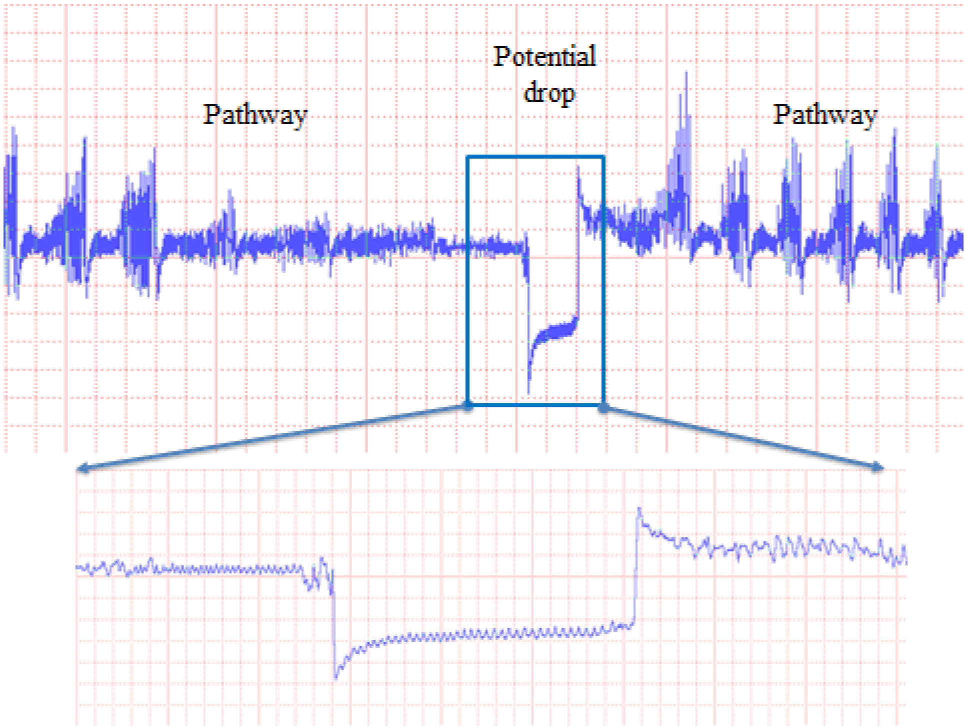

Figure 1.3. Adult whitefly (*B. tabaci B*) potential drops (intracellular punctures by stylet tips). Vertical axis is the voltage. Horizontal axis is time. Divisions on the horizontal axis: 2 sec per division. Inset: detailed view at 0.2 sec per division.

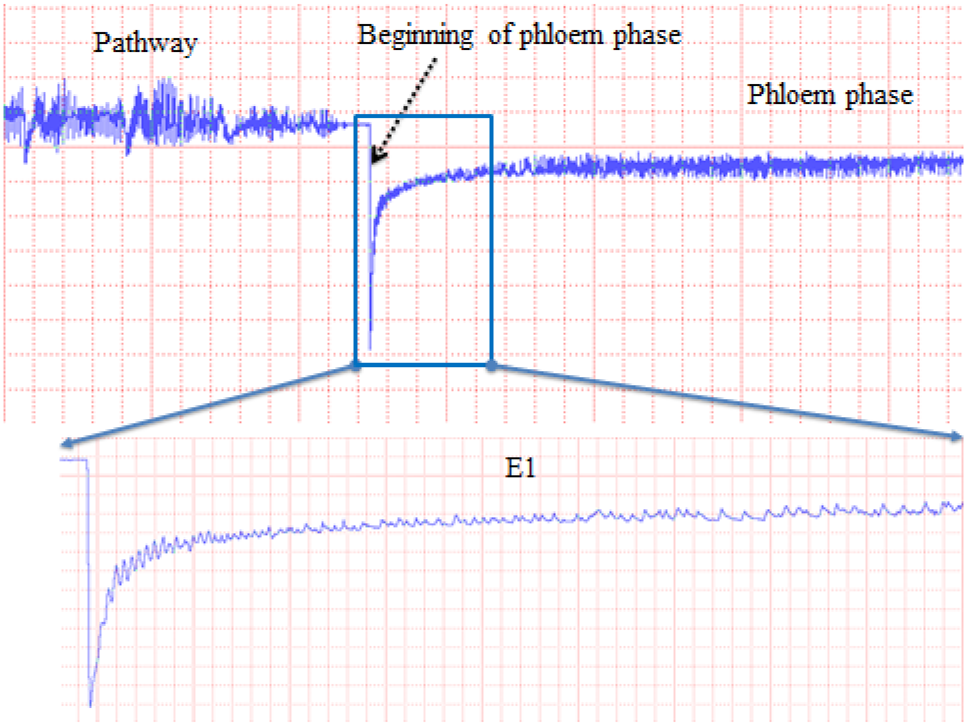

Figure 1.4. Adult whitefly (*B. tabaci B*) beginning of phloem phase and waveform E1 (salivation into a sieve element) waveform. Vertical axis is the voltage. Horizontal axis is time. Divisions on the horizontal axis: 2 sec per division. Inset: detailed view at 0.2 sec per division.

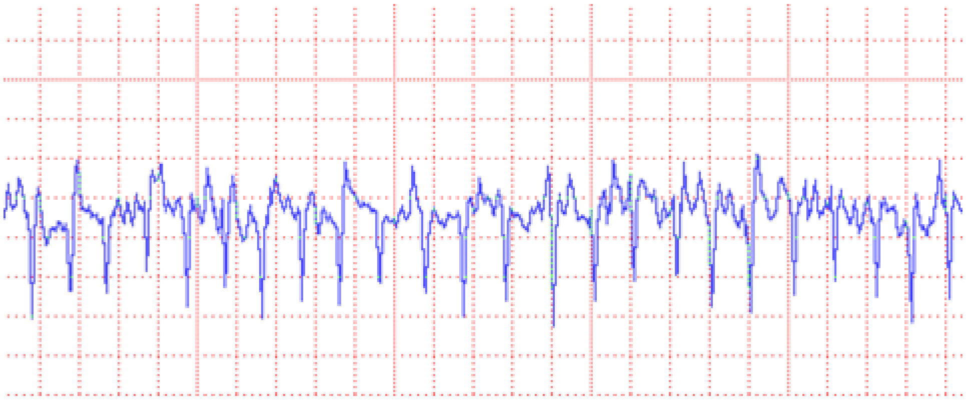

Figure 1.5. Adult whitefly (*B. tabaci B*) phloem sap-ingestion: waveform E2. E2 is always preceded by E1 and occurs at the same voltage level. An example of a "classic" E2 is shown. Other variations also occur (see Chapter 3). Vertical axis is the voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.2 sec per division.

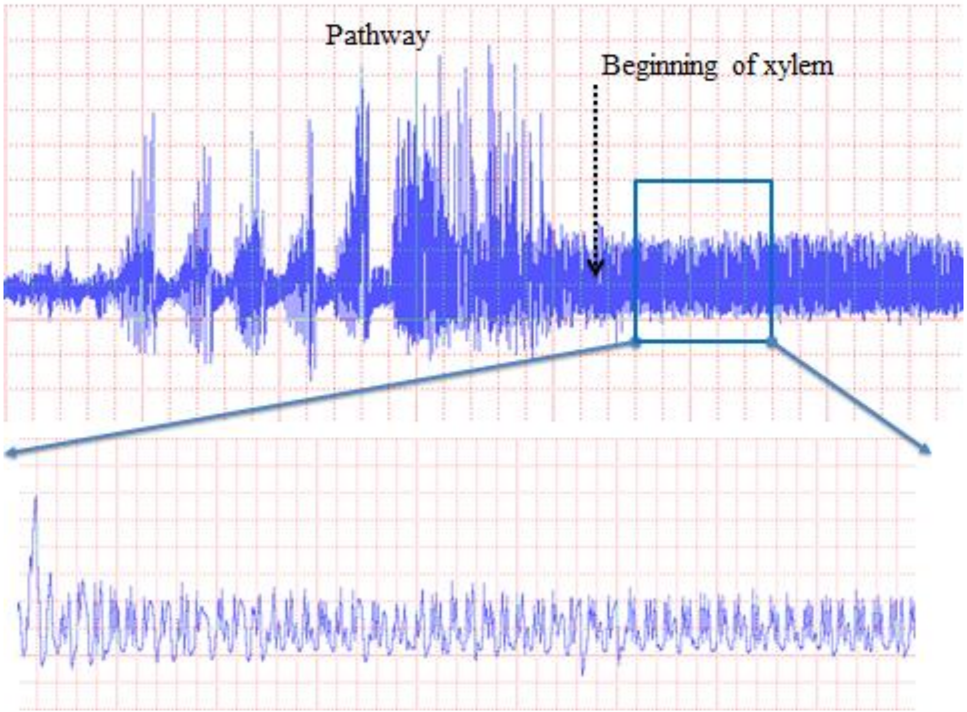

Figure 1.6. Adult whitefly (*B. tabaci B*) xylem sap-ingestion (waveform G). Vertical axis is the voltage. Horizontal axis is time. Divisions on the horizontal axis: 2 sec per division. Inset: detailed view at 0.2 sec per division.

Table 1.1: Whitefly adult feeding behavior variables and principle component values for each of five Arabidopsis genotypes (WT, *cim10*, *coi1*, *cev1*, and *npr1*) and for pooled "FastDev" genotypes (*cim10* + *coi1*) and pooled SlowDev genotypes (*cev1*+ *npr1*).

Variable	WT	*cim10* FastDev	*coi1* FastDev	*cev1* SlowDev	*npr1* SlowDev	P value (comparison of 5 genotypes)	FastDev *cim10* + *coi1*	SlowDev *cev1* + *npr1*	P value (R vs. S)
1. Time to 1st probe from start of EPG (min)	1.45± 2.29 (20)	2.66± 7.82 (20)	1.44± 2.69 (20)	1.98± 5.21 (20)	3.53± 6.19 (20)	0.1587[a]	2.05± 5.81 (40)	2.76± 5.70 (40)	0.0727[a]
2. Number of probes before the 1st E1	36.50± 28.78 (20)	31.05± 24.29 (20)	51.25± 46.38 (20)	36.40± 24.12 (20)	39.65± 32.88 (20)	0.4306[b]	41.15± 37.95 (40)	38.02± 28.51 (40)	0.9707[b]
3. Duration of 1st probe (sec)	556.82± 2115.6 (20)	493.28± 1554.47(20)	57.88± 63.47 (20)	108.47± 152.41 (20)	145.67± 303.48 (20)	0.4991[a]	275.58± 1108.05 (40)	127.07± 237.78 (40)	0.6826[a]
4. Duration of 2nd probe (sec)	126.58± 229.69 (20) **AB**	384.26± 748.48 (20) **A**	82.40± 114.89 (20) **AB**	102.42± 146.85 (20) **AB**	55.20± 122.23 (20) **B**	**0.0149[b]**	233.33± 550.21(40) **A**	78.81± 135.48(40) **B**	**0.0164[b]**
5. Duration of the shortest pathway before E1 (min)	9.60± 10.28 (20)	11.31± 6.96 (20)	6.89± 9.20 (20)	12.93± 15.36 (20)	7.81± 5.55 (20)	0.2706[a]	9.10± 8.36 (40)	10.37± 11.69 (40)	0.8966[a]
6. Duration of the second nonprobe period (min)	1.52± 1.73 (20)	1.10± 1.50 (20)	2.78± 4.84 (20)	2.70± 7.05 (20)	1.19± 2.09 (20)	0.0732[b]	1.94± 3.64 (40)	1.95± 5.19 (40)	0.1351[b]
7. Total duration of nonprobe periods before the 1st phloem phase (min)	87.05± 74.91 (20)	55.74± 42.26 (20)	116.31± 99.37 (20)	70.42± 64.93 (20)	87.07± 83.56 (20)	0.4436[a]	86.03± 81.37 (40)	78.75± 74.34 (40)	0.9783[b]
8. Mean duration of pds (sec)	4.88± 1.40 (20)	4.42± 1.15 (20)	3.98± 1.12 (20)	5.49± 2.09 (20)	4.59± 1.52 (20)	0.1654[b]	4.25± 1.14 (30)	5.01± 1.83 (30)	0.0846[b]
9. Average number of pds per probe	0.18± 0.28 (20)	0.25± 0.30 (20)	0.12± 0.19 (20)	0.36± 0.54 (20)	0.34± 0.36 (20)	0.1571 [b]	0.19± 0.26 (40) **B**	0.35± 0.45 (40) **A**	**0.0163[b]**

10. Time from start of EPG to 1st E (min)	220.17± 150.80 (20)	182.76± 126.10 (20)	252.19± 160.27 (20)	219.23± 161.58 (20)	207.80± 140.01 (20)	0.6662 [a]	217.48± 146.62 (40)	213.51± 149.34 (40)	0.9655[a]
11. Time from 1st probe to 1st phloem phase (min)	218.71± 151.02 (20)	180.09± 127.75 (20)	250.75± 160.46 (20)	217.24± 160.11 (20)	204.27± 138.03 (20)	0.5220[a]	215.42± 147.56 (40)	210.75± 147.69 (40)	0.9348[a]
12. Time from the beginning of that probe to 1st E (min)	18.95± 13.46 (14)	14.39± 8.31 (19)	16.03± 12.73 (11)	26.93± 17.43 (13)	14.16± 5.314 (16)	0.1171[b]	14.99± 9.97 (30)	19.89± 13.67 (29)	0.0776[b]
13. Number of G	0.45± 0.68 (20)	0.5± 0.60 (20)	0.45± 0.75 (20)	0.45± 0.68 (20)	0.6± 0.75 (20)	0.9178[a]	0.47± 0.67 (40)	0.52± 0.71 (40)	0.8159[a]
14. Mean duration of G (min)	43.05± 32.49 (7)	36.73± 28.54 (9)	45.11± 18.09 (7)	50.48± 23.24 (7)	42.70± 23.08 (9)	0.7743[c]	34.86± 18.79 (16)	35.16± 13.82 (16)	0.9603[d]
15. Total Duration of G (min)	10.99± 18.40 (20)	14.16± 20.41 (20)	13.73± 21.82 (20)	13.85± 21.19 (20)	14.26± 18.06 (20)	0.9579[a]	16.16± 25.02 (40)	18.44± 26.86 (40)	0.8748[a]
16. Number of probes shorter than 3 minutes after 1st E	16.4± 25.35 (20)	12.55± 15.99 (20)	22.05± 31.51 (20)	13.5± 23.27 (20)	20.95± 30.60 (20)	0.6815[a]	23.06± 26.68 (30)	23.75± 29.36 (29)	0.6419[a]
17. Number of E1	1.75± 1.40 (20)	2.45± 1.93 (20)	1.55± 1.70 (20)	1.75± 2.17 (20)	3.3± 2.73 (20)	0.0883[a]	2± 1.85 (40)	2.52± 2.56 (40)	0.6002 [a]
18. Number of E1 longer than 10 minutes followed by E2	0± 0 (20)	0± 0 (20)	0± 0 (20)	0± 0 (20)	0± 0 (20)	na	0 (40)	0 (40)	na
19. Number of single E1	0.05± 0.22 (20)	0.15± 0.36 (20)	0.05± 0.22 (20)	0.1± 0.30 (20)	0.25± 0.55 (20)	0.4675[a]	0.1± 0.30 (40)	0.17± 0.44 (40)	0.4861 [a]
20. Number of E2	1.7± 1.38 (20)	2.3± 1.83 (20)	1.5± 1.60 (20)	1.65± 2.09 (20)	3.05± 2.76 (20)	0.1694[a]	1.9± 1.75 (40)	2.35± 2.50 (40)	0.7985[a]
21. Number of sustained E2 (longer than 10 minutes)	1.3± 1.08 (20)	1.6± 1.78 (20)	1.1± 1.29 (20)	1.35± 1.49 (20)	1.8± 1.28 (20)	0.4030[a]	1.35± 1.56 (40)	1.57± 1.39 (40)	0.3181[a]
22. Duration of 1st E (min)	70.16± 92.61 (14)	64.71± 78.47 (19)	41.46± 57.76 (11)	113.33± 111.21 (13)	35.48± 67.05 (16)	0.1543[a]	56.18± 71.43 (30)	70.38± 96.23 (29)	0.8260[a]
23. Contribution of E1 to phloem phase (%)	2.36± 3.39 (14)	2.72± 3.25 (19)	8.00± 15.26 (11)	1.14± 0.85 (13)	3.30± 3.45 (16)	0.2546[b]	4.66± 9.67 (30)	2.33± 2.81 (29)	0.7099[b]
24. Duration the E1 followed by first	44.43± 28.26 (14)	24.96± 16.93 (16)	30.69± 11.13 (10)	60.25± 57.42 (13)	53.92± 78.21 (16)	**0.0327**[a]	27.16± 14.99 (26)	56.76± 68.56 (29)	0.0579[a]

sustained E2 (>10 min) (sec)	**A**	**B**	**AB**	**AB**	**AB**				
25. Duration the E1 followed by the first E2 (sec)	35.80± 17.78 (14) **AB**	24.74± 19.72 (19) **B**	27.86± 13.26 (11) **AB**	59.32± 58.19 (13) **A**	30.14± 18.09 (16) **AB**	**0.0335**[a]	25.89± 17.45 (30)	43.22± 42.95 (29)	0.0622[a]
26. Potential E2 index	58.44± 33.20 (14)	51.33± 33.66 (19)	34.83± 22.81 (11)	66.90± 28.83 (13)	50.27± 31.10 (16)	0.1617[a]	45.28± 30.79 (30)	57.72± 30.75 (29)	0.1600[a]
27. Total duration of E (min)	109.35± 104.41 (20)	118.46± 97.02 (20)	65.93± 89.41 (20)	126.36± 112.77 (20)	102.13± 97.15 (20)	0.3003[a]	92.20± 95.85 (40)	114.24± 104.61 (40)	0.4231[a]
28. Total duration of E1 (sec)	80.80± 86.7 (20)	67.33± 49.81 (20)	308.21± 1214.31 (20)	74.69± 77.01 (20)	122.30± 146.38 (20)	0.2678[a]	187.77± 857.00 (40)	98.50± 117.93 (40)	0.1786[a]
29. Total duration of E1 followed by sustained E2 (>10 min) (sec)	90.28± 66.50 (14)	49.83± 34.62 (16)	61.61± 24.08 (10)	96.97± 55.60 (13)	88.04± 86.32 (16)	0.0647[b]	54.36± 31.021 (26) **B**	92.047± 73.056 (29) **A**	**0.0156**[b]
30. Total duration of E1 followed by E2 (sec)	114.42± 82.31 (14)	66.16± 45.69 (19)	70.66± 25.96 (11)	111.25± 65.27 (13)	139.31± 145.80 (16)	0.0918[b]	67.81± 39.15 (30) **B**	126.73± 115.82 (29) **A**	**0.0120**[b]
31. Total duration of single E1 (sec)	0.70± 3.15 (20)	4.48± 13.03 (20)	269.35± 1204.57 (20)	2.38± 7.35 (20)	10.85± 34.83 (20)	0.4943[a]	136.91± 851.45 (40)	6.61± 25.21 (40)	0.5191[a]
32. Total duration of E2 (min)	108.00± 104.14 (20)	117.34± 96.9 (20)	60.79± 84.31 (20)	125.11± 112.03 (20)	100.09± 97.09 (20)	0.2348[a]	89.07± 94.11 (40)	112.60± 104.25 (40)	0.3955[a]
33. Mean duration of E1 (sec)	42.71± 17.14 (14) **AB**	28.77± 16.98 (19) **B**	125.10± 321.16 (11) **A**	57.85± 54.35 (13) **AB**	35.30± 19.94 (16) **AB**	**0.0403**[a]*	64.09± 194.87 (30)	45.41± 40.11 (29)	0.0853[a]
34. Mean duration of E2 (min)	84.37± 76.12 (14) **AB**	69.77± 71.67 (19) **AB**	43.85± 41.47 (11) **B**	121.39± 97.18 (13) **A**	53.00± 72.95 (16) **AB**	**0.0401**[a]*	60.27± 62.79 (30)	83.66± 89.98 (29)	0.1921[b]
35. Number of probes	56± 33.03 (20)	48.5± 23.38 (20)	77± 43.79 (20)	52± 28.75 (20)	64.05± 36.37 (20)	0.1053[c]	62.75± 37.53 (40)	58.02± 32.93 (40)	0.4917[c]
36. Number of C	57.55± 33.56 (20)	50.6± 22.85 (20)	78.55± 43.77 (20)	53.75± 29.21 (20)	67.1± 35.95 (20)	0.1054[c]	64.57± 37.25 (40)	60.42± 33.03 (40)	0.5350[c]
37. Number of short probes (pathway < 3 minutes)	45.2± 31.25 (20)	35.3± 21.05 (20)	65.1± 44.42 (20)	39.85± 27.32 (20)	53.15± 35.53 (20)	0.0670[c]	50.2± 37.48 (40)	46.5± 32.00 (40)	0.5751[c]

38. Number of np	56.25± 33.07 (20)	48.65± 23.54 (20)	77.5± 43.84 (20)	52.2± 28.73 (20)	64.25± 36.49 (20)	0.0996[c]	63.07± 37.68 (40)	58.22± 32.98 (40)	0.4841[c]
39. Number of pd	8± 8.52 (20)	9.35± 8.27 (20)	6.6± 8.49 (20)	11.35± 13.17 (20)	14.15± 14.11 (20)	0.3018[a]	7.97± 8.39 (40)	12.75± 13.55 (40)	0.1743[a]
40. Total duration of pathway (min)	176.64± 66.79 (20)	193.27± 74.61 (20)	188.20± 72.90 (20)	178.15± 91.91 (20)	173.79± 69.46 (20)	0.9053[d]	190.74± 72.86 (40)	175.97± 74.99 (40)	0.3745[d]
41. Total duration of non- phloem phase waveforms (including np) (min)	263.62± 89.25 (14)	295.43± 95.33 (19)	300.11± 89.86 (11)	225.74± 76.19 (13)	292.57± 92.15 (16)	0.2452[a]	297.15± 91.82 (30)	262.61± 90.45 (29)	0.1202[a]
42. Total duration of np (min)	118.14± 65.32 (20) **AB**	91.53± 43.62 (20) **B**	149.64± 76.39 (20) **A**	97.71± 60.16 (20) **B**	124.84± 76.98 (20) **AB**	**0.0484[d]**	120.58± 68.09 (40)	111.28± 69.56 (40)	0.4654[c]
43. Total duration of pd (sec)	47.48± 47.18 (16)	43.91± 31.83 (18)	48.80± 42.20 (12)	85.96± 66.15 (14)	75.98± 48.22 (16)	0.0646[c]	34.40± 36.78 (40)	60.48± 60.19 (40)	0.0852[a]
44. Total probing time (min)	301.75± 65.35 (20)	328.59± 43.70 (20)	270.32± 76.34 (20)	322.49± 59.96 (20)	295.34± 77.10 (20)	0.0512[d]	299.46± 68.12 (40)	308.91± 69.55 (40)	0.4443[a]
45. Mean duration of np (sec)	168.08± 148.66 (20)	123.37± 58.46 (20)	139.05± 104.14 (20)	132.99± 80.44 (20)	123.80± 53.68 (20)	0.9956[a]	131.21± 83.74 (40)	128.39± 67.66 (40)	0.9951[b]
46. Mean duration of pathway (min)	3.87± 2.14 (20)	4.27± 1.97 (20)	3.06± 1.96 (20)	4.15± 2.73 (20)	3.67± 3.48 (20)	0.1678[b]	3.66± 2.03 (40)	3.91± 3.10 (40)	0.9206[b]
47. Time from start of EPG to 1st sustained E2 (> 10 minutes) (min)	233.98± 146.43 (20)	228.4± 132.59 (20)	275.24± 160.09 (20)	224.49± 157.18 (20)	223.83± 142.10 (20)	0.8947[a]	251.82± 147.01 (40)	224.16± 147.89 (40)	0.4162[a]
48. Time from 1st probe to 1st sustained E2 (> 10 minutes) (min)	232.53± 146.70 (20)	225.73± 134.73 (20)	273.8± 160.46 (20)	222.50± 155.71 (20)	220.29± 140.27 (20)	0.7166[a]	249.76± 148.25 (40)	221.40± 146.28 (40)	0.3432[a]
49. Time from the beginning of that probe to 1st sustained E2 (> 10 minutes) (min)	21.49± 14.14 (14)	19.73± 14.86 (16)	16.58± 8.53 (10)	30.06± 20.25 (13)	17.42± 6.78 (16)	0.4098[b]	18.52± 12.69 (26)	23.08± 15.53 (29)	0.1913[b]
50. Time from start of EPG to 1st E2 (min)	220.58± 150.54 (20)	184.65± 126.28 (20)	252.45± 160.04 (20)	222.75± 158.83 (20)	208.59± 139.94 (20)	0.6958[a]	218.55± 146.37 (40)	215.67± 147.93 (40)	0.9310[a]
51. Time from 1st probe to 1st E2	219.13± 150.76	181.98± 127.95 (20)	251.00± 160.22	220.76± 157.37 (20)	205.06± 137.96 (20)	0.5439[a]	216.49± 147.32 (40)	212.91± 146.29 (40)	0.9501[a]

(min)	(20)		(20)						
52. Time from the beginning of that probe to 1st E2 (min)	19.55± 13.65 (14)	14.87± 8.47 (19)	16.49± 12.80 (11)	27.38± 18.03 (13)	15.15± 5.50 (16)	0.1464[b]	15.46± 10.08 (30)	20.63± 13.92 (29)	0.0662[b]
53. Total duration of np during the 1st hour (min)	25.56± 15.29 (20)	18.45± 14.74 (20)	28.74± 13.91 (20)	26.77± 10.95 (20)	27.19± 14.37 (20)	0.1718[a]	23.60± 15.07 (40)	26.98± 12.61 (40)	0.2798[d]
54. Total duration of np during the 2nd hour (min)	15.16± 13.66 (20)	10.81± 9.02 (20)	15.57± 14.45 (20)	7.83± 8.19 (20)	14.60± 13.49 (20)	0.3495[a]	13.19± 12.13 (40)	11.21± 11.53 (40)	0.3653[a]
55. Total duration of np during the 3rd hour (min)	12.99± 15.05 (20)	17.14± 14.87 (20)	10.03± 13.22 (20)	7.60± 10.21 (20)	17.89± 15.05 (20)	0.0849[a]	13.58± 14.35 (40)	12.74± 13.72 (40)	0.8657[a]
56. Total duration of np during the 4th hour (min)	17.18± 14.70 (20)	12.17± 13.95 (20)	17.62± 18.87 (20)	12.53± 14.92 (20)	16.90± 15.78 (20)	0.7861[a]	14.89± 16.61 (40)	14.72± 15.32 (40)	0.9807[a]
57. Total duration of np during the 5th hour (min)	20.16± 18.65 (20)	12.28± 16.08 (20)	21.74± 15.12 (20)	12.02± 16.10 (20)	13.71± .82 (20)	0.0728[a]	17.01± 16.13 (40)	12.87± 15.78 (40)	0.1833[a]
58. Total duration of np during the 6th hour (min)	16.04± 17.62 (20)	11.53± 12.10 (20)	26.49± 20.46 (20)	14.82± 19.34 (20)	18.10± 18.43 (20)	0.1578[a]	19.01± 18.24 (40)	16.46± 18.72 (40)	0.3639[a]
59. Number of pds during the 1st hour	0.35± 0.98 (20)	1.2± 3.20 (20)	0.45± 1.14 (20)	1.05± 2.18 (20)	1.7± 3.27 (20)	0.5411[a]	0.82± 2.40 (40)	1.37± 2.77 (40)	0.2088[a]
60. Number of pds during the 2nd hour	1.45± 2.79 (20)	0.65± 1.66 (20)	2± 2.91 (20)	3.35± 5.89 (20)	1.3± 2.07 (20)	0.6123[a]	1.32± 2.44 (40)	2.32± 4.48 (40)	0.5158[a]
61. Number of pds during the 3rd hour	2.4± 3.97 (20)	0.7± 2.05 (20)	0.8± 1.79 (20)	2.05± 3.56 (20)	1.25± 1.71 (20)	0.2501[a]	0.75± 1.90 (40)	1.65± 2.78 (40)	0.0566[a]
62. Number of pds during the 4th hour	0.95± 2.66 (20) **AB**	2.4± 3.76 (20) **A**	0.65± 2.08 (20) **B**	0.7± 1.26 (20) **AB**	2± 2.77 (20) **AB**	**0.0318[a]***	1.52± 3.12 (40)	1.35± 2.22 (40)	0.6146[a]
63. Number of pds during the 5th hour	0.75± 1.61 (20)	1.2± 2.19 (20)	0.6± 1.87 (20)	1.4± 2.43 (20)	2.5± 4.24 (20)	0.3033[a]	0.9± 2.03 (40)	1.95± 3.46 (40)	0.2732[a]
64. Number of pds during the 6th hour	0.75± 1.61 (20)	1.2± 2.19 (20)	0.6± 1.87 (20)	1.4± 2.43 (20)	2.5± 4.24 (20)	0.3033[a]	0.9± 2.03 (40)	1.95± 3.46 (40)	0.2707[a]
65. Average duration of pds during the 1st hour (sec)	3.25± 1.01 (3)	3.99± 0.99 (5)	5.04± 0.95 (3)	3.63± 2.23 (6)	3.39± 0.43 (7)	0.1672[a]	4.38± 1.06 (8)	3.50± 1.48 (13)	0.0852[b]

66. Average duration of pds during the 2nd hour (sec)	4.72± 1.37 (7)	3.31± 0.36 (5)	4.01± 1.07 (8)	5.42± 3.00 (8)	4.32± 0.98 (7)	0.1598[a]	3.74± 0.91 (13)	4.91± 2.28 (15)	0.0589[a]
67. Average duration of pds during the 3rd hour (sec)	5.08± 1.67 (9)	3.31± 0.83 (4)	5.11± 1.73 (4)	5.29± 2.19 (7)	4.54± 1.82 (9)	0.2632[b]	4.21± 1.59 (8)	4.87± 1.95 (16)	0.3591[b]
68. Average duration of pds during the 4th hour (sec)	5.06± 0.86 (3) **AB**	4.05± 0.81 (10) **B**	5.06± 2.28 (3) **AB**	7.12± 2.87 (6) **A**	3.74± 1.12 (10) **B**	**0.0097**[b]	4.28± 1.25 (13)	5.01± 2.52 (16)	0.6140[a]
69. Average duration of pds during the 5th hour (sec)	4.78± 1.55 (4) **AB**	4.04± 1.43 (8) **AB**	3.29± 0.86 (3) **B**	5.61± 1.20 (6) **A**	3.95± 0.58 (8) **AB**	**0.0450**[d]*	3.84± 1.30 (11)	4.66± 1.21 (14)	0.0747[b]
70. Average duration of pds during the 6th hour (sec)	3.78± 0.55 (2)	5.34± 1.02 (3)	6.63(1)	4.80± 2.02 (2)	4.08± 1.01 (5)	0.264[d]	5.66± 1.05 (4)	4.29± 1.22 (7)	0.0935[d]
71. Number of probes during the 1st hour	11.95± 7.03 (20)	10.9± 7.15 (20)	14± 6.34 (20)	15.45± 6.47 (20)	12.95± 5.35 (20)	0.1393[c]	12.45± 6.85 (40)	14.2± 6.00 (40)	0.2280[d]
72. Number of probes during the 2nd hour	7.95± 6.37 (20)	6.65± 4.55 (20)	9.95± 9.59 (20)	7.15± 5.87 (20)	8.8± 6.69 (20)	0.8995[a]	8.3± 7.59 (40)	7.97± 6.27 (40)	0.8543[a]
73. Number of probes during the 3rd hour	7.2± 7.28 (20)	9± 6.36 (20)	6.95± 6.05 (20)	6.3± 6.33 (20)	10.7± 7.89 (20)	0.2019[a]	7.97± 6.22 (40)	8.5± 7.40 (40)	0.9652[a]
74. Number of probes during the 4th hour	9.1± 9.03 (20)	5.85± 4.00 (20)	9.85± 9.82 (20)	7.55± 7.64 (20)	9.7± 6.50 (20)	0.5504[a]	7.85± 7.67 (40)	8.62± 7.09 (40)	0.5096[a]
75. Number of probes during the 5th hour	10± 9.46 (20) **AB**	6.65± 7.19 (20) **B**	15.8± 13.07 (20) **A**	6.8± 7.43 (20) **AB**	9.15± 11.62 (20) **AB**	**0.0469**[a]	11.22± 11.40 (40)	7.97± 9.70 (40)	0.2136[a]
76. Number of probes during the 6th hour	7.9± 9.87 (20)	7.6± 7.32 (20)	12.6± 13.69 (20)	6.35± 8.66 (20)	9.7± 10.23 (20)	0.4731[a]	10.1± 11.13 (40)	8.02± 9.51 (40)	0.3290[a]
77. Time from the beginning of the 1st probe to first pd	181.55± 138.25 (20)	170.86± 138.7 (20)	233.42± 175.50 (20)	198.84± 172.35 (20)	163.19± 150.14 (20)	0.6301[a]	202.14± 159.31 (40)	181.02± 160.56 (40)	0.5127[a]

(min)									
78. Time from the end of the last pd to the end of the probe (min)	81.81± 101.73 (16)	60.58± 73.98 (18)	46.18± 63.58 (12)	85.01± 113.01 (14)	51.51± 69.15 (16)	0.8906^{a}	54.82± 69.23 (30)	67.15± 92.13 (30)	0.9000^{a}
79. Duration of the longest E2 (min)	123.92± 86.12 (14) **AB**	87.67± 72.71 (19) **AB**	75.97± 59.34 (11) **B**	152.83± 84.82 (13) **A**	80.68± 82.83 (16) **AB**	**0.0384**^{c*}	83.38± 67.29 (30)	113.02± 89.95 (29)	0.1387^{c}
80. Duration of np just after the probe of the first sustained E2 (> 10 min) (sec)	194.64± 189.06 (10)	100.40± 78.19 (11)	82.59± 50.18 (9)	112.66± 107.99 (9)	287.84± 598.63 (13)	0.4609^{b}	92.39± 66.03 (20)	216.17± 465.82 (22)	0.6043^{b}
81. % of probing spent in C	62.13± 26.54 (20)	60.46± 25.19 (20)	72.75± 25.75 (20)	58.04± 28.07 (20)	62.99± 26.00 (20)	0.4194^{a}	66.61± 25.90 (40)	60.52± 26.82 (40)	0.3306^{a}
82. % of probing spent in E1	0.44± 0.52 (20)	0.33± 0.23 (20)	1.46± 5.65 (20)	0.36± 0.37 (20)	0.64± 0.73 (20)	0.2256^{a}	0.89± 3.98 (40)	0.50± 0.59 (40)	0.1386^{a}
83. % of probing spent in E2	32.11± 29.22 (20)	34.08± 26.62 (20)	19.16± 25.74 (20)	35.50± 29.95 (20)	29.77± 26.07 (20)	0.2766^{a}	26.62± 26.93 (40)	32.64± 27.87 (40)	0.4635^{a}
84. % of probing spent in G	5.06± 9.22 (20)	5.02± 8.38 (20)	6.42± 11.63 (20)	5.89± 9.42 (20)	6.49± 8.52 (20)	0.9719^{a}	5.72± 10.03 (40)	6.19± 8.87 (40)	0.7984^{a}
85. % E2 >10 min	56.25± 43.16 (20)	65.27± 41.64 (20)	39.58± 44.00 (20)	58.83± 45.92 (20)	56.50± 37.80 (20)	0.4111^{a}	52.43± 44.24 (40)	57.66± 41.53 (40)	0.6401^{a}
86. PCA Factor 1	0.0604± 1.0145 (20)	-0.2701± 0.7891 (20)	0.3234± 1.1380 (20)	0.0189± 0.9877 (20)	-0.1327± 1.0382 (20)	0.5200^{a}	0.0266± 1.0122 (40)	-0.0568± 1.0031 (40)	0.7039^{a}
87. PCA Factor 2	-0.1165± 1.0533 (20)	-0.1565± 0.7016 (20)	0.3368± 0.9584 (20)	-0.2344± 1.1283 (20)	0.1706± 1.0810 (20)	0.3214^{d}	0.0901± 0.8659 (40)	-0.0318± 1.1098 (40)	0.5850^{d}
88. PCA Factor 3	-0.0816± 1.0246 (20)	-0.2353± 1.0982 (20)	0.0048± 0.6768 (20)	0.0420± 1.0018 (20)	0.2700± 1.1614 (20)	0.6111^{d}	-0.1152± 0.9085 (40)	0.1560± 1.0767 (40)	0.2360^{c}
89. PCA Factor 4	0.1420± 0.8348 (20)	0.0862± 0.9983 (20)	0.2022± 0.7999 (20)	-0.0689± 0.9586 (20)	-0.3616± 1.3202 (20)	0.5844^{a}	0.1442± 0.8948 (40)	-0.2152± 1.1484 (40)	0.1087^{a}
90. Number of whiteflies to reach phloem phase.	14(20) **AB**	19(20) **A**	11(20) **B**	13(20) **B**	16(20) **AB**	**0.0285**^{e}	30(40)	29(40)	0.7994^{e}

Variables were calculated from 7-hour EPG recordings using the EPG analysis workbook of Sarria et al. (2009). Table entries are means ± standard deviations (n). P values are from a) Non-parametric Wilcoxon Test b) Parametric one-way ANOVA or t-test on log10 transformed data c) Parametric one-way ANOVA or t-test on square root transformed data d) Parametric one-way ANOVA or t-test on untransformed data e) Chi-square test. Significant P-values (< 0.05) are highlighted in bold. For comparisons among the 5 genotypes, values that are significantly different ($P < 0.05$, stat test) are indicated by different letters. The letter “A” indicates a higher numerical value than letter “B”. *Mean separation tests did not detect any significant differences among the five genotypes at the $\alpha = 0.05$ level despite a significant ($P < 0.05$) ANOVA. Since ANOVA is more powerful than means separations, the highest mean (A) was designated as significantly different from the lowest mean (B), with all other means intermediate (AB).

Table 1.2: Principal Component Analysis with 18 selected variables. Each variable is proceeded by the same identification number as in Table 1.1. Second row provides proportion of variance accounted for by the first four PCA factors. Third row provides the eigenvalues for the four PCA factors. Rows 5-22 provide the loading for each variable for PCA factors 1-4. Asterisks indicate loading > 40% or < -40%.

	Factor	Factor 2	Factor 3	Factor 4
Proportion of total variance accounted for by PCA Factor	0.44	0.14	0.08	0.07
Eigenvalues	7.85	2.55	1.40	1.31
Variable [a]				
2. Number of probes before the 1st E1[b]	76*	52*	-6	-12
3. Duration of 1st probe (sec)[b]	-18	12	-18	72*
6. Duration of the second nonprobe period (min)[b]	0	-9	20	70*
7. Total duration of nonprobe periods before the 1st phloem phase (min)[b]	91*	9	18	-4
9. Average number of pds per probe[c]	-44*	-46*	55*	2
11. Time from 1st probe to 1st phloem phase (min)[c]	94*	12	-17	-1
14. Mean duration of G (min)[c]	2	6	-55*	2
19. Number of single E1[c]	-16	9	4	-52*
20. Number of E2[c]	-70*	16	49*	4
21. Number of sustained E2 (longer than 10 minutes)[c]	-70*	1	55*	-3
32. Total duration of E2 (min)[c]	-61*	-47*	22	-16
35. Number of probes[d]	23	93*	2	-2
37. Number of short probes (pathway < 3 minutes)[b]	22	91*	9	-6
39. Number of pd[c]	-53*	14	72*	-1
45. Mean duration of np (sec)[b]	43*	-61*	26	9

48. Time from 1st probe to 1st sustained E2 (> 10 minutes)(min)[c]	89*	12	-23	6
51. Time from 1st probe to 1st E2 (min)[c]	94*	11	-17	-2
77. Time from the beginning of the 1st probe to first pd (min)[c]	83*	14	-18	11

[a] Variable numbers are same as in Table 1 to facilitate cross referencing.
[b] Data were log 10-transformed prior to analysis.
[c] Data were not transformed prior to analysis.
[d] Data were square root-transformed prior to analysis.

Chapter 2

Bemisia tabaci B Nymph Feeding Behaviors on Arabidopsis Salicylic Acid- and Jasmonic Acid-Signaling Mutants

Abstract

The electrical penetration graphs (EPGs) technique was used to study the feeding behavior of *Bemisia tabaci* B nymphs on five *Arabidopsis thaliana* genotypes. Studies in Zarate et al. (2007) demonstrated that, compared to wild-type (WT) Arabidopsis, whitefly nymphal development was accelerated (FastDev) on Arabidopsis mutants where JA perception was blocked (*coi1*) or where SA-regulated signaling was constitutively active (*cim10*). Conversely, whitefly nymphal development was delayed (SlowDev) on mutants where SA-regulated signal transduction is impaired (*npr1*) or where JA-regulated and ET-regulated defenses are constitutively active (*cev1*). Based on those findings, we compared feeding behaviors among WT, *coi1, cim10, npr1*, and *cev1* in order to identify if changes in nymph feeding behaviors were associated with the SlowDev and FastDev phenotypes. Surprisingly, the EPG studies revealed that the five Arabidopsis genotypes did not differ significantly in nymph feeding behavior. Also, when data from the two FastDev genotypes (*coi1* and *cim10*) were pooled and compared to pooled data from the two SlowDev genotypes (*cev1* and *npr1*), no significant differences in feeding behaviors were detected. Since there were no differences in feeding behavior correlated with nymphal development, the results suggested that the accelerated or delayed nymphal

development observed by Zarate et al. (2007) was due to differences in phloem sap quality.

Introduction

The whitefly *Bemisia tabaci* B (also known as *B. tabaci* biotype B, *Bemisia argentifolii*, and Middle East-Asia Minor 1) is a difficult agricultural pest to control since its introduction into the United States in the 1990s (Barinaga 1993). *B. tabaci* B is part of the *Bemisia* species complex consisting of at least 24 morphologically indistinguishable species (De Barro et al. 2011). The phloem-feeding *B. tabaci* B causes damage to plants by depleting nutrients the phloem sap, transmitting of a wide range of plant viruses, producing of copious amounts of honeydew that supports the growth of sooty mold fungi on the plant surfaces to reduce the crop's market value and can increase processing costs, and inducing developmental disorders in crops and horticultural plants (Gamble 2002; Jones 2003; McKenzie and Albano 2009).

Both whitefly nymphs and adults cause extensive damage to plants. The whitefly life cycle consists of the egg, four nymphal instars and the adult stage (Walker et al. 2010). Adult female whiteflies deposit eggs, usually on the abaxial side of a leaf, by inserting the pedicel of the egg into the leaf tissue. The first instars (crawlers) that hatch from the eggs have well-developed legs and travel 2 to 15 mm on the leaf surface searching for a suitable settling site (Simmons 2002). Nymphs in their second, third and fourth instars have reduced legs and are sessile. Consequently, once the crawler finds a feeding site, the nymph stays in the same location throughout its development until it emerges as an adult. When molting between nymphal instars, the nymph retracts its stylets, molts, and re-inserts stylets back into the plant. Thus, the nymphal stage, which constitutes over half of the whitefly life cycle, has a prolonged and intimate interaction with the host plant.

Whiteflies feed on phloem sap using highly flexible mouthparts called stylets (Pollard 1955). *B. tabaci* B adult stylets are approximately 217 μm in length and nymph stylets range from 113 to 200 μm in length (Freeman et al. 2001). Penetration through the plant tissue follows mostly an intercellular pathway. The stylets weave between cells causing minimal mechanical damage while searching for a feeding site; stylets ultimately penetrate a sieve element from which they ingest phloem sap (Freeman et al. 2001). The stylets have a food canal for ingesting plant sap and a salivary canal for delivery of watery and gelling saliva into plant tissues (Pollard 1955). Gelling saliva is secreted incrementally into the intercellular space as the stylets advance through plant tissue, and soon after secretion, it gels and forms a "salivary sheath" around the stylets (Miles 1999). In addition to the gelling saliva, it is presumed that whiteflies secrete watery saliva into the plant apoplast as the stylets move along their way to the phloem; this is a behavior documented in another hemipteran pest - aphids (Moreno et al. 2011). Upon penetrating a phloem sieve element, whiteflies first inject watery saliva into the sieve element before ingesting phloem sap (Jiang et al. 1999). Whitefly nymphs feed from the same sieve element throughout an entire instar stage and alternate between bouts of sap ingestion and periods of non-ingestion, which is presumed to be reflective of salivation (Jiang and Walker 2003; Lei et al. 1996).

To feed successfully, whiteflies have to circumvent the plant's constitutive and induced defense mechanisms. The mechanical movement of the whitefly's stylets and its salivary secretions are signals that may be perceived by the plant to alert the plant to whitefly attack. Insect herbivore-associated molecular patterns (HAMPs), herbivore-associated elicitors (HAEs), and effectors in the saliva of phloem-sap feeding insects are recognized by the plant's defense signaling machinery (Bonaventure 2012). Recognition of insect HAMPs and elicitors leads to changes in plant defense hormone levels and activation or suppression of defense signal-transduction cascades to influence the expression of resistance traits or susceptibility factors (Hogenhout and Bos 2011).

At the core of these innate immunity responses are the defense hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). These defense-signaling pathways may act antagonistically, cooperatively, or synergistically depending on the cues perceived during pathogen or pest attack (Derksen et al. 2013; Pieterse et al. 2012). In addition, insect effectors alter the timing and the magnitude of defense gene activation by interrupting key steps in innate immunity (Thaler et al. 2012). Although exceptions exist, phloem sap-feeding insects (eg., whiteflies and aphids) and biotrophic microbial pathogens have been shown to activate SA-regulated defense pathways (Walling 2008; Walling 2009; Zhu-Salzman et al. 2004). In contrast, tissue-damaging herbivores and pathogens (e.g., nectrotrophs) most often activate the JA/ET- regulated defense pathways (Erb et al. 2012).

The changes in plant gene expression in response to whitefly feeding and the role of specific defense-signaling pathways have been most extensively studied with *B. tabaci* B and *Arabidopsis thaliana* (Kempema et al. 2007; Zhang et al. 2012; Zhang et al. 2013). Transcriptome analyses detected an increase in SA-biosynthesis and SA-regulated RNAs in whitefly-infested leaves and in apical non-infested leaves; concurrently, there was no change or a decrease in transcript levels of JA- and ET-regulated RNAs (Kempema et al. 2007). While mutations in the *phytoalexin-deficient4 (pad4-1* mutant*)* gene and ET-defense signaling did not impact whitefly nymphal development (Kempema 2007; Kempema et al. 2007), nymphal development rates were altered in mutants that influenced SA- and JA-signaling (Zarate et al. 2007). Nymphal development was accelerated (FastDev) in Arabidopsis lines that had SA-signaling constitutively active (*cim10*) or could not perceive JA (*coi1*) (Zarate et al. 2007). Whereas, in lines where JA- and ET-signaling is constitutively active (*cev1*) or SA-signaling was impaired (*npr1, NahG*) nymphal development was slow (SlowDev) (Zarate et al. 2007). In addition, the importance of JA-regulated defense traits in retarding whitefly nymphal development was also demonstrated by extreme delays in nymphal development on methyl jasmonate (MeJA)-treated *npr1* plants (Zarate et al. 2007). Collectively, these studies by Kempema et al. (2007) and Zarate et al. (2007) indicate that JA-regulated defenses are a critical component of the innate immune response that interferes with *B. tabaci* B nymphal development. Similar conclusions were made by Zhang et al. (2013).

In this Chapter, we examined the whitefly second instar feeding behaviors on four Arabidopsis defense mutants (*coi1*, *npr1*, *cev1*, and *cim10*) and WT plants using the DC

Electrical Penetration Graph (EPG) technique (Tjallingii 1988). Since whitefly nymphal development was accelerated *cim10* and *coi1* plants but delayed in *npr1* and *cev1* plants, we hypothesized that there would be substantive changes in the ability to consume phloem sap between FastDev (*cim10* and *coi1*) vs SlowDev (*npr1* and *cev1*) genotypes that would influence nymphal feeding behaviors.

Materials and Methods

Plant Growth, Infestation and Insect Rearing

The whitefly (*Bemisia tabaci* B) colony was maintained on *Brassica napus* in a growth room at 27^{o}C, 55% relative humidity, and long-day (16-hr light and 8-hr dark) conditions. *Brassica napus* var 'Florida Broad Leaf' (W. Atlee Burpee & Co.) plants were grown in 15-cm diameter pots containing UC Soil Mix Number 3 (Matkin 1957) and fertilized with Miracle-Gro (Scotts, Marysville, Ohio). Growth room conditions were 24°C, 150 to 250 µE m^{-2} s^{-1} lights with a 12-hr light and 12-hr dark photoperiod.

Five Arabidopsis (*Arabidopsis thaliana*) plant genotypes were used in the experiments: wild-type ecotype Columbia-0 (WT), and the mutants *coronatine insensistive 1* (*coil*) (JA-defense impaired), *non-expressor of PR genes1* (*npr1*) (SA-defense impaired), *constitutive expression of VSP1* (*cev1*) (JA- and ET-dependent defenses constitutively active), and *constitutive immunity 10* (*cim10*) (SA-defense constitutively active) (Zarate et al. 2007). Seeds were pipetted onto Murashige & Skoog agar plates (10 g per L sucrose and 0.8% [w/v] agarose) and were allowed to germinate and grow for one week. Mutant *cev1* plants were plated one week prior to all other genotypes due to their slow growth rate (Zarate et al. 2007). For *coi1*, seeds were supplemented 30 µM methyl jasmonate (MeJA)/0.01% ethanol to select for homozygous seedlings; after one week, MeJA-insensitive homozygous *coi1* seedlings were selected based on elongated root length and green cotyledons (Feys et al. 1994). After one week on Murashige & Skoog agar plates, seedlings were transplanted into 10-cm pots containing Sunshine Mix Number 1 soil (JM McConkey company, Inc, Cat# SUSMLC1), which was supplemented with fertilizer (Osmocote 14–14–14; Scott Horticulture Solutions). Plants were grown for three to four weeks (see below) under fluorescent and incandescent lights (180 µE m^{-2} s^{-1}) at 22℃ under short-day (8-hr light and 16-hr dark) conditions.

To infest the plants, whiteflies were collected into 15-ml tubes, 100 whiteflies per tube. Each pot, containing a three-week-old plant (or four-week-old for *cev1*), was covered with a white mesh bag to prevent whiteflies from escaping, and a whitefly-containing tube was inserted into the soil of the pot. The cap of the tube was then removed, releasing the whiteflies onto the plant. After one week of infestation, adult whiteflies were aspirated off the plants. At 10 days post infestation, plants with second-instar nymphs were used for EPG experiments.

Electrical Penetration Graph (EPG)

All EPG experiments were performed on intact plants using two Giga-4 DC-EPG monitors, each with 1 Giga-ohm input resistance (EPG systems, Wageningen, The Netherlands). The substrate voltage was applied to the plant via a copper wire inserted into the soil. At the beginning of each recording, the substrate voltage for each channel was adjusted so that the waveforms fit in the +5 to -5 volt frame of the WINDAQ recording software. Output was analogue-to-digitally converted at 100 samples per second per channel with a DI 720-P analog-to-digital converter, and recorded using WINDAQ software (hardware and software from DATAQ Instruments, Akron, Ohio, USA).

Wiring Second Instar Nymphs and Experimental Procedure

With the aid of a stereomicroscope, a 1-cm length of 10-micron diameter gold wire (Sigmund Cohn Corp., Mt. Vernon, New York) was attached to each nymph using water-based silver glue (see Walker and Medina-Ortega (2012) for the silver glue formulation). To begin wiring, a leaf infested with nymphs was gently lifted to expose the abaxial surface with nymphs; the leaf was held in place by securing the tip of the leaf with a thin piece of masking tape. A small 1-cm x 1-cm plastic sheet containing a fresh drop of water-based silver glue was placed near a randomly selected nymph in its second instar so that both the glue and the nymph were in the same field of view under the microscope. With the blunt end of a thin metal probe, a droplet of water-based silver glue was transferred from the plastic sheet to the nymph's dorsum. Then using forceps to hold the 1-cm length of gold wire, one end of the wire was dipped into water-based silver glue multiple times to create a droplet at the tip of the wire. This end of the wire was quickly placed on the spot of silver glue on the nymph's dorsum. This two-step process allowed a good electrical connection between the nymph and gold wire.

To connect the nymph to the EPG monitor, a 3-mm diameter nail was inserted into the Bayonet Neill–Concelman (BNC) input of the EPG's head-stage amplifier. Fresh water-based silver glue was applied to the nail head, and the head-stage amplifier was positioned to bring the head of the nail (with silver glue) in contact with the free end of the gold wire attached to the nymph. The glue bonded the gold wire to the nail. EPGs were recorded for eight hours. There were five treatments (WT, *coi1*, *cev1*, *npr1*, and *cim10*). Each recording used a different plant and different nymph. The order of genotypes recorded was randomized, and recordings were made until there were 20 replications of each Arabidopsis genotype. In a given recording session, up to eight nymphs, each on a different plant, were recorded simultaneously. There was a 40% failure rate with either too much background noise for interpretation or nymph molting.

Waveform Annotation and Statistical Analysis

EPG recordings were viewed and annotated using the WinDaq Waveform browser. Two waveforms were identified: waveform H (ingestion of phloem sap from a sieve element) and waveform L (an unknown non-ingestion behavior, possibly corresponding to salivation with the stylet tips inside a sieve element) (Jiang and Walker 2003; Lei et al. 1996). Hours 2 through 8 of each recording were annotated by marking the beginning of each waveform as waveform H or L. The first hour of the recording was not annotated to allow the nymph an hour to acclimatize to the wire before its behavior was analyzed. The annotated files were imported into Microsoft Excel, which was used to calculate six feeding behavior variables for each recording. The feeding behavior variables were: 1) number of bouts of waveform L; 2) number of bouts of waveform H; 3) total time of waveform L; 4) total time of waveform H; 5) average duration of bouts of waveform L; and 6) average duration of bouts of waveform H.

The six variables were compared among the five Arabidopsis genotypes using JMP software (SAS Institute, Cary, North Carolina). Means and standard deviations of each variable were calculated for each genotype. Each variable was analyzed by one-way ANOVA for a completely randomized design with genotype as the independent variable. Each variable was tested to determine if it met the requirements of homogeneity of variances and normality of the residuals. For variables that violated these assumptions, data were log10- or square root-transformed and re-analyzed by one-way ANOVA. If the transformed data still violated the assumptions, the data were then analyzed by the nonparametric Wilcoxon test. For significant ($P < 0.05$) parametric ANOVAs, Tukey-Kramer's All Pairs Comparison was used to compare means among genotypes and for significant ($P < 0.05$) Wilcoxon tests. Dunn All Pairs for Joint Ranks Mean Comparison was used to compare means among genotypes. Significance level for mean comparisons was $\alpha = 0.05$.

The six variables also were subjected to principal component analysis using the factor procedure of PC/SAS software (SAS Institute, Cary, North Carolina). The first two principle component factors, which had eigenvalues greater than 1 and accounted for approximately 87% of the total variance, were then compared among the five genotypes as described previously for the six variables calculated directly from the EPG recordings.

In addition, data from the two FastDev genotypes (*cim10* and *coi1*) were pooled and data from the two SlowDev genotypes (*cev1*and *npr1*) were pooled, and the six variables calculated directly from the EPG recordings plus the first two principle component factors were compared between the pooled SlowDev and pooled FastDev genotypes using a t-test when the assumption of normality was met. For variables that violated this assumption, data were log10- or square root-transformed and re-analyzed with a t-test. If the transformed data still violated this assumption, the data were then analyzed by the nonparametric Wilcoxon test.

Results

All EPG recordings were made on whiteflies in their second instar. The whitefly nymph stylets had already penetrated a phloem sieve element. For this reason, the only EPG waveforms that were observed were the two phloem-phase waveforms: H (sap ingestion) and L (unknown non-ingestion behavior) (Jiang and Walker 2003; Lei et al. 1996) (Fig. 2.1-2.3).

Principal component analysis (PCA) analyses were performed on six variables including the number, duration and total time spent in H or L behaviors for nymphs residing on the four mutant lines and WT plants. The first two PCA factors combined accounted for 86% of the total variance with PCA factor 1 accounting for 63% and PCA factor 2 accounting for 23% of the variance (Table 2.1). Table 2.1 displays the loadings of the six variables analyzed. Variables with high positive loading (> 40%) to PCA factor 1 were total time of L (variable 3) and average duration of L (variable 4).

Variables with high negative loading (< -40%) to PCA factor 1 included total time of H (variable 5) and average duration of H (variable 6). Variables with high positive loading to PCA factor 2 were number of L (variable 1) and the number of H (variable 2). Average duration of H (variable 6) was the only high negative loading (< -40%) to PCA factor 2.

None of the six variables calculated directly from the EPG recordings nor the first two PCA factors differed significantly between the pooled FastDev (*cim10* and *coi1*) and pooled SlowDev genotypes (*cev1* and *npr1*) (Table 2.2). Similarly, none of the six variables nor the two PCA factors differed significantly among the five genotypes (Table 2.2).

Discussion

As in previous studies (Jiang and Walker 2003; Lei et al. 1996), only two EPG waveforms (H and L) were produced when whitefly nymphs have their stylet tips in a phloem sieve element. In the present study, several variations of waveform H (phloem sap ingestion) were detected (Fig. 2.1). The correlation studies with variant waveforms and honeydew production described in Chapter 3 showed that all of these H waveform variations are associated with phloem sap ingestion. The feeding behavior associated with waveform L (Fig. 2.3) is not yet known but it is hypothesized that whitefly nymphs salivate during waveform L (Jiang and Walker 2003; Lei et al. 1996).

The first two PCA factors each had eigenvalues greater than 1 and accounted for a combined total variance of 86%, and consequently both were retained for interpretation.

The total time of L (variable 3) and average duration of L (variable 4) had high positive loading (> 0.40) to PCA factor 1. Also, total time of H (variable 5) and average duration of H (variable 6) had a high negative loading (< -0.40) to PCA factor 1 (Table 2.1). It is logical that if total time in L had a high positive loading then total time in H would have a high negative loading as these are the only two waveforms produced. These loadings indicate that high values for PCA factor 1 are indicative of less time spent ingesting phloem sap.

Number of L (variable 1) and the number of H (variable 2) both had high positive loadings to PCA factor 2. Also, the average duration of H (variable 6) had high negative loading to PCA factor 2. Since L and H are the only two feeding waveforms in phloem phase, the high values for PCA factor 2 indicated frequent transitions between waveform L and H, and short durations in phloem sap ingestion. Neither PCA factor1 nor PCA factor 2 differed significantly among the five genotypes, and between the pooled FastDev and pooled SlowDev genotypes. Also, when PCA factor 2 was plotted against PCA factor 1, the genotypes do not segregate into discrete clusters indicating that there is no significant difference among the five genotypes (Fig. 2.4).

Kempema et al. (2007) and Zarate et al. (2007) showed that nymph development was accelerated on the FastDev genotypes (*coi1* and *cim10*) and delayed on the SlowDev genotypes (*cev1* and *npr1*). Consequently, we hypothesized that nymphs would spend more time ingesting phloem sap on the FastDev mutants than on the SlowDev (*npr1* and *cev1*) mutants. However, the genotypes did not differ significantly for any of the variables from the EPG recordings, nor for two PCA factors (Table 2.2). Furthermore, there were no significant differences for any of the variables in the pooled FastDev and pooled SlowDev data (Table 2.2).

Consequently, the differences in developmental rate among the genotypes observed by Zarate et al. (2007) do not appear to be due to differences in time spent ingesting phloem sap. Three alternative hypotheses to explain the different developmental rate among the genotypes can be posed; (1) the volume of sap ingested per unit time may differ among the genotypes, as was observed for whiteflies feeding on a resistant alfalfa genotype (Jiang and Walker 2007); 2) nutritional quality of the phloem sap may differ among the genotypes; and/or 3) phloem sap in SlowDev genotypes may contain component(s) that are deleterious to the whitefly or its essential endosymbionts, but are sublethal (no difference in mortality was observed by Zarate et al. 2007). The first hypothesis does not seem likely as the amount of sap ingested on the five genotypes was examined using the "honeydew clock" technique (Jiang and Walker 2007), and appeared to be similar among the 5 genotypes (unpublished data). The second hypothesis of different nutritional quality of the phloem sap appears to be the most likely explanation for the differences in developmental rate among the five genotypes. This hypothesis would predict that relative to WT or the SlowDev genotypes (*cev1* and *npr1*), the phloem of FastDev genotypes has higher levels of essential nutrients (e.g., amino acids) or lower levels of metabolites that interfere with insect development.

Plant phloem sap is not an ideal food source for meeting the general nutritional requirements of animals. Carbohydrates, predominantly sugars or sugar alcohols are in far greater concentration than is needed for animal nutrition while nitrogenous nutrients, such as amino acids, are often relatively dilute (Douglas 2006). Additionally, the ratio of essential amino acid to non-essential amino acids is very low in phloem sap; only about 1:4-1:20, in contrast to the 1:1 ratio of essential to non-essential amino acids in animal protein (Douglas 2006). Consequently, amino acid concentration, especially essential amino acid concentration, in the phloem sap can affect the performance of phloem sap-feeders (Blackmer and Byrne 1999; Crafts-Brandner 2002).

Blackmer and Byrne (1999) examined amino acid concentration in phloem sap and whitefly performance over a range of leaf and plant ages in cantaloupe (*Cucumis melo* L). Using PCA, they found that concentration of the amino acids histidine and orthithine in phloem sap was correlated with faster *B. tabaci* B nymphal developmental rate, whiles high levels of mostly essential amino acids were correlated with greater body weight of the emerging adults (Blackmer and Byrne 1999). Bentz et al. (1995a) measured phloem sap N-amino content and whitefly performance on young, mature and old leaves of poinsettia (*Euphorbia pulcherrima* Willdenow). Both N-amino content and *B. tabaci* B survival were significantly greater on mature leaves than on the other two leaf ages; however nymphal development rate did not differ significantly among the three leaf ages despite the difference in N-amino content.

Several studies have manipulated nitrogen fertilization to plants and observed effects on whitefly performance. In most studies, at least one aspect of whitefly performance was positively correlated with nitrogen fertilization (Bentz and Larew 1992; Bentz et al. 1995b; Bi et al. 2001; Blua and Toscano 1994; England et al. 2011). Several studies have attempted to correlate whitefly performance with amino acid or soluble protein content in leaves or petioles with varying results (Bi et al. 2001; England et al. 2011), but amino acid and soluble protein levels in leaves or even in petioles are unlikely to be accurately represent levels in phloem sap, which is the actual diet of whiteflies.

While differences in nutritional quality of phloem sap is likely to affect whitefly nymphal development rate, as far as we are aware, the nutritional quality of phloem sap has not been compared among plants with activated or deactivated JA- and SA- defense pathways. Nor is it clear why activation of the pathways would have a differential effect on phloem sap nutritional quality. Perhaps a more likely reason for the differences in nymphal development rate observed by Zarate et al. (2007) would be defensive constituents in the phloem sap that have an adverse but non-lethal effect on the whiteflies; such constituents could act directly on the whiteflies or indirectly via their essential symbionts. Our results rule out an effect on feeding behavior as the mechanism resulting in the differences in nymphal development rate as observed by Zarate et al. (2006). Consequently, future studies should focus on differences in phloem sap nutritional and defensive constituents among these genotypes.

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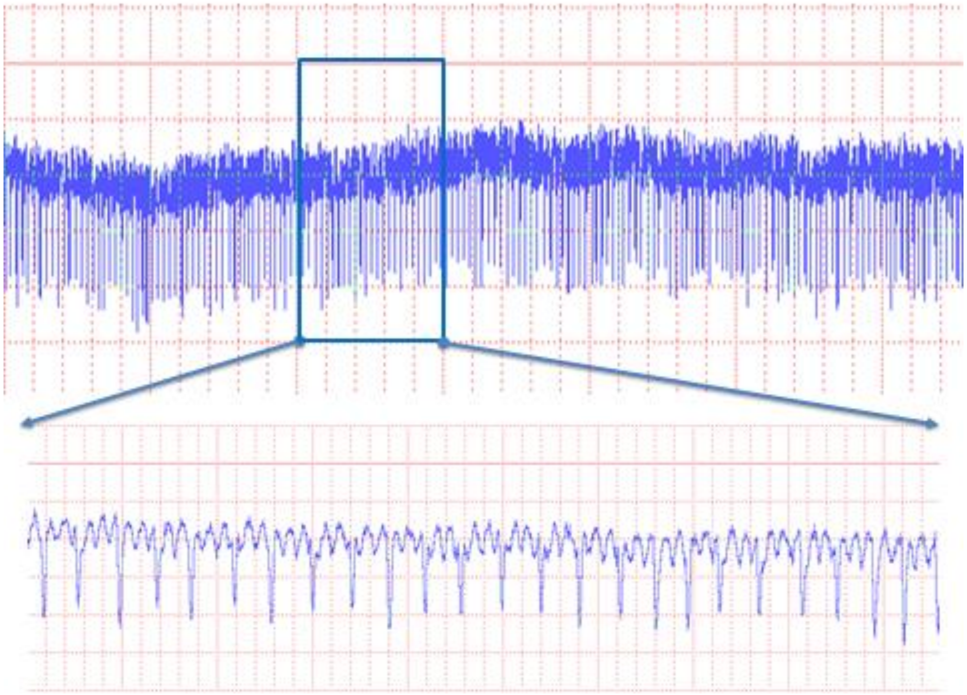

Figure 2.1. *B. tabaci B* second instar nymph waveform H. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis in upper figure: 2 sec per division. Lower figure shows details at 0.2 sec per division of the section indicated in the upper figure.

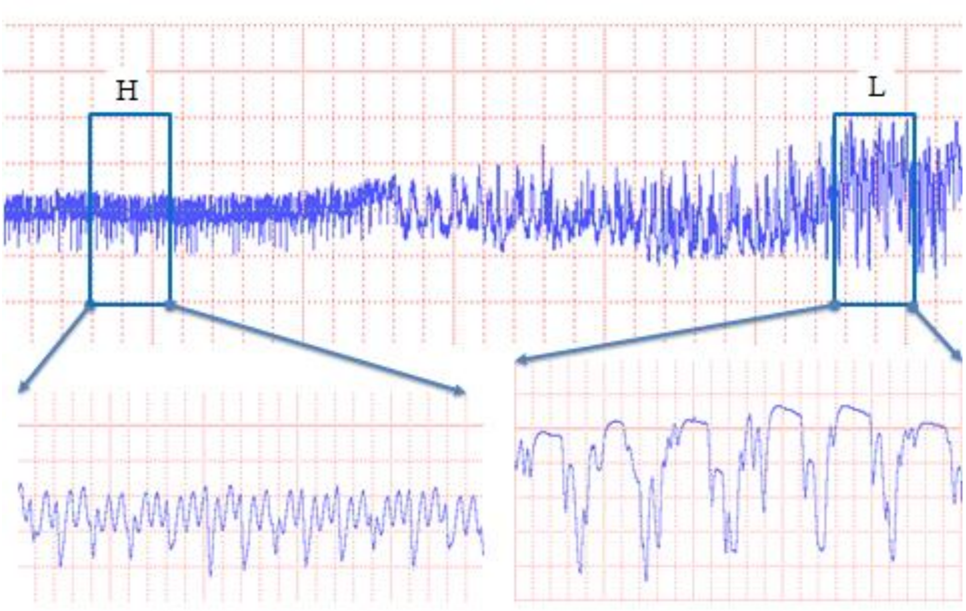

Figure 2.2. *B. tabaci B* second instar nymph transition from waveform H to L. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis in upper figure: 2 sec per division. Lower two figures shows details at 0.2 sec per division of the sections indicated in the upper figure.

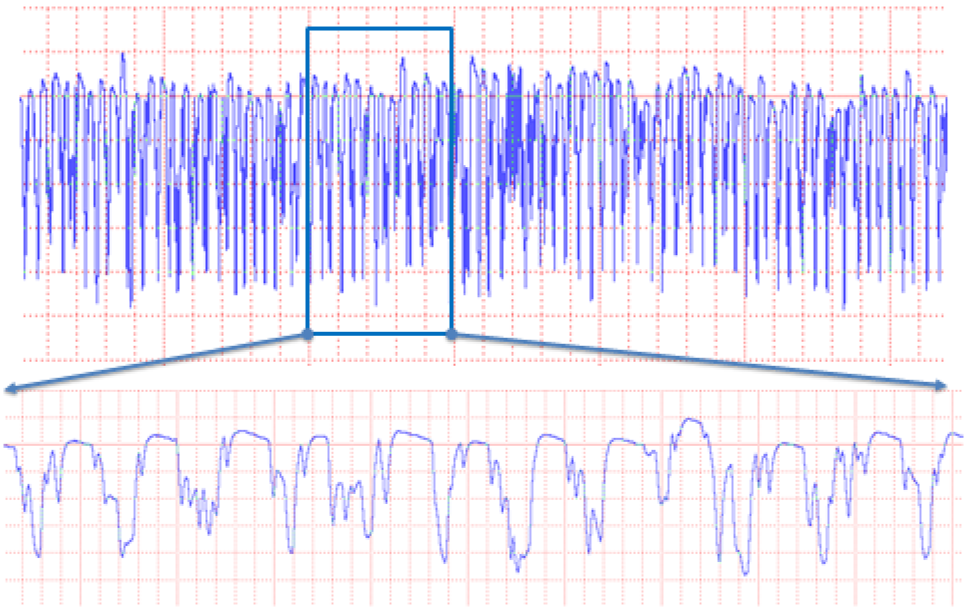

Figure 2.3. *B. tabaci B* second instar nymph waveform L. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis in the upper figure: 2 sec per division. Lower figure shows details at 0.2 sec per division of the section indicated in the upper figure.

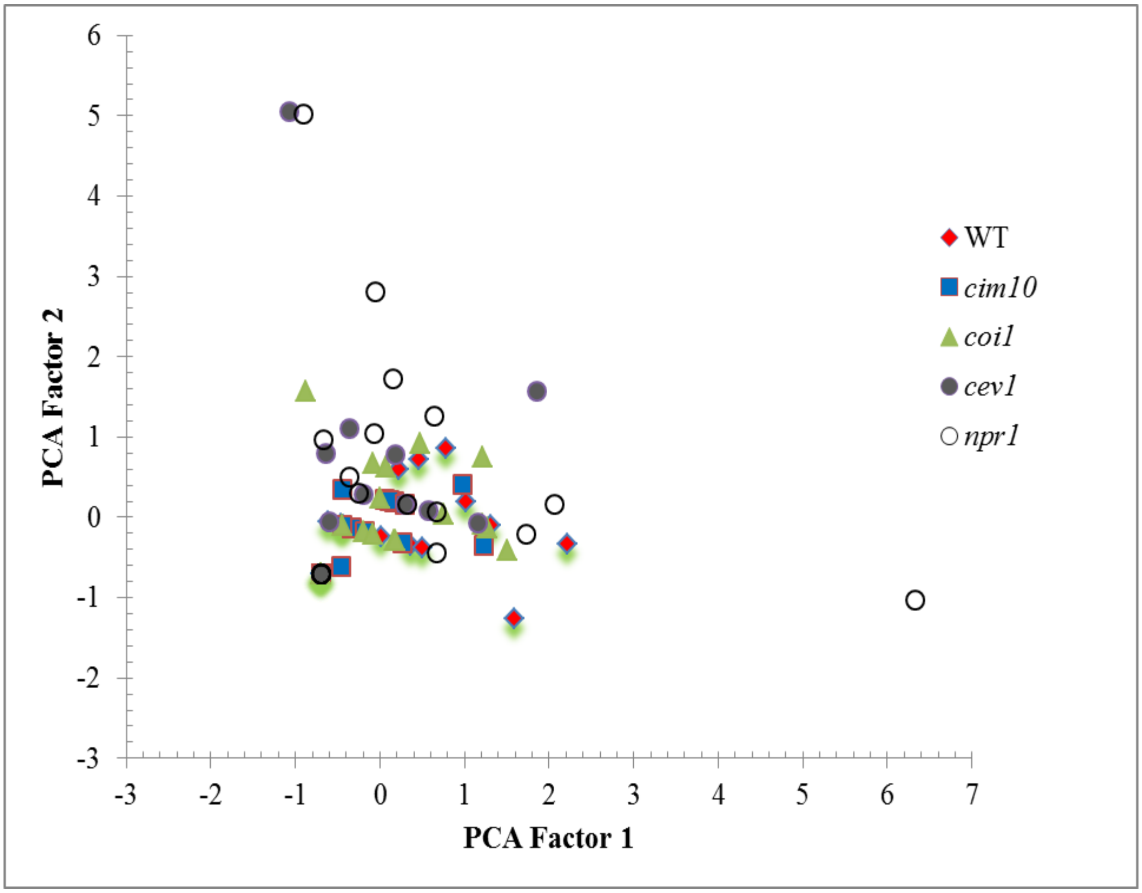

Figure 2.4. Scattered plot for principal component analysis (PCA) factor 1 (x-axis) and factor 2 (y-axis) with five genotypes (WT, *cim10*, *coi1*, *cev1* and *npr1*).

Table 2.1: Principal Component Analysis with 6 variables. Second row provides proportion of variance accounted for by the first two PCA factors. Third row provides the eigenvalues for the two PCA factors. Rows 5-10 provide the loading for each variable for PCA factors 1-2. Asterisks indicate loading > 40% or < -40%.

	Factor 1	Factor 2
Proportion of total variance	0.63	0.23
Eigenvalues	3.83	1.40
Variable		
1. Number of L	18	97*
2. Number of H	13	98*
3. Total time of L (min)	89*	36
4. Average duration of L (min)	95*	60
5. Total time of H (min)	-81*	-20
6. Average duration of H (min)	-54*	-69*

Table 2.2: Whitefly nymph feeding behavior variables and principle component factors for five Arabidopsis genotypes, and for pooled genotypes.

Variable	WT	*cim10* "FastDev"	*coi1* "FastDev"	*cev1* "SlowDev"	*npr1* "SlowDev"	P value (5 genotypes)	"FastDev" *cim10* +*coi1*	"SlowDev" *cev1*+ *npr1*	P value (FastDev vs. SlowDev)
1. Number of L	1.25± 1.33 (20)	1.00± 0.97 (20)	1.75± 1.51 (20)	2.10± 3.58 (20)	3.10± 3.76 (20)	0.2129^{a}	1.37± 1.31 (40)	2.60± 3.66 (40)	0.3448^{a}
2. Number of H	2.15± 1.18 (20)	1.90± 0.96 (20)	2.60± 1.50 (20)	3.00± 3.49 (20)	3.85± 3.60 (20)	0.2345^{a}	2.25± 1.29 (40)	3.42± 3.52 (40)	0.2767^{a}
3. Total time of L (min)	29.42± 38.27 (20)	18.68± 25.37 (20)	35.12± 36.70 (20)	27.65± 46.60 (20)	57.86± 78.04 (20)	0.2343^{a}	26.90± 32.23 (40)	42.76± 65.26 (40)	0.6897^{a}
4. Average duration of L (min)	13.92± 16.40 (20)	10.12± 11.32 (20)	15.57± 13.71 (20)	7.65± 11.36 (20)	18.41± 26.77 (20)	0.7410^{b}	12.84± 12.71 (40)	13.03± 21.01 (40)	0.3937^{a}
5. Total time of H (min)	390.52± 38.23 (20)	401.30± 25.37 (20)	384.88± 36.70(20)	392.35± 46.60 (20)	362.13± 78.04 (20)	0.2517^{a}	393.09± 32.23 (40)	377.24± 65.26 (40)	0.8887^{a}
6. Average duration of H (min)	246.66± 138.12 (20)	274.55± 137.69 (20)	209.66± 133.17 (20)	261.68± 166.49 (20)	188.49± 160.85 (20)	0.2058^{a}	242.10± 137.68 (40)	225.08± 165.78 (40)	0.4094^{a}
7. PCA Factor 1	0.1170± 0.8886 (20)	-0.2176± 0.5770 (20)	0.0854± 0.7626 (20)	-0.2886± 0.7453 (20)	0.3038± 1.6413 (20)	0.2729^{a}	-0.0661± 0.6849 (40)	0.0076± 1.2934 (40)	0.3568^{a}
8. PCA Factor 2	-0.2419± 0.5361 (20)	-0.2846± 0.4221 (20)	-0.0172± 0.6355 (20)	0.1373± 1.3581 (20)	0.4065± 1.4681 (20)	0.5947^{a}	-0.1509± 0.5494 (40)	0.2719± 1.4025 (40)	0.7247^{a}

Variables and principle component factors for five Arabidopsis genotypes (WT, *cim10*, *coi1*, *cev1*, and *npr1*) and for pooled "FastDev" genotypes (*cim10* + *coi1*) and pooled "SlowDev" genotypes (*cev1* + *npr1*). Table entries are means ± standard deviations (n). P values are from a) Non-parametric Wilcoxon Test b) Parametric one-way ANOVA on Log10 transformed data.

Chapter 3

Bemisia tabaci B Electrical Penetration Graph Phloem Sap Ingestion (E2) Waveforms

Abstract

The electrical penetration graph (EPG) monitor is a powerful tool used to study the feeding behavior of piercing-sucking insects. Unlike chewing insects with feeding behaviors visible by eye, the feeding behavior of piercing-sucking herbivores such as whiteflies requires the aid of EPGs. Waveforms generated by the EPG monitor indicate the location of whitefly's stylets in plant tissue, as well as specific feeding behaviors occurring at that location. Five categories of waveforms are produced by the whitefly: pathway, xylem sap ingestion, potential drop, phloem salivation and phloem sap ingestion. Although many whitefly waveforms have been correlated with biological behaviors, many other waveform patterns remain elusive and the biological behaviors associated with the waveforms are not yet known. In Chapters 1 and 2, adults and nymphs of *Bemisia tabaci* B feeding on five Arabidopsis genotypes generated uncharacterized waveform patterns during phloem phase. To determine which of these waveforms were associated with phloem sap ingestion or non-ingestion, EPGs were recorded from adults and second instar nymphs of *B. tabaci* B on wild-type *Arabidopsis thaliana*, while simultaneously video-recording the whiteflies to observe honeydew production, which is indicative of phloem sap ingestion. With adult whitefly, four ingestion waveforms (classical, second, third, and fourth types of E2) correlated with honeydew production. Surprisingly, the second type of E2 waveform, which resembled

E1 waveform in aphid and whitefly, also correlated with honeydew production. These studies also showed that whiteflies ingesting phloem occasionally terminated ingestion, entered the pathway phase for a short period of time, and re-inserted stylets into the same sieve element to reinitiate E2, without E1 waveform. This is the first demonstration of whiteflies directly entering E2. Lastly, whitefly nymphs generate two phloem-phase waveforms (waveforms H and L) in Arabidopsis, and only waveform H was correlated with honeydew production.

Introduction

Studies of feeding behavior of piercing-sucking insects such as the whiteflies require the use of the electrical penetration graph (EPG). To observe a whitefly's feeding behavior on a plant, a voltage is applied to the plant through an output wire inserted into the soil of a potted plant. The whitefly is glued to a platinum or gold wire connected to the EPG's input resistor. When the whitefly's stylets insert into plant tissue, a circuit is completed and the current flows from the applied voltage source into the plant, through the whitefly, and back into an input resistor (Tjallingii 1988; Walker 2000). The whitefly's feeding behavior produces fluctuations in electrical resistance and voltage in the circuit. The EPG generates waveform patterns associated with these fluctuations. Different feeding behaviors produce distinctly different waveforms; consequently EPGs can be used to monitor many different aspects of feeding behavior (Tjallingii 1988; Walker 2000).

For EPG output to be interpretable, the different waveforms need to be correlated with the insect's stylets location in different plant tissue layers and its behavior in that tissue. The use of transmission electron microscopy, stylectomy, and the observation of honeydew production are several techniques used to elucidate stylet location in plant tissues or biological behaviors associated with the waveforms.

EPGs have been used to study adult whitefly feeding behavior for four whitefly species: *B. tabaci* B, *B. tabaci* Q, *Trialeurodes vaporariorum* (Westwood) and *Parabemisia myricae* Kuwana (Janssen et al. 1989; Walker and Perring 1994; Lei et al. 1997; Lei et al. 1998; Jiang et al. 1999; Johnson and Walker 1999; Lei et al. 1999; Jiang et al. 2000b; Jiang and Walker 2001; Lei et al. 2001; Liu et al. 2012). In these studies, the main adult whitefly waveforms produced are categorized as: pathway (whitefly stylets penetrate between epidermal and/or mesophyll cells), potential drops (intracellular punctures with their stylet tips into plant cells), xylem sap ingestion, sieve element salivation, and phloem sap ingestion.

Lei et al. (1997) was the first to report two distinct waveform types during phloem phase of whiteflies (*T. vaporariorum*), which they referred to as E(phloem)1 and E(phloem)2. Jiang et al. (1999) and Liu et al. (2012) reported two similar phloem-phase waveforms for *B. tabaci* B and *B. tabaci* Q, which they named E(pd)1 and E(pd)2. Jiang et al. (1999) noted that phloem phase could consist of only E(pd)1 or both E(pd1) and E(pd2), in which case E(pd)2 always followed E(pd)1. Phloem phase never consisted of only E(pd)2. Jiang et al. (2000a) then correlated E(pd)1 with inoculation of a circulative plant virus. Circulative plant viruses are inoculated via salivation; consequently, this result also correlated E(pd)1 with salivation into the sieve element. Honeydew production has been correlated with phloem phase (Walker and Perring 1994; Lei et al. 1997). By process of elimination, E(pd)2 has been widely accepted as correlated with phloem sap ingestion, but prior to the present study, this correlation has not been directly tested for whiteflies. In this Chapter, the two phloem-phase waveforms will be referred to as E1 and E2, rather than E(pd)1 and E(pd)2, as a reflection of their great similarity to the better-studied aphid phloem phase waveforms E1 and E2 (Tjallingii 2006).

EPGs have been also used to study the feeding behavior of whitefly nymphs for two species: *B. tabaci* B, and *T. vaporariorum* (Lei et al. 1996a; Jiang and Walker 2003; 2007). Lei et al. (1996a) reported two phloem phase waveforms produced by *T. vaporariorum* on cucumber, which they named waveforms H and L referring to the relatively high frequency and low frequency of voltage fluctuations in the two waveforms. Jiang and Walker (2003) reported two similar waveforms produced by *B. tabaci* B feeding on alfalfa and retained the names H and L. They also described a very brief waveform, J, that occurs at the beginning of each phloem phase. Using honeydew production as an indicator of phloem sap ingestion, both Lei et al. (1996a) and Jiang and Walker (2003) correlated waveform H with ingestion of phloem sap. Honeydew production was not associated with waveform L or J. At present, all that can be said about the L and J waveforms is that they are correlated with non-ingestion behavior while the stylet tips are in a sieve element. Both Lei et al. (1996a) and Jiang and Walker (2003) speculated that this non-ingestion behavior may be salivation.

In Chapter 1, EPG studies of adult *B. tabaci* B feeding on wild type and four defense mutant Arabidopsis identified three new phloem-phase waveforms that did not resemble published examples of E(pd)1 or E(pd)2. To interpret these EPG recordings, it was necessary to determine if the new adult phloem-phase waveforms were correlated with phloem-sap ingestion. In addition it was investigated if any of the new waveforms were strictly associated with WT or one or more of the defense mutants (*coi1, cim10, npr1,* and *cev1*) of Arabidopsis studied in Chapters 1 and 2. In addition, periods of phloem-phase interruptions have provided a new perspective of the relationship between E1 and E2. Finally, the two waveforms recorded with nymph feeding were assessed for their correlation with phloem-sap ingestion.

Materials and Methods

Plant Growth, Whitefly Infestation and Insect Rearing

Wild-type (WT) *Arabidopsis thaliana* ecotype Columbia-0 seeds were plated on onto Murashige & Skoog agar plates (10 g per L sucrose and 0.8% [w/v] agarose) to germinate and grow for one week. After one week, seedlings were transplanted into 10-cm pots containing Sunshine Mix Number 1 soil, which was supplemented with fertilizer (Osmocote 14–14–14; Scott Horticulture Solutions). Plants were grown under fluorescent and incandescent lights (180 µE m^{-2} s^{-1}) at 22°C under short-day (8-hr light and 16-hr dark) conditions. Adult whitefly EPG and video recordings of honeydew production were made on Arabidopsis plants three weeks after transplanting to soil.

To assess nymph behaviors by EPG and video recordings, Arabidopsis plants were infested with 100 adult whiteflies per plant two weeks after transplanting. A white mesh fabric enclosed the pots and plants to prevent whiteflies from escaping. After one week of infestation, adult whiteflies were aspirated off of the plants. At 10 days post infestation, plants with second instar nymphs were used for EPG and video recordings of honeydew production.

The whitefly (*Bemisia tabaci* B) colony was maintained on *Brassica napus* in a growth room at 27℃, 55% relative humidity, 150 to 250 µE m^{-2} s^{-1} lights with 16-hr light and 8-hr dark conditions. *Brassica napus* var 'Florida Broad Leaf' (W. Atlee Burpee & Co.) plants were grown in 15-cm diameter pots containing UC Soil Mix Number 3(Matkin 1957) and fertilized with Miracle-Gro (Scotts, Marysville, Ohio) under 24℃, 150 to 250 µE m^{-2} s^{-1} lights with 12-hr light and 12-hr dark conditions.

Wiring Adult Whiteflies

Female adult whitefly were glued to the ends of 2.5-µm diameter platinum wires (Wollaston process wire, Sigmund Cohn Corp., Mt. Vernon, New York) using electrically conductive silver glue (Electrodag 503, Ladd Research Industries, Williston, Vermont). The platinum wire is encased in a silver sheath that needs to be removed prior to use. To prepare the wire, one end of a 2-cm silver-encased platinum wire was attached to the head of a 3-mm diameter nail using silver glue. Then, a small drop of silver glue was brushed onto tip of the other end of the wire. When the glue was dried, the free end of the wire was submerged in 40% nitric acid to dissolve the silver casing and expose approximately 1 cm of the 2.5-µm diameter platinum core. The exposed core was gently submerged in water twice to remove residual nitric acid and the wire was ready for wiring a whitefly.

Whiteflies were collected in 15-ml centrifuge tubes and placed at -20℃ for 2 minutes to immobilize the whiteflies. After 2 minutes, the whiteflies were transferred to a Petri dish, which was chilled on a cold plate (Sigma-Aldrich Techware, Milwaukee, WI). The cold plate and Petri dish were placed under a stereomicroscope to aid attachment of wires to the whiteflies. A cardboard windscreen was placed around the Petri dish to shield the wire from air currents during attachment of wires to a whitefly. A 1 cm x 1 cm plastic sheet containing a fresh drop of silver glue was placed inside the Petri dish in the same field of view as the whiteflies. Using forceps to hold the nail with the platinum wire, the tip of the wire was dipped into the glue and quickly placed onto the thoracic dorsum of an adult whitefly. The previously-applied dried silver glue at the apex of the wire facilitated this process by serving as a nucleus for the fresh wet glue.

For EPG recordings, a leaf was gently turned over exposing the abaxial surface, and the tip of the leaf was gently taped down to keep the abaxial side of the leaf facing upwards. The nail with the platinum wired whitefly was inserted into the input of the head-stage amplifier of the EPG, and the whitefly was placed in contact with the abaxial leaf surface.

Wiring Whitefly Nymphs

Second instar nymphs were attached to a 1-cm long piece of 10-µm diameter gold wire (Sigmund Cohn Corp., Mt. Vernon, New York) using water-based silver glue with recipe describe in Walker and Medina-Ortega (2012). To wire a second-instar nymph, a leaf infested with nymphs was gently turned over exposing the abaxial surface of the leaf. The tip of the leaf was gently taped down using a thin piece of masking tape to hold the abaxial side of the leaf facing upwards with the nymph in view. A small 1-cm x 1- cm plastic sheet holding a fresh drop of water-based silver glue was placed near a second-instar nymph so that both the glue and the nymph were in the same field of view when observed with a stereomicroscope. Using a thin wire, a small drop of water-based silver glue was transferred from the plastic sheet to the nymph's dorsum. Then using forceps to hold the 1-cm gold wire, the end of the gold wire was gently dipped into the silver glue multiple times to create a small droplet at the tip of the wire. Before the silver glue dried, the end of the wire was quickly placed in contact with the previously applied glue on the nymph's dorsum. The wet glue on the wire readily fused with the dry glue on the whitefly's dorsum creating a good electrical contact. After attaching the gold wire to the nymph, the other end of the wire was glued (same silver glue) to a nail that was inserted into the input of the head-stage amplifier of the EPG.

Electrical Penetration Graph (EPG)

All EPG experiments were performed on intact plants using two Giga-4 DC-EPG monitors, each with 1 Giga-ohm input resistance (EPG systems, Wageningen, The

Netherlands). The substrate voltage was applied to the soil of the potted plant via a copper wire. At the beginning of each recording, the substrate voltage for each channel was adjusted to fit the +5 to -5 volt frame of the WINDAQ recording software. Output was analogue-to-digitally converted at 100 samples per second per channel with a DI 720-P A-D converter, and recorded using WINDAQ software (hardware and software from DATAQ Instruments, Akron, Ohio, USA).

Video Recording of Honeydew Production

Honeydew production was correlated with EPG waveforms by simultaneously recording EPGs and high magnification video of the same whitefly. During EPG recording of whitefly nymphs, the high magnification video image of the nymph was displayed on a video screen, and a simple motion detector (Fig. 3.1) was placed close (about 1 mm) to the screen right over the image of the nymph's vasiform orifice (anus). The output of the motion detector was fed into the same DI-720 A-D converter that received the EPG signal; thus two channels were recorded by the Windaq software, one with the EPG and the other with the output of the motion detector. Excretion of honeydew produced a distinct voltage spike on the motion detector channel; consequently, the Windaq recording contained both the EPG and a record of each drop of honeydew so that honeydew production could be correlated with specific EPG waveforms (Table 3.1).

The motion detector system used for nymphs could not be used for adults because adults do not stay motionless when feeding (e.g., they groom, pivot on their stylets, etc) and thus honeydew production would not stand out from other movements in the motion detector channel. Consequently, a system similar to that used by Jiang and Walker (2003) was used for correlating honeydew production with adult whitefly EPG waveforms. Briefly, two video cameras were used, one with a close-up lens focused on the whitefly and the other focused on the computer screen that was displaying the EPG in real time. The two cameras were linked via a Video Effector (TK-C5OU; JVC, Tokyo) to produce a split-screen recording where the highly magnified image of the nymph was displayed on one half of the screen and the corresponding EPG was displayed on the other half. The split screen display was recorded on DVD and was then played back to determine when honeydew was produced, number of honeydew drops, duration between successive drops, and the waveform being produced during honeydew production (Table 3.2). The Windaq display on the computer screen does not display a timer in recording mode, so a timer (displaying hour, minutes, and seconds) that was synchronized with the beginning of the Windaq EPG recording was displayed on the computer screen along with the EPG, and thus was part of the split screen EPG/video that was recorded to DVD. This facilitated locating any point of interest in the split-screen EPG/video recording (such as honeydew production) with the same point in the Windaq file. This was helpful because more waveform detail could be gleaned from the Windaq recording than from the split screen EPG/video recording.

Results and Discussion

Adult Whitefly: Four Types of Ingestion Waveforms

Four phloem-phase waveforms produced by *B. tabaci* B adults were correlated with honeydew production (Figs 3.2 - 3.10). Rather than propose formal names for the new forms, at this time, they will be referred to simply as different types of waveform E2 (Table 3.2). The first type is the “classical” E2, which is very similar to previously published images of the adult whitefly phloem sap ingestion waveform (Lei et al. 1996a; Lei et al. 1997; Jiang et al. 1999; Liu et al. 2012). We refer to it as "classical" because it is very similar to aphid waveform E2, images of which have appeared in many aphid EPG studies (Tjallingii 2006). The classical E2 waveform is characterized by very regular sharp downward peaks. The intervening periods between the sharp downward peaks have relatively low amplitude voltage fluctuations and are at a higher voltage level (i.e., more positive) than the downward peaks. The intervening periods may or not include an upward peak (e.g., with small upward peaks and without small upward peaks: Fig. 3.2, 3.3, 3.11 and 3.12). When upward peaks are present, they are smaller in amplitude than the downward peaks (Fig. 3.2). The frequency of downward peaks may increase or decrease but the amplitude of the peaks are consistent throughout phloem phase (Fig. 3.2-3.3). This is the most frequently observed type of E2 that was observed in adult *B. tabaci* B.

Four EPG/video recordings of classical E2 waveforms correlated with honeydew were observed (Table 3.2). The phloem phase for each replicate ranged between 1239 to

21,934 seconds and the duration of this waveform ranged between 1227 to 21897 seconds. In the EPG/Video recordings, the whitefly produced 1, 1, 9, and 9 number of honeydew drops respectively for each replicate, and the higher number of honeydew drops produced corresponded with longer phloem phase (Table 3.2). The time from beginning of the waveform to the first honeydew drop ranged between 639 to 1247 seconds, and the average time between honeydew drops range between 353.25±139.13 to 447.37±123.90 seconds. The time from E1 to first honeydew drop and time from beginning of the waveform to the first honeydew drop were at most 37 seconds apart indicating that the whitefly made the classical E2 waveform quickly after E1.

The remaining types of E2 waveforms have not been reported in previous studies. The second type of E2 is characterized by very regular frequency of sharp upward peaks (Fig. 3.4-3.8). Distinct sharp downward peaks, characteristic of classical E2, also may occur in this type of E2 (Fig. 3.4 and 3.6), or they may be barely perceptible (Fig. 3.5), or completely absent (Fig. 3.7 and 3.8). In all of these variations of second type of E2 waveforms, the frequency may increase or decrease, but the amplitude remains steady.

Four EPG/video recordings of the second type of E2 waveforms correlations with honeydew production were observed (Table 3.2). The phloem phase for each replicate ranged between 657 to 3275 seconds. In the EPG/Video recordings, the whitefly produced 2 to 4 honeydew drops after the appearance of the waveform. The duration of the waveforms ranged between 437 to 3215 seconds. The time from beginning of the waveform to the first honeydew drop ranged between 653 to 2203 seconds, and the average time between honeydew drops range between 26.00±0.00 to 473.00±0.00 seconds. The time from E1 to first honeydew drop and time from beginning of the waveform to the first honeydew drop were at most 60 seconds apart indicating that the whitefly made the second type of E2 waveform quickly after E1.

Dominant upward peaks during phloem phase are characteristic of aphid waveform E1 and whitefly waveform E(pd)1 where saliva is secreted into the sieve element (Prado and Tjallingii 1994; Jiang et al. 1999; Tjallingii 2006; Liu et al. 2012). Consequently, it was surprising that the second type of E2, which was dominated by upward spikes, was correlated with honeydew secretion. This was especially surprising for the variations where downward spikes, characteristic of phloem sap ingestion, were barely perceptible or absent (Fig. 3.5-3.7). The second type of E2 waveform was present in all five Arabidopsis genotypes (*coi1*, *npr1*, *cev1*, *cim10*, and WT) that were examined in Chapter 1, and was not specific to any particular genotype (Table 3.3). This E2 waveform occurred in only 3 to 11% of phloem phases that were observed in Chapter 1 (Table 3.3).

The third type of E2 has regular downward peaks, somewhat similar to "classical" E2, but the intervening periods have high amplitude fluctuation (Fig. 3.9) rather than the low amplitude fluctuation in the characteristic of the plateaus between downward peaks in the "classical" E2 (Figs. 3.3, 3.11). However, unlike the other E2 waveforms, the downward peaks vary in frequency, amplitude and sharpness. Two EPG/video recordings of the third type of E2 waveforms correlations with honeydew production were observed (Table 3.2). The duration of the phloem phases for the two replicates were 974 and 6938 seconds, and the number of honeydew drops for each was 1 and 4 respectively. The duration of the waveforms was 954 and 1293 seconds for each replicate. The time from beginning of the waveform to the first honeydew drop was 444 and 4659 seconds, and the average time between honeydew drops was 327.33±180.58 seconds for one of the replicate.

The fourth type of E2 is characterized by large fluctuations in the overall voltage level and distinct patterns of voltage fluctuation are not readily apparent (Fig. 3.10). The fourth type of E2 waveform may be simply a noisy recording of one of the previously described types, or it may be a new E2 waveform. One EPG/video recordings of the fourth type of E2 waveforms correlated with honeydew were observed (Table 3.2). The duration of the phloem phase was 16,546 seconds and the duration of the waveform was 12, 693 seconds. The time from beginning of waveform to the first honeydew drop was 245 seconds. The average time between honeydew drops was 269.55±71.74 seconds and 30 honeydew drops were observed.

For adult whiteflies, the four types of E2 waveforms observed in this study were strictly correlated with honeydew production (Table 3.2). It is important to note that within the same phloem phase, different types of E2 waveforms may appear. Frequently, the classical E2 waveform appears with one other type of ingestion waveform. Rarely, three types of ingestion waveforms were observed in one phloem phase.

Adult Whitefly Phloem Phase with Pathway Interruptions

In adult whitefly EPG recordings of Arabidopsis (*coi1*, *npr1*, *cev1*, *cim10*, and WT), we observed that during phloem ingestion, the whiteflies occasionally make brief interruptions into pathway phase (Fig. 3.11). The brief interruptions into pathway phase averaged 31 ± 19 seconds (mean ± SD, Table 3.4). Pathway interruptions were not common. Only 23 out of a total of 205 phloem phases that produced the E2 waveform had a pathway interruption. More than one pathway interruption per phloem phase was never observed. The frequency of occurrence of pathway interruptions was similar among the five Arabidopsis genotypes (Table 3.4). When phloem phase resumed after the brief pathway interruptions, phloem phase reinitiated with the phloem sap ingestion waveform E2, and only the classical E2 waveforms were observed with pathway interruptions (Fig. 3.11). This result was unexpected as in both aphids and adult whiteflies, E1 has always been reported to be the first phloem-phase waveform following pathway phase (Jiang et al. 1999; Tjallingii 2006) (Fig. 3.12). Salivation during waveform E1 at the beginning of phloem phase has been hypothesized to function by suppressing the plant's defensive sieve element occlusion response (Tjallingii 2006), but recent experimental evidence provided no support for that hypothesis (Walker and Medina-Ortega 2012; Medina-Ortega KJ 2013).

Nonetheless, while the function of E1 salivation at the beginning of phloem phase remains unresolved, E1 was not needed after the brief pathway interruptions. Once phloem sap ingestion resumed after a pathway interruption, it continued for a prolonged time (median = 41.6 minutes, Table 3.4). The duration of these brief pathway interruptions (range from 22.34±5.50 to 39.61±28.67 seconds) suggests that the stylets re-insert into the same sieve element that they were in prior to the interruption. If that is the case, then E1 salivation may not be necessary when the sieve element is re-punctured because whatever function E1 salivation plays was already accomplished during E1 after the initial penetration of the sieve element. The reason why whiteflies make occasional pathway interruptions during phloem phase is unknown.

Second Instar Nymph Correlations: High (H) and Low (L) Frequency Waveforms

B. tabaci B second instar nymphs on Arabidopsis WT produced two distinct waveforms: low (L) and high (H) frequency waveforms (Table 3.1). Both L and H frequency waveforms were observed in previous studies (Lei et al. 1996b; Jiang and Walker 2003) but this is the first time nymph waveforms have been examined on Arabidopsis. Waveform H for *B. tabaci* B nymphs feeding on Arabidopsis (Fig. 3.13) was very similar to waveform for *B. tabaci* B nymphs feeding on alfalfa (*Medicago sativa*) (Jiang and Walker 2003) and for *T. vaporariorum* nymphs feeding on cucumber (*Cucumis sativus)* (Lei et al. 1996b).

Waveform H was characterized by very regular downward voltage spikes at a relatively constant amplitude (Fig 3.13). The frequency varied from approximately 5 to 10 Hertz during long bouts of waveform H, but changes in frequency were always very gradual (Table 3.1). As (Lei et al. 1996b) and (Jiang and Walker 2003), waveform H for *B. tabaci* B nymphs feeding on Arabidopsis was correlated with honeydew production, and thus is a phloem sap ingestion waveform. Thirteen EPG/video recordings of the H waveforms were made and all were correlated with honeydew production (Table 3.1). The duration of the waveforms observed ranged from 1869 to 7186 seconds, and the total number of honeydew drops ranged from 24 to 84. The average time between honeydew drops was 99.07 ± 25.85 seconds.

During phloem phase, nymphs transition between waveforms H and L (Fig. 3.14). Three EPG/video recordings of the L waveforms were observed and this waveform was not correlated with honeydew production (Table 3.1). Waveform L usually had higher amplitude than waveform H. This made identification of the transitions from H to L and L to H easy to detect when scanning the EPG recording with a compressed time scale (Fig 3.14). Waveform L was characterized by sharp downward peaks with intervening upward rounded humps that repeat at a low frequency (Fig. 3.15). The duration of waveform L ranged from 113 to 774 seconds. The frequency was 2.5 Hertz during bouts of waveform L.

Conclusions

The use of the EPG monitor is an important tool in studying the feeding behavior of piercing-sucking insects. Although many waveforms have been correlated with insect behavior, there is still a lot that is unknown about the different waveforms patterns. One of the most difficult tasks and time consuming part of EPG experiments is to identify and annotate EPG waveforms. Thus, it is important to distinguish between phloem waveforms that are ingestion waveforms from those that are non-ingestion waveforms.

In Chapters 1 and 2, adults and nymphs of *B. tabaci* B feeding on five Arabidopsis genotypes generated uncharacterized ingestion waveform patterns during phloem phase. The objective of this Chapter was to observe whether or not the waveforms correlated with honeydew production.

In this Chapter, we have correlated four adult whitefly ingestion waveforms (classical, second, third, and fourth types of E2) with honeydew production. Given the similarities to aphid E2 waveforms, previous adult whitefly-EPG have presumed that the classical E2 waveform is the ingestion waveform (Walker and Perring 1994; Lei et al. 1997; Jiang et al. 1999; Liu et al. 2012). In addition to the classical E2 waveforms, we identified three additional E2 waveforms correlated with honeydew production and these waveforms have not been observed in previous studies.

The second type of E2 waveform was especially unexpected to correlate with honeydew production because it resembled the E1 waveform of aphid and E(pd)1 of whitefly when saliva is secreted into the sieve element upon entering the phloem (Prado and Tjallingii 1994; Jiang et al. 1999; Tjallingii 2006; Liu et al. 2012). Moreover, we observed that during phloem ingestion whiteflies make brief interruptions into pathway phase and re-insert stylets into the same sieve element without E1 waveform. This result was not expected because the E1 waveform has always been reported to be the first phloem-phase waveform following pathway. This observation indicates that E1 salivation is not necessary when the sieve element is re-punctured.

In Chapter 2, we showed that the whitefly nymphs also produce H and L waveforms on Arabidopsis plants. These data were consistent with previous studies with *T. vaporariorum* and *B. tabaci* B (Lei et al. 1996b; Jiang and Walker 2003; 2007) that showed whitefly nymphs generate two phloem phase waveforms (waveforms H and L) on alfalfa and cucumber. The waveform J, which is brief, infrequent and appears at the beginning of phloem phase, was not found in whitefly-Arabidopsis interactions and therefore may be unique to alfalfa. EPGs in conjunction with honeydew clocks or video recordings have shown that honeydew production is correlated with H waveforms (Lei et al. 1996a; Jiang and Walker 2003; 2007). In this Chapter, using coupled EPG and video recordings, we showed that similar to feeding on alfalfa (Jiang and Walker 2003), only the H waveform is correlated with honeydew production during whitefly feeding on Arabidopsis. These observations support the hypotheses of Lei et al. (1996a) and Jiang and Walker (2003) that non-ingestion waveform L may be salivation waveform.

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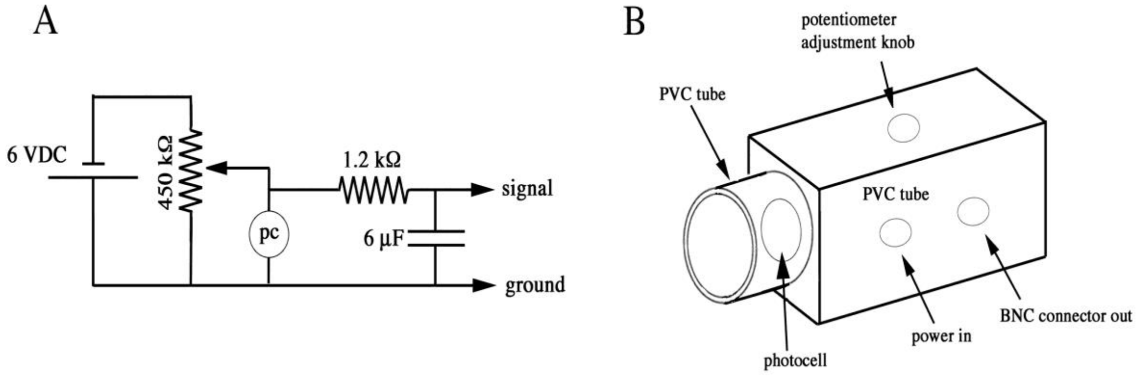

Figure 3.1. Design of a motion detector used to detect honeydew production. A. Circuit diagram; pc, photocell. B. Assembled motion detector. The PVC tube (X mm diameter) surrounds the photocell; the open end of the PVC tube is placed approximately 1 mm away from the screen displaying the real time video of the nymph and is positioned over the screen image of the whitefly nymph's vasiform orifice. The signal output goes to the same A-D converter that receives the EPG signal, but in a different channel than the EPG. Thus the Windaq software records both the EPG of the whitefly and the output of the motion detector.

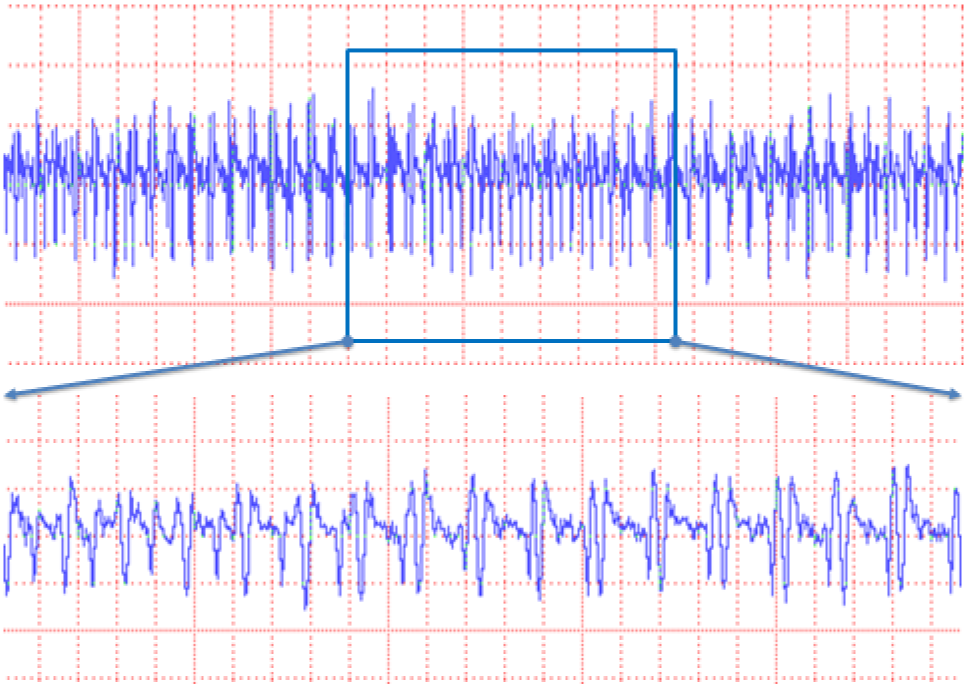

Figure 3.2. *B. tabaci B* adult "classical" E2 waveform. Waveform with regular alternation of high amplitude plateaus and sharp downward peaks. Vertical axis is the voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

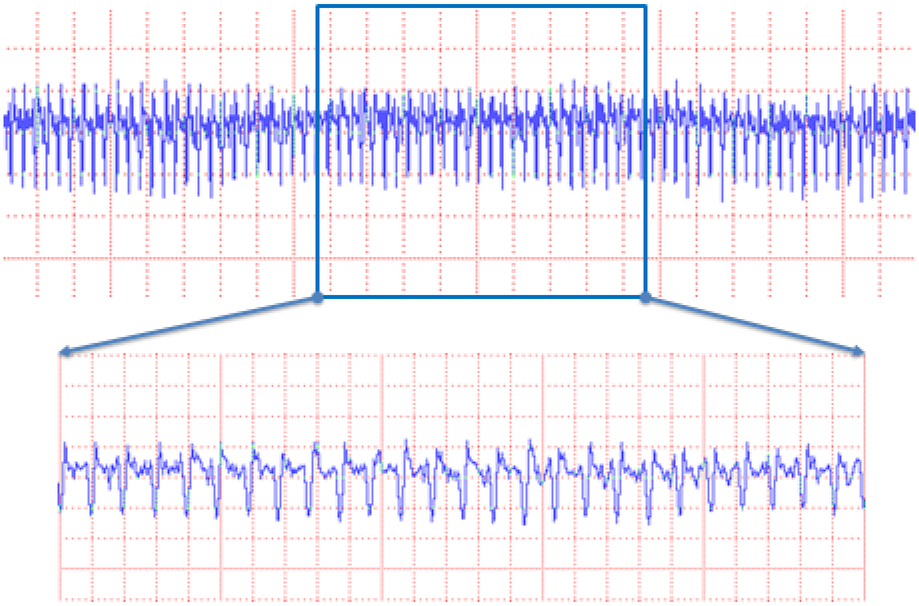

Figure 3.3. *B. tabaci B* adult "classical" E2 waveform. Waveform with regular alternation of low amplitude plateaus and sharp downward peaks. Vertical axis is the voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

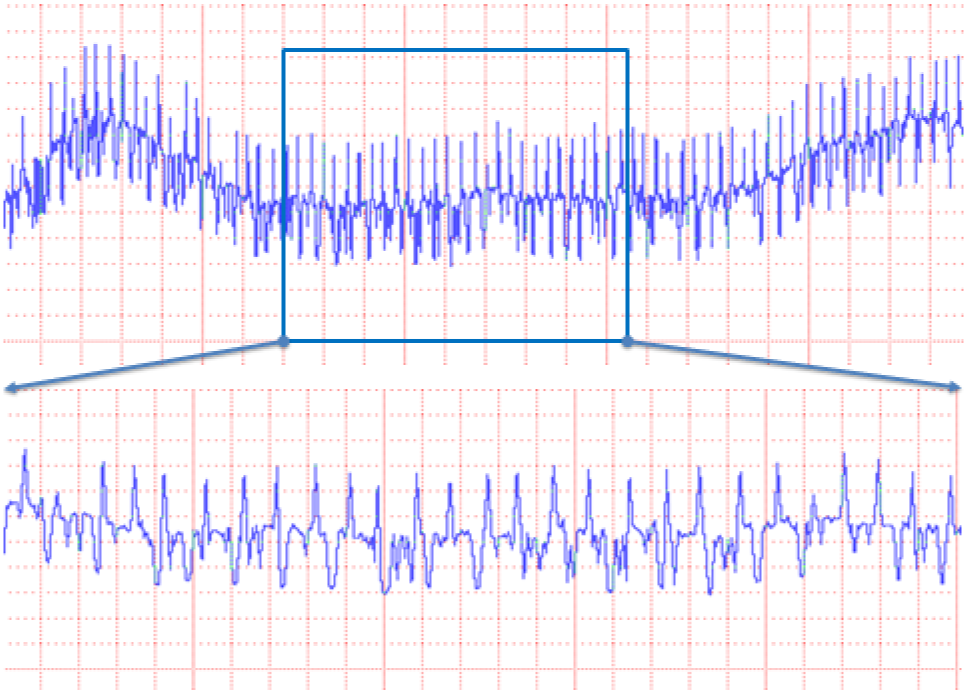

Figure 3.4. *B. tabaci B* adult second type of E2 waveform. This variation of E2 is characterized by dominant upward and downward voltage peaks. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

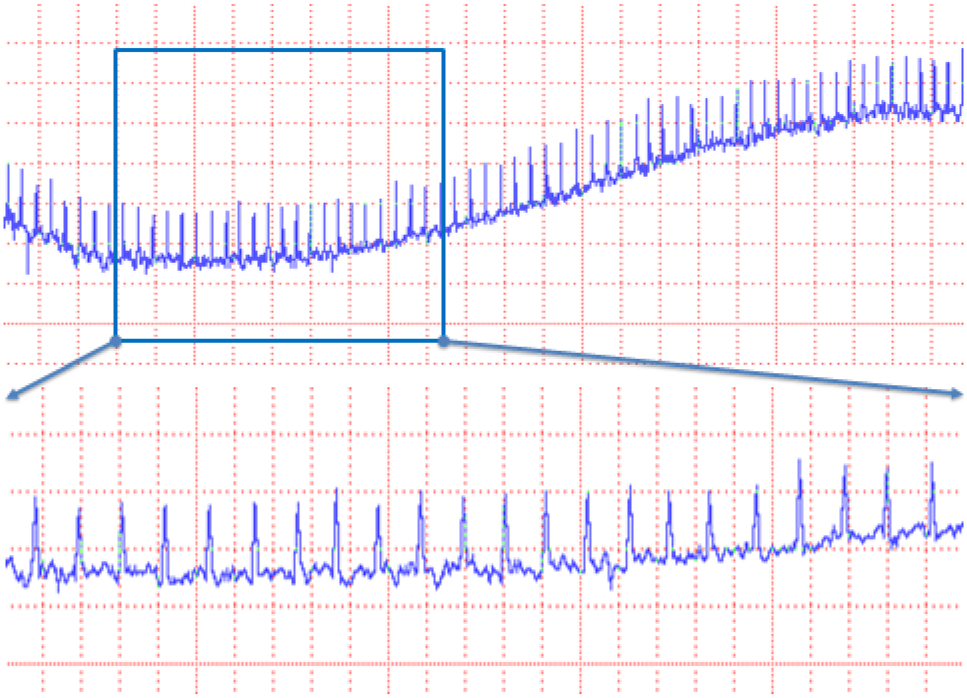

Figure 3.5. *B. tabaci B* adult second type of E2 waveform. This variation of E2 is characterized by dominant upward peaks with intervening low amplitude plateaus at a voltage level lower than the upward peaks. The plateaus may have barely perceptible small regular downward peaks. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

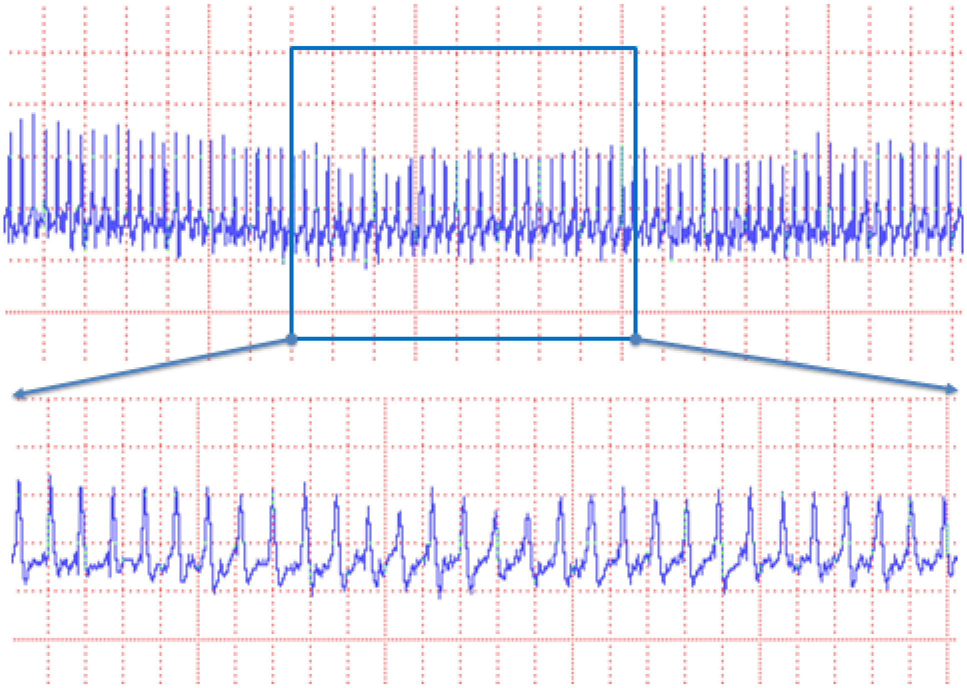

Figure 3.6. *B. tabaci B* adult second type of E2 waveform. This variation of E2 is characterized by dominant upward peaks followed immediately by downward peaks giving the waveform a sawtooth appearance. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

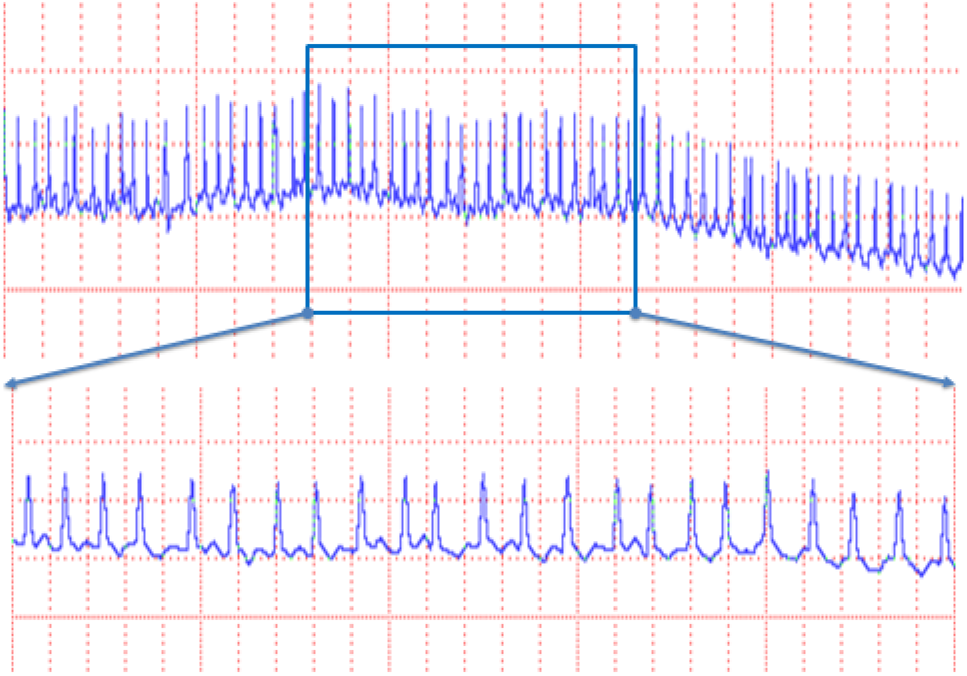

Figure 3.7. *B. tabaci B* adult second type of E2 waveform. This variation of E2 is characterized by dominant upward peaks with intervening low amplitude plateaus at a voltage level lower than the upward peaks. Unlike the variation shown in Fig 3.2B, the plateaus in this variation lack any trace of regular small downward spikes. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

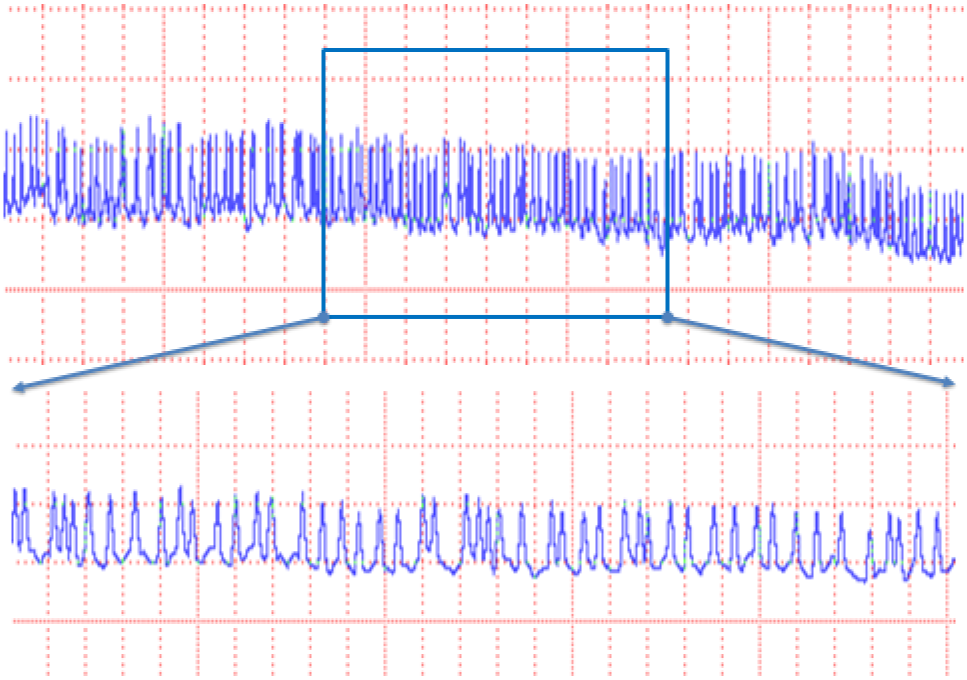

Figure 3.8. *B. tabaci B* adult second type of E2 waveform. This variation of E2 is characterized by dominant upward peaks with intervening low amplitude plateaus at a voltage level lower than the upward peaks. This variation differs from the one shown in Fig 3.2D by the upward spikes occurring at a higher and less regular frequency. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

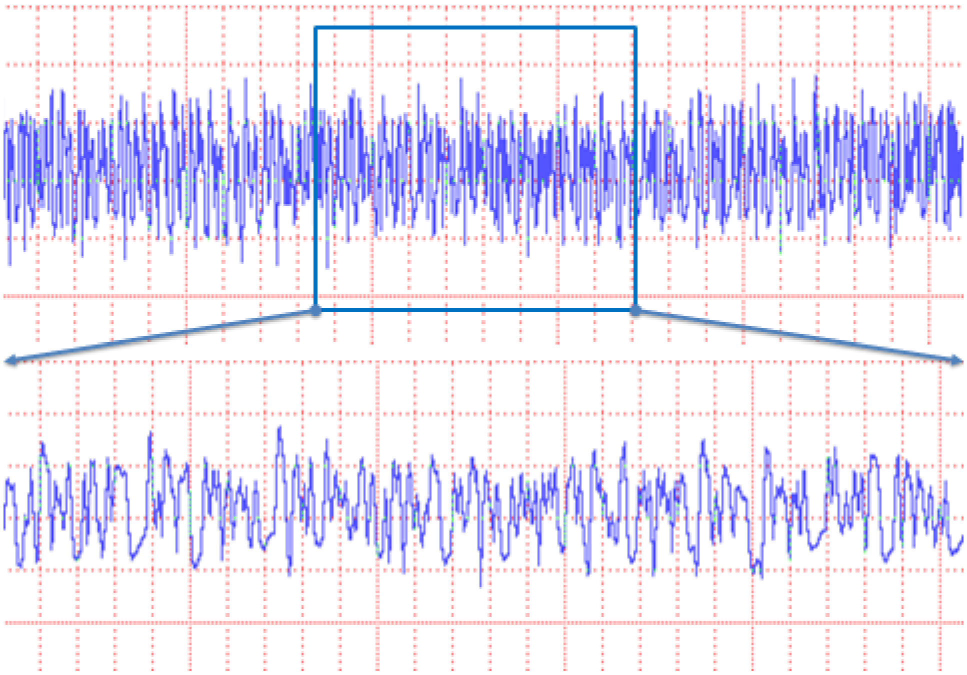

Figure 3.9. *B. tabaci B* adult third type of E2 waveform. This variation of E2 is characterized by regular downward peaks, somewhat similar to "classical" E2, but the intervening periods have high amplitude fluctuation (Fig. 3.3) rather than the low amplitude fluctuation in the characteristic of the plateaus between downward peaks in the "classical" E2 (Figs. 3.1, 3.5) Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

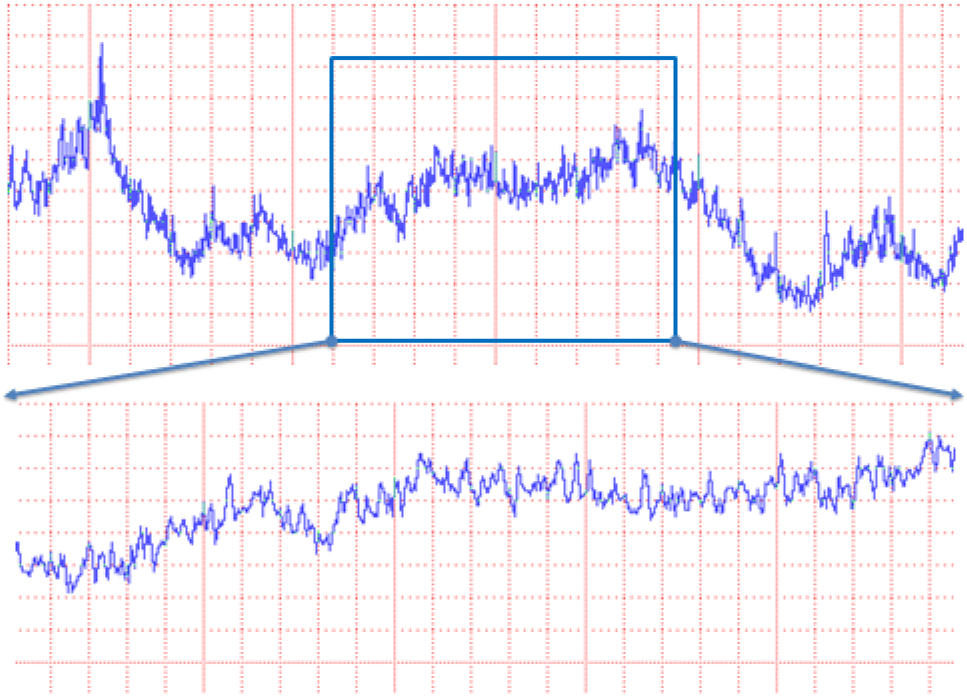

Figure 3.10. *B. tabaci B* adult noisy E2 waveform. This variation of E2 is characterized by large fluctuations in the overall voltage level and a lack of distinct patterns of voltage fluctuation. This may simply be a noisy recording of one of the previously described types of E2, or possibly be an additional variation of E2. Vertical axis is the voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

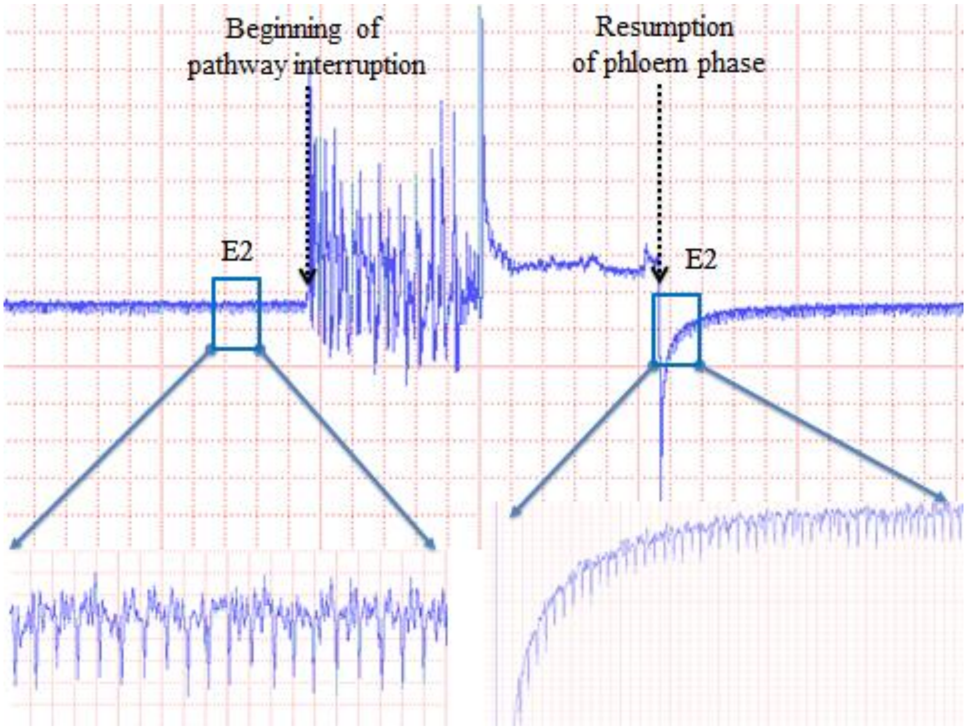

Figure 3.11. *B. tabaci B* adult pathway interruptions in phloem phase. Note the sharp voltage drop which indicates a change in position of the stylet tips from an extracellular to an intracellular position at the transition from the pathway interruption to the resumption of phloem phase. Also note that phloem phase resumes with waveform E2. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 2 sec per division. Inset detailed view at 0.2 sec per division.

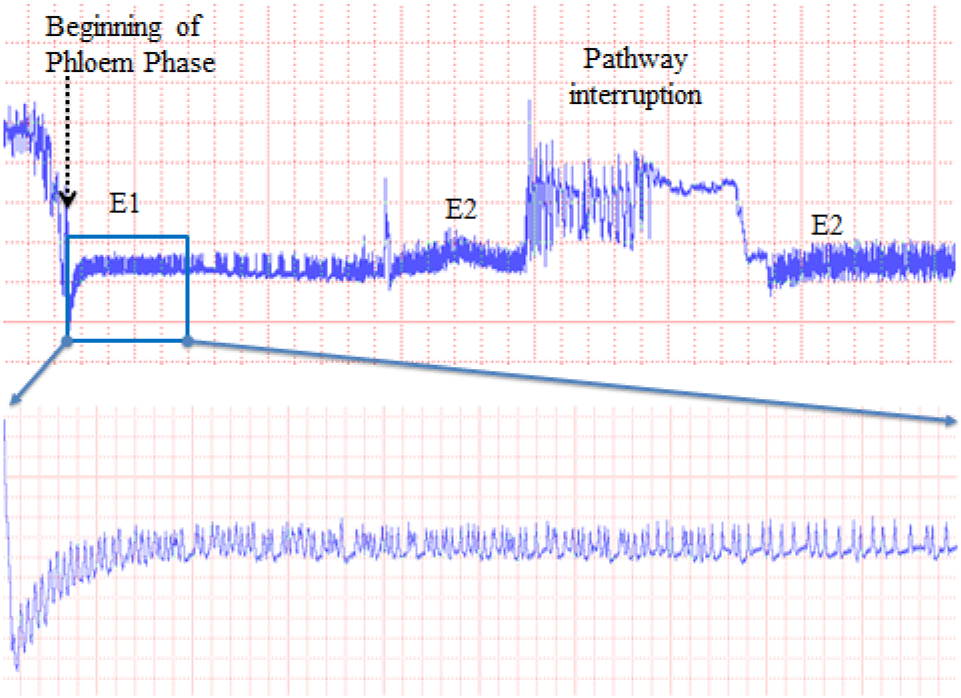

Figure 3.12. *B. tabaci B* adult E1 before E2, pathway interruption, and resumption into phloem phase (E2). Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 2 sec per division. Inset detailed view of E1 at 0.2 sec per division.

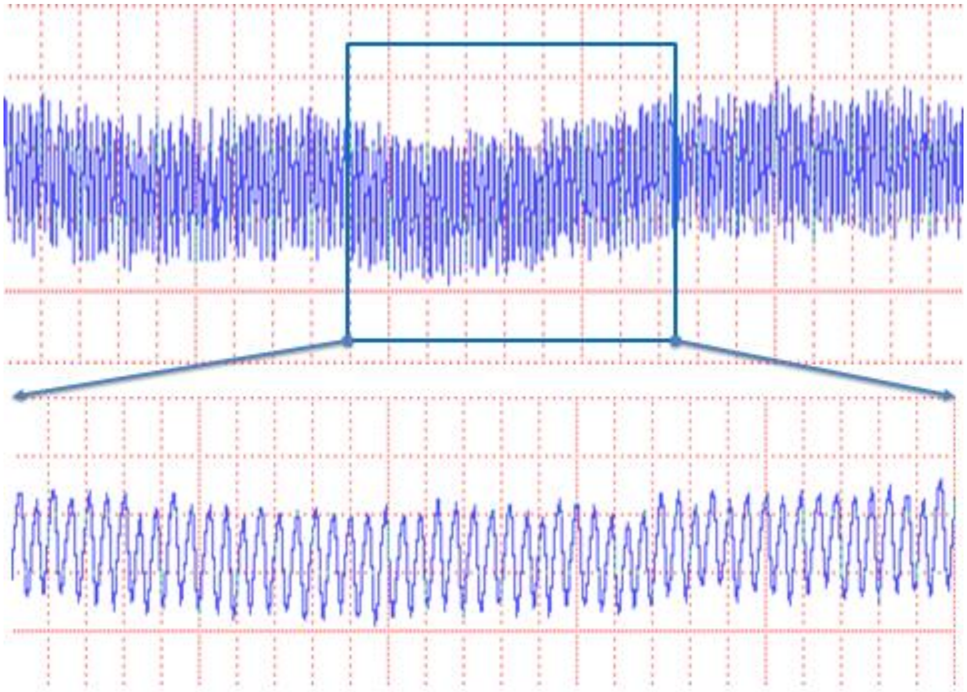

Figure 3.13. *B. tabaci B* second instar nymph high (H) frequency waveform. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

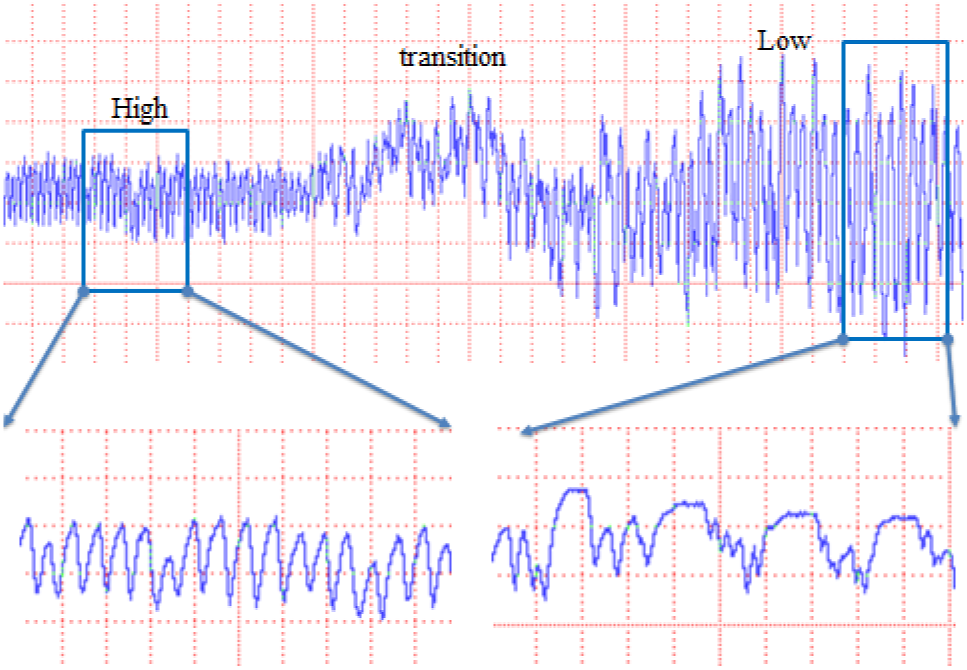

Figure 3.14. *B. tabaci* second instar nymph high (H) to low (L) frequency waveform transition. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

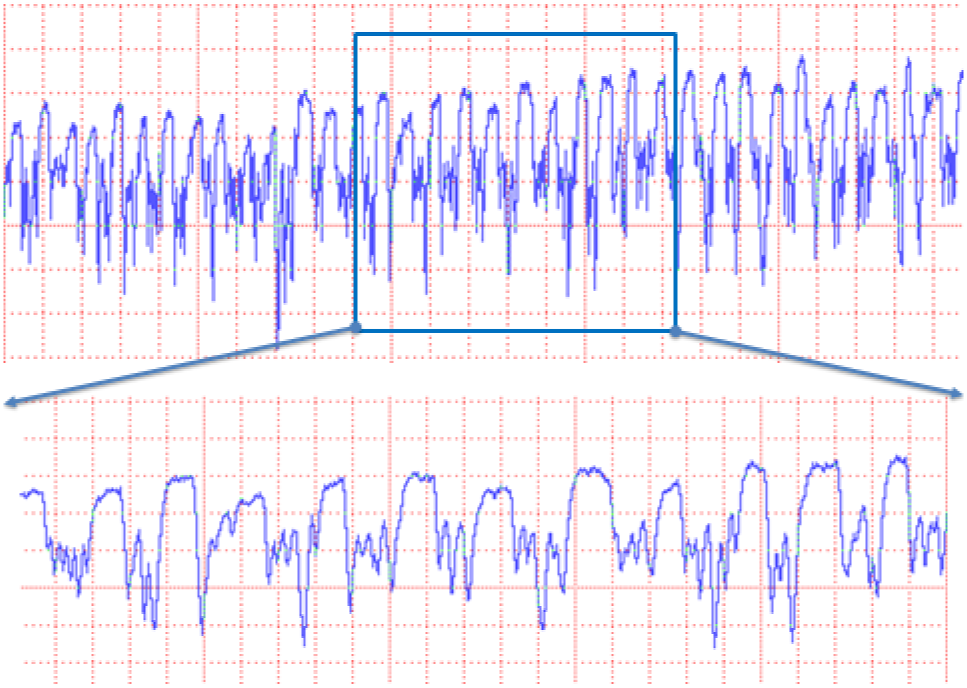

Figure 3.15. *B. tabaci B* second instar nymph low (L) frequency waveform. Vertical axis is voltage. Horizontal axis is time. Divisions on the horizontal axis: 0.6 sec per division. Inset: detailed view at 0.2 sec per division.

Table 3.1: Nymph waveforms and correlations with honeydew production.

Waveform[A]	Duration of waveform (sec)	Total number of honeydew drops	Frequency of waveform (Hertz)
H	3443	24	5
H*	1869	29	7.5
H	3599	34	8.7
H	4858	82	10
H	2906	30	10
H	6132	79	6.2
H	7060	57	8.7
H	7097	61	7.5
H	7186	79	7.5
H	7186	84	10
H	7186	63	6
H	4399	32	5
H	7186	82	10
L*	468	None	2.5
L	113	None	2.5
L	774	None	2.5

[A] The high (H) and low (L) amplitude nymph waveforms measured by EPG were correlated with or without honeydew production (video recording). Each row represents a different EPG/Video recording correlation. In one case, the H and L waveforms were from the same EPG/Video recording and this is indicated with an asterisk.

Table 3.2: Characteristics of four type of adult whitefly ingestion waveforms

Waveform[A]	Duration of phloem phase (sec)	Time from E1 to first honeydew drop (sec)	Duration of waveform (sec)	Time from beginning of waveform to first honeydew drop (sec)	Total number of honeydew drops	Mean ± SD of time between honeydew drops (sec)
Classical E2	1239	1107	1227	1107	1	N/A
Classical E2	21934	676	21897	639	9	353.25±139.13
*Classical E2	4387	908	4350	871	9	447.37±123.90
Classical E2	1562	1271	1538	1247	1	N/A
Second Type of E2	1408	1302	1384	1278	2	104.00±0.00
*Second Type of E2	657	670	502	653	2	26.00±0.00
*Second Type of E2	1234	762	437	718	2	473.00±0.00
*Second Type of E2	3275	2263	3215	2203	4	355.33±40.12
Third Type of E2	974	464	954	444	1	N/A
*Third Type of E2	6938	5814	1293	4659	4	327.33±180.58
*Fourth Type of E2	16546	4098	12693	245	30	269.55±71.74

[A] Each row represents a different EPG/Video recording correlation with an adult whitefly. The asterisk indicates figures taken from the EPG recordings for Figures 3.1-3.4.

Table 3.3: *B. tabaci B* adult phloem sap-ingestion the second type of E2 waveform for WT, *cim10*, *coi1*, *cev1*, and *npr1* Arabidopsis.

Genotype	Total number of whiteflies to reach phloem phase[A]	Total number of phloem phases	Number of phloem phases with the second type of E2 waveform	Percent of phloem phase with second type of E2 waveform
WT	14	36	4	11
cim10	19	47	3	6
coi1	11	30	3	10
cev1	13	32	1	3
npr1	16	60	2	3

[A] Data from chapter 1 of dissertation.

Table 3.4: *B. tabaci* B adult pathway interruptions during phloem phase on WT, *cim10*, *coi1*, *cev1*, and *npr1* Arabidopsis[A]

Genotype	Duration (sec)	Average Duration (Mean ± SD sec)	Time from beginning of phloem phase to beginning of pathway interruption (sec)	Average time from beginning of phloem phase to beginning of pathway interruption (mean ± SD sec)	Time from resumption of phloem phase after the pathway interruption to the end of phloem phase (sec)	Average time from resumption of phloem phase after the pathway interruption to the end of phloem phase (mean ± SD sec)
WT	28	22.34±5.50	10495	4262.66±5510.82	6985	6317.2±5994.66
WT	20		2259		11950	
WT	18		34		16	
cim10	12	26.04±7.92	1069	3564±2893.5	948	5022.5±5354.80
cim10	25		4611		3266	
cim10	32		370		15590	
cim10	26		8493		4213	
*cim10**	26, 35		3459, 3382		4409, 1709	
coi1	18	30.83±13.76	122	378±406.73	1258	1286.66±1196.25
coi1	45		165		2497	
coi1	29		847		105	
cev1	28	33.79±23.16	1778	6551±7622.69	7790	5812±3917.41
cev1	15		19625		1097	
cev1	73		4220		3323	
*cev1**	31, 20		786, 6346		11199, 5651	
npr1	26	39.61±28.67	293	874±657.37	2002	1618.66±1901.16
npr1	22		2143		5249	
npr1	36		591		1347	
npr1	24		949		394	
*npr1**	97, 31		557, 711		404, 316	

[A] Each row is from a different whitefly EPG recording. In rows where genotype is marked with an asterisk, there were two pathway interruptions, each in a different phloem phase. In all other rows, there was only a single pathway interruption.

Conclusion

Whiteflies cause extensive damage by depleting plant nutrients, transmitting over hundreds of plant viruses, and secreting honeydew onto plant surfaces which reduces crop quality and creates challenges during crop harvesting (Gamble 2002; Jones 2003). The *Bemisia tabaci* B whitefly is a difficult pest to control due to its ability to rapidly acquire resistance to insecticides and due to the relative ineffectiveness of biocontrol strategies (Martinez-Carrillo et al. 2006). With a wide range of plant hosts, *B. tabaci* B is either evading or suppressing effective plant defenses (Walling 2008). In the absence of strong gene-for-gene resistance in most crop plants, there is a strong need to develop new methods of controlling this whitefly. Understanding the basics of whitefly-plant interactions, and the defense traits that influence whitefly life history parameters, including adult and nymph feeding, will provide important foundations for new mechanism for controlling this devastating pest.

To date, studies have primarily focused on the role of plant's innate immune responses in whitefly-plant interactions. As summarized in the *Introduction* of this Dissertation, these studies have spanned responses of different host plants (eg., squash, tomato, Lima bean, and Arabidopsis) and their interactions with one or more whitefly species (*B. tabaci* B, *B. tabaci* A, *B. tabaci* Q, and *Trialeurodes vaporariorum*). These studies have primarily focused on the defenses regulated by the three key defense hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET), which are at the core of innate immune responses to pathogens and pests.

Of primary importance to this Dissertation was the discovery that nymphal development was accelerated on *cim10* and *coi1* plants, and delayed on *npr1*, *NahG* and *cev1* plants relative to each other and WT plants (Zarate et al. 2007). These genetic data and others, as well as the severe delays in nymphal development that occur on MeJA-treated plants, indicate the importance of JA in controlling defense traits that slow whitefly nymphal development (Zarate et al. 2007; Zhang et al. 2013b). The finding that *B. tabaci* B suppresses expression of effective JA-regulated defenses indicated that this whitefly manipulates its host to promote its own success (Kempema et al. 2007; Zarate et al. 2007). Furthermore, whitefly co-infestations with other herbivores have also shown that whiteflies suppress JA-regulated indirect defenses (i.e., volatiles) in both Arabidopsis and Lima bean (Zhang et al. 2013a; Zhang et al. 2009).

The goal of this Dissertation was to determine the tissue location of resistance traits that influence *B. tabaci* B adults and nymphs feeding in four defense mutants (*cim10, cev1, coi1,* and *npr1)* using the electrical penetration graph (EPG) technique. We hypothesized that adult whiteflies would encounter fewer deterrents at the surface, in the apoplast and in the phloem of the *cim10* and *coi1* mutants that accelerate nymph development (herein FastDev genotypes). Reciprocally, we hypothesized whiteflies would encounter more surface, pathway and phloem deterrents and therefore would have difficulty in accessing and consuming phloem sap on the *cevl* and *npr1*mutants that delay nymph development (herein SlowDev genotypes).

In Chapter 1, adult whitefly feeding behaviors were examined on WT and the mutants *cim10*, *cev1*, *coi1*, and *npr1*. Feeding behaviors on each genotype were compared in two manners. First, behaviors were compared among the five genotypes. Second, behaviors from pooled data from the two FastDev genotypes (*cim10* and *coi1*) versus the two SlowDev genotypes (*cev1* and *npr1*) were compared. In both of these comparisons, a small number of adult feeding variables differed significantly among the five genotypes. Surprisingly, whitefly adult feeding behaviors were not strictly correlated with the genotype-dependent changes in nymphal development observed in Zarate et al. (2007). The suite of variables that are most strongly associated with host plant acceptance/suitability were not consistently associated with how each genotype affected nymphal development. For most of the feeding variables where the ANOVA detected significant differences among the five genotypes, the significant differences were due to *cim10* differing significantly from one or more of the other genotypes. These data might suggest that feeding behavior of whiteflies differed more between *cim10* plants and the other genotypes than did the other genotypes differ among themselves.

Therefore, we must conclude that the alterations in plant biochemistry that are imparted by the *cim10*, *cev1*, *coi1*, and *npr1* mutants and which strongly impact nymphal development, have little impact on adult feeding behavior. The changes in defense traits in the JA- and SA-defense mutants did not consistently impact adult behaviors along the path to the phloem and within the phloem. These data indicate that the phloem-localized and JA-regulated defense traits that deter nymph development are in insufficient quantities to impact adults, do not influence adults, or these changes in phloem chemistry are not perceived by adults.

During annotation of the EPG waveforms of adult *B. tabaci* B feeding, several new waveform patterns were observed, and in Chapter 3, these waveforms were correlated with honeydew production. Using simultaneous EPG and video recordings, we showed that the classical E2 waveform and three novel E2-related waveforms (the second, third, and fourth E2 forms) were positively correlated with honeydew production in adults. Of particular interest was the second type of E2 waveform. This waveform was correlated with honeydew production but it strongly resembled the E1 waveform of aphids and E(pd)1 of whiteflies, which correlates with saliva secretion into the sieve element upon entering the phloem (Jiang et al. 1999; Liu et al. 2012; Prado and Tjallingii 1994; Tjallingii 2006).

In addition, we observed that during phloem ingestion, whiteflies make brief interruptions by transiently withdrawing their stylets from the sieve element, entering pathway phase, and then quickly reinserting their stylets back into the sieve element to resume phloem ingestion (Chapter 1, Chapter 3). This behavior occurs without an E1 waveform. This result was surprising because the E1 waveform has always been reported to be the first waveform upon entry into phloem-phase (Jiang et al. 1999; Tjallingii 2006). This observation indicates that E1 salivation is not always necessary when a sieve element is re-punctured.

B. tabaci B nymph feeding on WT and the defense mutants (*coi1*, *npr1*, *cev1*, *cim10)* was also examined using EPGs (Chapter 2). Similar to previous studies with *T. vaporariorum* on cucumber and *B. tabaci* B on alfalfa (Jiang and Walker 2003; 2007; Lei et al. 1996b), two waveforms (H and L) were produced on Arabidopsis plants. Using EPG and video recordings, we showed only the H waveform is correlated with honeydew production during nymph feeding on Arabidopsis (Chapter 3). These observations support the hypotheses of Lei et al. (1996a) and Jiang and Walker (2003) that waveform L is a non-ingestion waveform and may be salivation waveform. Also, the analyses of H and L feeding parameters did not differ significantly among the individual genotypes nor when the pooled FastDev and pooled SlowDev data were compared. The results indicate that the differences in developmental rate among the genotypes observed by Zarate et al. (2007) was not due to differences in time spent ingesting phloem sap.

Thus, possible hypotheses to explain the different nymphal developmental rate among the genotypes are (1) the volume of sap ingested per unit time may differ among the genotypes, (2) nutritional quality of the phloem sap may differ among the genotypes, and (3) phloem sap in SlowDev genotypes may contain component(s) that are deleterious to the whitefly or its essential endosymbionts, but are sublethal (no difference in mortality was observed by Zarate et al. 2007). Among these hypotheses, the second hypothesis appears to be the most likely explanation for the differences in developmental rate among the five genotypes. This hypothesis predicts that compared to WT or the SlowDev genotypes, the phloem of FastDev genotypes has increased levels of essential nutrients (e.g., amino acids) or decreased levels of metabolites that interfere with insect development. Thus, future studies should be directed towards differences in phloem sap nutritional and defensive constituents among these genotypes. While JA-regulated defenses appear to be at the core the plant immune response to *B. tabaci* nymphs on Arabidopsis (Zarate et al. 2007), the complex intertwining of JA and SA defense networks with each other and additional phytohormone pathways and their impact on phloem quality should be addressed (Erb et al. 2012; Pieterse et al. 2012).

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