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Categorization and Indentification of Mechanisms of Sugarcane Resistance to the Sugarcane Aphid (Hemiptera: Aphididae)

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CATEGORIZATION AND IDENTIFICATION OF MECHANISMS OF SUGARCANE
RESISTANCE TO THE SUGARCANE APHID (HEMIPTERA: APHIDIDAE)

A Dissertation
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the formal
requirements for the degree of
Doctor of Philosophy
in
The Department of Entomology

by
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May 2010

Dedicated to
my dearest parents
&
beloved wife
whose sincere prayers and sacrifices have made this milestone achievable for me

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ABSTRACT

Sugarcane in Louisiana is colonized and sometimes heavily infested by two aphid species, the sugarcane aphid, *Melanaphis sacchari* (Zehntner), and the yellow sugarcane aphid, *Sipha flava* (Forbes). *Melanaphis sacchari*, the main vector of sugarcane yellow leaf virus, is distributed throughout Louisiana's sugarcane-growing regions. Five cultivars representing 90% of the commercial acreage in Louisiana (LCP 85-384, HoCP 91-555, Ho 95-988, HoCP 96-540, L 97-128) were evaluated for resistance to aphids in the greenhouse. Antibiosis was the primary category of resistance to both aphid species. Based on the intrinsic rate of natural increase, L 97-128 and HoCP 91-555 were comparatively susceptible and resistant cultivars, respectively. In plant and ratoon cane field experiments, the fewest number of aphids occurred on HoCP 91-555, indicating resistance under field conditions. *Melanaphis sacchari* was more abundant than *S. flava* in both plant and ratoon cane. Laboratory studies indicated that *Diomus terminatus* (Coleoptera: Coccinellidae) could have additional role in managing *M. sacchari*.

Categories of resistance were also assessed by studying the feeding behavior of *M. sacchari* on LCP 85-384, HoCP 91-555, and L 97-128 using the electrical penetration graph technique. Differences among cultivars were not detected in the time interval that aphids initiate sieve element feeding; however, *M. sacchari* ingested phloem sap of L 97-128 twice as long as on HoCP 91-555. Differences between L 97-128 and HoCP 91-555 were not detected in levels of total phenolics and available carbohydrates, and in water potential. Free amino acid analyses of phloem sap extracts indicated that two essential amino acids (histidine and arginine) were absent in the phloem sap of HoCP 91-555. Analyses of honeydew collected from aphids feeding on both cultivars showed that two free essential (leucine and isoleucine) and two free nonessential (tyrosine and proline) amino acids were absent in the honeydew of aphids feeding on HoCP 91-555. These studies suggest that the absence of particular free essential amino acids in the phloem

sap of HoCP 91-555 and inability of *M. sacchari* to derive specific free essential and nonessential amino acids are underlying mechanisms responsible for reduced feeding time and lower growth potential on this cultivar.

CHAPTER 1: GENERAL INTRODUCTION

Contemporary commercial sugarcane is an interspecific hybrid of *Saccharum* spp., a member of the grass family Poaceae, and native to tropical and subtropical regions of Papua New Guinea. Sugarcane is damaged by a wide range of insect groups in many parts of the world, but Louisiana sugarcane is infested mainly by a stalk borer and several sap feeders. The major insect pest is the sugarcane borer, *Diatraea saccharalis* F. (Lepidoptera: Crambidae), which is responsible for more than 90% of the insect related damage to sugarcane (Reagan 2001). Common sap feeders include aphids, the West Indian canefly, *Saccharosydne saccharivora* Westwood, the pink sugarcane mealybug, *Saccharococcus sacchari* Cockerell, the sugarcane delphacid, *Perkinsiella saccharicida* Kirkaldy, and the sugarcane tingid, *Leptodictya tabida* Herrich-Schaeffer (White et al. 1995, Woolwine 1998, Setamou et al. 2005). Hemipterans in general are considered problematic because of disease transmission; however, the role of hemipteran-vectored diseases is less critical in North American sugarcane than in other sugarcane production areas of the world (Reagan 1995). Historically, integrated pest management (IPM) programs in Louisiana sugarcane have focused on *D. saccharalis* (Reagan and Martin 1989, Bessin et al. 1990, Bessin et al. 1991, Showler and Reagan 1991, White 1993, White et al. 2004, Reay-Jones et al. 2005a, Posey et al. 2006). Since 2000, Louisiana State University Agricultural Center sugarcane entomological research has focused primarily on the Mexican rice borer, *Eoreuma loftini* (Dyar) (Lepidoptera: Crambidae), another devastating pest of sugarcane (Reay-Jones et al. 2003, 2005, 2008). Within the aphid complex, only two aphid species, the sugarcane aphid, *Melanaphis sacchari* Zehntner, and the yellow sugarcane aphid, *Sipha flava* Forbes (Hemiptera: Aphididae), have been identified colonizing sugarcane in Louisiana. The common problems associated with these aphid species are transmission of viruses and development of black sooty mold on honeydew deposits, which can interfere with

photosynthesis (Hall and Bennet 1994). *Melanaphis sacchari* is an important vector of sugarcane yellow leaf virus (ScYLV), the causal agent of yellow leaf disease of sugarcane (Schenck and Lehrer 2000). Field surveys have shown that ScYLV infections occur at varying levels in all Louisiana sugarcane-growing areas, with some fields having up to 25% infected plants (McAllister et al. 2005). Sugar yield losses of 11 and 14% resulting from ScYLV have been documented in first and second Louisiana ratoon crops, respectively (Grisham et al. 2001). In order to minimize its spread, absence of ScYLV has been added to certification standards for micropropagated seedcane (McAllister et al. 2008). Effective management strategies are needed to reduce area wide populations of *M. sacchari*, but literature is sparse on the insect's biology and ecology.

Evaluation of commonly-grown commercial sugarcane cultivars for resistance to *M. sacchari* and *S. flava* was the primary goal of this research. *Melanaphis sacchari*, being the vector of ScYLV, is economically more important than *S. flava*; therefore laboratory and greenhouse studies were conducted to determine feeding behavior and performance of this aphid on different sugarcane cultivars (Chapters 3, 4). Plant resistance to insects is relative and highly variable (Smith 2005). Resistance recorded under greenhouse conditions may not be occurring under field conditions. Therefore, cultivars used in the greenhouse experiments were also evaluated under field conditions for two years to corroborate greenhouse results (Chapter 6). A noticeable activity of *Diomus terminatus* (Coleoptera: Coccinellidae) was observed during the peak population times of *M. sacchari* in the field study. Therefore, a biological control component was also included in the study in which the potential of this predator to control *M. sacchari* was assessed under laboratory conditions (Chapter 7).

Primary and secondary plant metabolites, such as amino acids and phenolics, can affect host plant acceptance, colonization, and population increase of aphids (Douglas 1998, Kessler

and Baldwin 2002). Levels of these metabolites were measured in susceptible and resistant cultivars to ascertain their possible roles in resistance against aphids (Chapter 5). Aphids have the advantage of overcoming nutritional deficiencies of host plant with or without the symbiotic association of bacteria of the genus *Buchnera* (Douglas 1998, Telang et al. 1999). Honeydew of aphids feeding on selected resistant and susceptible cultivars was analyzed for free amino acids to determine if amino acids were implicated in differences in feeding behavior and performance on these cultivars (Chapter 5).

This project helped identify potential sources of aphid resistance in Louisiana sugarcane germplasm. Use of the electrical penetration graph technique facilitated identification of sugarcane tissues that influence resistance to *M. sacchari*. Free amino acid work has enhanced our understating of underlying causal factors associated with *M. sacchari* population increase on different sugarcane cultivars. Field studies have provided critical information on aphid scouting time, which can be helpful in making appropriate management decisions.

CHAPTER 2: LITERATURE REVIEW

2.1. General Aphid Biology

Aphids (Hemiptera: Aphididae) are small, soft bodied insects that feed on plant phloem sap. Aphids are unique among insects in terms of their life cycle because both sexual (holocyclic) and asexual (anholocyclic) reproduction are common (Dixon 1985). Most aphids produce several parthenogenetic generations during summer, a single sexual generation in autumn, and overwinter as eggs. Polymorphism, the development of apterous (wingless) and alate (winged) forms, is also common in aphids. Alate forms help aphids disperse to alternate host plants (Dixon 1985, Guldemon 1990). Almost 10% of aphid species alternate host plants and many of these belong to the subfamily Aphidinae (Powell and Hardie 2001). Aphids that live on a single host species are termed autoecious, while aphids spending fall, winter and spring on a primary host (woody tree or shrub), and summer on a secondary host (a herbaceous plant) are called heteroecious (Dixon 1985). Aphids detect specific volatile cues from plants to successfully migrate back to their primary host plant (Powell and Hardie 2001). Because of complexity and variation in aphid life cycles, aphids are often described as “facultatively opportunistic” (Tatchell 1990). The ability to develop winged forms and reproduce asexually gives aphids the advantages of rapid dispersal and exponential reproduction.

Many aphids are crop pests, with more than 90% of them being host specific, whereas some are polyphagous (Risebrow and Dixon 1987). Common problems associated with aphids are direct feeding damage, galling, transmitting plant viruses, and producing honeydew. Black sooty mold develops on honeydew deposits and results in reduced photosynthesis and thus decreased yield of a plant (Risebrow and Dixon 1987, Tatchell 1990). In large numbers, aphids can deplete plant vigor and may even cause plant death (Risebrow and Dixon 1987). Aphid feeding can not only have immediate *i.e.*, within hours or days effects on host plant (Webster et

al. 1987, Puterka and Peters 1988, Behle and Michels 1990), but long term effects such as delayed plant development, reduced plant height, delayed pollen shed and silking and reduced grain fill are also possible (Bing et al. 1991). Deol et al. (1997) documented that greenbugs, *Schizaphis graminum* Rondani, feeding on sorghum, *Sorghum bicolor* L., leaves for one day caused continuous damage to the plant tissue for up to 22 days after the aphid removal.

2.2. Sugarcane Aphid Taxonomy, Distribution, and Host Plants

The sugarcane aphid, *Melanaphis sacchari* Zehntner, belongs to the order Hemiptera, suborder Sternorrhyncha, superfamily Aphidoidea and family Aphididae. *Melanaphis sacchari* is distributed in South Africa, India, Sri Lanka, Laos, China, Philippines, Australia, Hawaii, Central and South America (Blackman and Eastop 2000). It is an important pest of sorghum and sugarcane, *Saccharum* spp., in many parts of the world, and can also be found on hosts in the genera *Echinochloa*, *Oryza*, *Panicum*, and *Pennisetum* (Blackman and Eastop 2000).

Melanaphis sacchari was first discovered in the United States in Florida sugarcane in 1977 (Mead 1978). The first finding in Louisiana was reported on 9 September 1999 on the USDA-ARS Ardoyne Research Farm near Houma and a subsequent survey showed that eight out of 21 sugarcane-growing parishes were infested (White et al. 2001).

2.3. Sugarcane Aphid Morphology and Biology

Melanaphis sacchari is a small (1.1-2.0 mm) ant-tended aphid with variable body colors depending on the host plant and environmental conditions (Blackman and Eastop 2000). Pale yellow, yellow brown, purple or even pinkish colors have been documented (Blackman and Eastop 2000), but it is generally whitish under Louisiana conditions. *Melanaphis sacchari* body is ovate, siphunculi are a little longer than cauda, and terminal processes are more than three times longer than the base of the last antennal segment (Blackman and Eastop 2000). They are generally asexual (Blackman and Eastop 2000) but sexual forms have also been reported on

sorghum (David and Sandhu 1976) and sugarcane (Yadava 1966). *Melanaphis sacchari* change body morphs and both alate and apterous forms have been recorded. It has four nymphal stadia which are completed in four to twelve days. Adults survive from 10 to 37 days and may produce 34 to 96 nymphs per female (Singh et al. 2004).

Melanaphis sacchari can reproduce parthenogenetically year-round in Florida (Hall and Bennett 1994). Populations fluctuate over the sugarcane-growing season with low infestations recorded in spring that increase during May and June. Population outbreaks occur in mid-July and crash shortly thereafter. *Melanaphis sacchari* overwinter parthenogenetically on ratoon sorghum or wild alternate hosts such as *Sorghum verticilliflorum*, *S. halepense*, *Panicum maximum*, and *Setaria* spp. (Singh et al. 2004).

2.4. Yellow Sugarcane Aphid Taxonomy, Distribution, and Host Plants

The yellow sugarcane aphid, *Sipha flava* Forbes, belongs to the order Hemiptera, suborder Sternorrhyncha, superfamily Aphidoidea and family Aphididae. Forbes (1884) first described *S. flava* from sorghum fields in Illinois. Numerous species of Gramineae are suitable hosts including *Digitaria*, *Hordeum*, *Panicum*, *Paspalum*, *Pennisetium*, *Saccharum*, *Sorghum*, and *Triticum* (Blackman and Eastop 2000). The geographical range of *S. flava* includes much of North America (as far north as New York State and west to Washington State), the Caribbean, Central America, and South America (Blackman and Eastop 2000).

2.5. Yellow Sugarcane Aphid Morphology and Biology

Sipha flava body size ranges from 1.3 to 2.0 mm. The apterous forms are small, oval, and yellow with numerous long bristle-like hairs on the body. Winged forms have yellow abdomens with variable patterns of dorsal markings (Blackman and Eastop 2000). This aphid exhibits no host alternation, but parthenogenetic cycle is interrupted by annual sexual forms in areas with low winter temperatures. In contrast, in areas with warm winters, the aphid continues to

reproduce parthenogenetically (Blackman and Eastop 2000). *Sipha flava* colonizes the lower surfaces of leaves, usually on the lower to middle leaves of the stalk. However, during large outbreaks, upper leaves may also become infested (Hall and Bennett 1994).

2.6. Sugarcane Aphid and Yellow Sugarcane Aphid Economic Importance

Feeding by *M. sacchari* causes a slight loss of leaf greenness; however, heavily infested leaves turn black from sooty mold developing on honeydew deposits (Hall and Bennet 1994). Significant reductions in nitrogen, phosphorous, potassium, total sugar, and chlorophyll content in sorghum have been documented to be associated with infestations of *M. sacchari* (Singh et al. 2004). Factors such as host plant development stage and nutritional status, time and duration of infestation, interval between infestations, and environmental stress can affect the magnitude of yield losses due to *M. sacchari* infestation (Singh et al. 2004). A major problem associated with *M. sacchari* is the transmission of the persistent sugarcane yellow leaf virus (ScYLV), millet red leaf virus, and certain strains of the non-persistent sugarcane mosaic potyvirus (Blackman and Eastop 2000). In Hawaii *M. sacchari* is considered to be the most important and efficient vector of ScYLV. ScYLV was prevalent in 11 to 71% of clones of four *Saccharum* species in Hawaii with cultivars showing differential susceptibility to the disease that ranged from 0-95% (Schenck and Lehrer 2000). In South Africa *M. sacchari* is also commonly called the sorghum aphid because of severe losses incurred in sorghum. If no chemical treatment is applied, wilting/curling and chlorosis of leaves are common and yield losses of 46-78% have been recorded in sorghum (Van den Berg et al. 2001).

In Louisiana field surveys have shown that ScYLV infections occur at varying levels in all sugarcane-growing areas, with some fields having up to 25% infected plants (McAllister et al. 2005). In order to minimize its spread, absence of ScYLV has been added to certification standards for micropropagated seedcane (McAllister et al. 2008). Sugar yield losses of 11 and

14% resulting from ScYLV have been documented in first and second Louisiana ratoon crops, respectively (Grisham et al. 2001). However, direct yield losses due to *M. sacchari* feeding have not been recorded.

Sipha flava has been an important pest of sugarcane in the United States and elsewhere (Hall and Bennett 1994), causing reddish leaf discoloration from the injection of a toxin that leads to chlorosis and necrosis (Breen and Teetes 1986, Webster 1990). In addition to direct feeding damage, another concern associated with this aphid is the possibility of transmitting non-persistent sugarcane mosaic potyvirus (Hall and Bennett 1994, Blackman and Eastop 2000). Hall (2001) reported that the heights of sugarcane plants infested with *S. flava* in a greenhouse were reduced by 36.2 %, and infested plants produced fewer leaves and tillers. At the end of the study, infested plants had 71.7% less dry matter when compared to non-infested ones.

2.7. Sugarcane Aphid and Yellow Sugarcane Aphid Management

Early planting, high plant density, destruction of overwintering hosts (ratoon sorghum, millet, etc) and mulching are some of the cultural practices that might help lower populations of *M. sacchari* in sorghum (Singh et al. 2004). Climatic factors, such as heavy rains, can also help wash away aphids (Cichocka et al. 2002). However, chemical treatments are generally the main solution to prevent heavy population increases. Several insecticides including triazimate (Aphistar[®]), acephate (Orthene[®]), carbofuran (Furadan[®]), lambda cyhalothrin (Karate[®]), pyriproxyfen (Knack[®]) and fenpropathrin (Danitol[®]) were tested at small plot level against *M. sacchari* and *S. flava* in Louisiana (Posey et al. 2001). All but fenpropathrin and pyriproxyfen significantly reduced the number of aphids when compared to untreated checks. McAllister et al. (2003) also reported significant reduction in *M. sacchari* numbers three days after insecticide treatment. However, caution is necessary in selecting a proper insecticide because some might adversely affect beneficial insects and inadvertently increase aphid populations. Applications of

insecticides for sugarcane borer, *Diatraea saccharalis* F., control in Louisiana, for example, resulted in increased populations of *S. flava* (Showler et al. 1987). Similar results were documented from the use of pyrethroid insecticides fenvalerate (Pydrin[®]), cypermethrin (Cymbush[®]) and cyfluthrin (Baythroid[®]) against *D. saccharalis* (Bessin et al. 1988). Different chemicals can also have opposite effects on controlling the same aphid species. Lambda cyhalothrin, for example, suppressed populations of *S. flava*, but esfenvalerate (Asana[®]) enhanced its populations (Rodriguez et al. 1995). Similar caution is necessary when selecting fungicides because certain products might protect aphids from infection by entomopathogenic fungi. Nanee and Radcliffe (1971) documented an increase in green peach aphid, *Myzus persicae* Sulzer, populations on potatoes, which was associated with decrease in incidence of diseased aphids in fungicide treated plots.

Singh et al. (2004) listed more than 47 species of biological control agents effective in lowering *M. sacchari* populations in different countries (Singh et al. 2004). These include pathogens (*Verticillium lecanii* Zimmerman), parasitoids (Hymenoptera [Aphelinidae, Elasmidae, and Braconidae]), and predators (Diptera [Syrphidae, Cecidomyiidae, Chamaemyiidae], Neuroptera [Chrysomelidae, Hemerobiidae], Coleoptera [Coccinellidae], Hemiptera [Lygaeidae, Anthocoridae]). Among these groups, ladybeetles (Coccinellidae), lacewings (Chrysopidae), and hover flies (Syrphidae) are believed more important (Singh et al. 2004).

2.8. Aphid-Plant Interaction

Aphid arrival at the host plant is a chance event largely dependent on wind, but once in the close proximity of potential host, visual and olfactory cues play dominant roles (Niemeyer 1990). After alighting on the host, surface chemicals play an important role in host acceptance. When these chemicals are suitable, aphids start probing to taste inner plant components. Once

the aphid stylet reaches phloem, the final site of aphid feeding, it will accept or reject the host plant depending on the absence or presence of toxic compounds and required nutrients in the phloem (Auclair 1963, Risebrow and Dixon 1987). Bisges et al. (1990) studied within-plant dispersal of the spotted alfalfa aphid, *Therioaphis maculata* Buckton, on alfalfa, *Medicago sativa* L., and noticed that aphids preferred feeding on leaf blades of nodes near plant crowns, which indicated that aphids not only select particular host plants but also specific plant parts. Although the process of host selection depends on a combination of visual, olfactory and gustatory cues, the relative importance of each stimulus depends on the feeding habits of particular aphid species. For polyphagous aphids, visual stimuli are more important, while for oligophagous and monophagous aphids, olfactory cues play dominant role in host selection (Niemeyer 1990). Aphids also have the ability to develop winged forms in response to unfavorable environmental conditions, such as crowding or poor host plant nutritional quality, which facilitate their dispersal to other host plants (Muller et al. 2001).

2.9. Host Plant Resistance

Reginald H. Painter, the father of host plant resistance (HPR), defined HPR as “the relative amount of heritable qualities possessed by the plant that influence the ultimate degree of damage done by the insect” (Painter 1951). Smith (2005) redefined HPR as the “sum of constitutive, genetically inherited qualities that result in a plant of one cultivar or species being less damaged than a susceptible plant lacking these qualities.” A noteworthy example of HPR is the control of grape phylloxera, *Phylloxera vitifolia* Fitch, in the late nineteenth century in France (Painter 1951). The wine industry in France was devastated by this pest because of susceptible grape cultivars, and the industry was saved from collapse by the introduction of resistant American cultivars. Since then, HPR has been widely studied and established as a viable strategy for insect pest control (Painter and Peters 1956, Dahms and Wood 1957, Chada

1959, Wood Jr. 1961, Schuster and Starks 1973, Starks et al. 1983, Webster et al. 1987, Jackson and Sisson 1990, Dixon et al. 1990, Flinn et al. 2001, Zhu et al. 2005).

The possibility of insecticide resistance development in aphids and environmental concerns from pesticide use in the present era has rendered chemical control as a less favorable option. In the absence of natural enemies and chemicals, Brewer et al. (1998) argued that present and future populations of aphids depend largely on host plants because resistant cultivars sustain lower numbers of aphids with mostly green leaves, whereas susceptible plants sustain large numbers of aphids showing leaf curling and chlorosis. Setamou et al. (2005) also suggested that in the absence of parasitism, preference of sugarcane cultivars was the main reason for observed differences in sugarcane lace bug, *Leptodictya tabida* Herrich-Schaeffer, populations.

One advantage of growing resistant cultivars is the reduced need for insecticides for aphid control (Webster and Starks 1984, Hill et al. 2004). In addition, a combination of host plant resistance and chemical control can help reduce not only the cost of chemical control, but also the residue problems associated with chemicals (Smith 2005). Other advantages of planting resistant cultivars include enhanced chemical, biological, cultural control, and a decrease in the spread of plant disease vectors (Smith 2005). However, longer periods of resistant cultivar development (3-5 years for a single insect and 10 or more years for multiple insects), geographical limitations on expression of resistance, and the chances of development of resistance-breaking biotypes are the main disadvantages of HPR (Smith 2005). Thus, resistant varieties do not guarantee absolute and long-term control, especially for aphids that have the ability to develop biotypes in situations where resistance is controlled by a single major gene (Cartier 1963, Starks and Merkle 1977, Webster and Starks 1984, Dixon et al. 1990, Reese et al. 1994b, Zhu et al. 2005).

Because of variations in resistance levels of different crops, Painter (1951) suggested three scenarios for using resistant varieties: (1) as a principal control method, (2) as an adjunct to

other strategies, or (3) as a safeguard against the release of more susceptible varieties than those already present in the cropping system. Plants differ in their response to the same aphid species (Webster et al. 1987, Girusse et al. 1990, White 1990, Flinn et al. 2001, Cichocka et al. 2002, Hill et al. 2004) and different aphid species (Painter 1958). Aphids also have a differential ability to infest the same host plant (Gellner et al. 1990); omugi barley, *Hordeum vulgare* L, for example, was resistant to *S. graminum* but was susceptible to the corn leaf aphid, *Rhopalosiphum maidis* Fitch (Painter 1951). Similarly, alfalfa plants resistant to *T. maculata* were susceptible to pea aphid, *Acyrtosiphon pisum* Harris. Therefore, host plant effects on aphids can not be generalized and each aphid-plant interaction needs to be studied independently.

2.10. Factors Affecting Plant Resistance to Aphids

Plant resistance to arthropods is relative and highly variable, dependent upon several interacting factors including the insect, plant, and environment (Smith 2005). The plant variables include plant density, height, tissue age and type, phenology, infection of plant tissue by disease, evaluation of excised and intact plant tissues, and pre-assay damage to the tissues. Insect variables such as age, gender, density and duration of infestation level, pre-assay conditioning, activity period, and biotypes can affect expression of plant resistance. In addition, variations in environmental factors such as photoperiod, temperature, soil nutrients and moisture, agrochemicals, relative humidity, and atmospheric fluctuations also affect plant resistance to insects (Smith 2005).

Aphid populations do not increase uniformly over time or by cultivars (Hesler et al. 1999). Russian wheat aphid, *Diuraphis noxia* Mordvilko, densities and subsequent damage to susceptible wheat varieties was greater than that on resistant ones at five, 10 and 15 days after infestation (Quisenberry and Shotzko 1994). Several factors including duration of infestation and architectural features affect aphid potential to cause damage. Oat, *Avena sativa* L., varieties with

S. graminum infestation for short duration had little injury, but an extended infestation caused a marked reduction in yield (Dahms and Wood 1957). The role of plant architectural features in insect-plant interactions also changes with age. Low plant height rendered pea, *Pisum sativum* L., varieties more susceptible to *A. pisum* infestation at early growth stages, however, at full-growth stage, increased plant height resulted in reduced aphid populations (Cartier 1963). Taller plants with longer internodes and less dense foliage exposed aphids to more parasites, predators, direct sunlight, wind and rain.

Plants exhibit resistance to aphids at different growth stages. Karley et al. (2002) reported that *M. persicae* and the potato aphid, *Macrosiphum euphorbiae* Thomas, did not perform as well on tuber-filling plants (9-11 weeks old), *Solanum tuberosum* L., as on pre-tuber-filling plants (3-5 weeks old) of potato. Significant differences were documented in the preference of biotype E of *S. graminum* among 16 sorghum entries at both seedling and mature plant stages (Dixon et al. 1990). However, differences at the seedling stage were more distinct than those at maturity. Kazemi and van Emden (1992) compared bird cherry-oat aphid, *Rhopalsiphum padi* L., fecundity on wheat varieties of Iranian and UK origin. Ommid, an Iranian variety, was resistant to the aphid at all growth stages, but Moghan 2, another Iranian variety, showed resistance only at the tillering stage. Resistance has also been reported in all plant stages of the Dowling cultivar of soybean, *Glycine max* (L.) Merr., which provided season-long protection against the soybean aphid, *Aphis glycines* Matsumura, equal to the use of a systemic insecticide in a field test (Hill et al. 2004). Comparison of yield parameters such as height, dry mass, number of pods, number of seeds, seed yield, and seed weight under heavy aphid infestation with and without imidacloprid treatment revealed no differences for Dowling cultivar of soybean.

2.11. Aphid Biotype and Host Plant Resistance

Aphids can develop biotypes which differ in their potential to cause damage to the host plant (Cartier 1963, Puterka and Peters 1988). Cultivars also differ in their response to various

biotypes (Cartier 1963, Starks and Merkle 1977, Webster and Starks 1984, Zhu et al. 2005). The risk with biotypes is that several years of research can be nullified. Therefore, close collaboration among entomologists, plant breeders, chemists and geneticists is needed to avoid such a scenario (Jackson and Sisson 1990, Webster 1990).

2.12. Categories of Host Plant Resistance

The three classical bases, now termed categories (Smith 2005), of HPR are preference (or nonpreference), antibiosis, and tolerance (Painter 1951). Either single or multiple categories of resistance operate together to influence the population increase of insect pests (Dixon et al. 1990, Unger and Quisenberry 1997, Flinn et al. 2001, Hill et al. 2004). Although multiple categories make it difficult to determine the individual role of each category (Unger and Quisenberry 1997), such cultivars provide resistance that is presumably more stable and prolonged (Smith 2005). Knowledge of resistance categories in host plant can aid in the development of more effective IPM strategies.

2.12.1. Antixenosis

Antixenosis, a term to replace Painter's nonpreference (Kogan and Ortman 1978), involves plant characteristics that attract or repel insects (Painter 1951). Other components of antixenosis include excitant, suppressant, or deterrent effects of host plants (Kogan 1994). Antixenosis is important because it influences the initial stage of plant infestation, and has been identified as one of the main categories of cereal crop resistance to aphids (Gallun et al. 1966, Webster et al. 1987, Dixon et al. 1990, Webster et al. 1994, Flinn et al. 2001, Andarge and Westhuizen 2004). In all these studies, varieties with strong antixenosis attracted the fewest insect pests. Antixenosis in several different germplasm accessions of sorghum against *M. sacchari* have been recorded in various countries (Singh et al. 2004). A significant biotype-variety interaction was reported by Webster and Starks (1984) in their antixenosis tests, in which

differences were not detected for *S. graminum* biotype E preference to Wintermalt and Post varieties of barley, but biotype C showed significantly less preference to Post. Antixenosis has been documented as a major category of resistance in tobacco, *Nicotiana tabacum* L., against the tobacco aphid, *Myzus nicotianae* Blackman, mainly due to low levels of cuticular components (Jackson and Sisson 1990). However, antixenosis is not always the dominant category of resistance. Choice tests with different wheat cultivars, for example, did not show cultivar preference by *R. padi* (Hesler et al. 1999). Similarly, Webster (1990) screened three different lines of sorghum for *S. flava* resistance and concluded that antixenosis was not a category of resistance expressed in sorghum.

2.12.2. Factors Affecting Antixenosis

Plant characteristics such as leaf pubescence are important to antixenosis in several crops including sugarcane (Gallun et al. 1966, Roberts et al. 1979, Roberts and Foster 1983, Sosa 1990). Webster et al. (1994) concluded that leaf pubescence was an important factor in determining the preference and reproduction of *S. flava* and *S. graminum* on wheat. However, leaf pubescence may not always be repellent to insect pests (Starks and Merkle 1977), or it might provide resistance against one type of aphid pest and not the other (Webster et al. 1994). Soybean cultivars with dense pubescence were more susceptible to *A. glycines* than glabrous or normal cultivars, possibly due to the additional protection from predators and parasitoids provided by feeding under trichomes (Hill et al. 2004).

Other plant characters, such as leaf color, also play an important role in initial plant selection by aphids. At early seedling stages of pea, the color of foliage is positively correlated with the alighting response of winged aphids (Cartier 1963). The greatest numbers of *A. pisum* were recorded on varieties with yellowish-green foliage, lesser on varieties with green foliage, and the least on varieties with deep-green foliage. A red tint in wheat seedlings was thought to be

a visual stimulus responsible for antixenosis in the accession P.I.225245 against *D. noxia* (Unger and Quisenberry 1997). Leaf glossiness and the epicuticle also have variable effects on plant infestations by different insect species. Glossy lines of wild cabbage, *Brassica oleracea* L., consistently had fewer cabbage aphids, *Brevicoryne brassicae* L., and cabbageworms, *Artogeia rapae* L., but numbers of diamondback moths, *Plutella xylostella* L., were variable (Stoner 1990). Webster and Inayatullah (1988) recorded more *S. graminum* selecting plants oriented towards the sun, and reported a directional response of *S. graminum* in antixenosis tests on barley.

Several studies with *M. sacchari* have shown that traits such as small, narrow, or fewer leaves; low leaf bending at the seedling stage; greater plant height; more distance between two leaves; and waxy lamina and epicuticular wax on lower surfaces are responsible for reduced susceptibility of sorghum to this aphid species (Singh et al. 2004).

2.12.3. Antibiosis

Antibiosis refers to adverse effects on insect biology from feeding on resistant plants (Painter 1951). These adverse effects on aphids include, but are not limited to, reduced nymphal weight (Starks and Merkle 1977), reproductive rate (Dixon et al. 1990, Webster 1990, Robinson 1993), survivorship (Zeng et al. 1993), relative growth rate, body size (Fuentes-Contreras and Niemeyer 1998), adult longevity (Hill et al. 2004), and increased proportion of alates (Hesler et al. 1999), and prenymphepositional period (Andarge and Westhuizen 2004). Antibiosis has been well documented as a primary category of resistance to aphids in several studies (Webster and Starks 1984, Dixon et al. 1990, White 1990, Hill et al. 2004, Zhu et al. 2005). Different sorghum lines exhibited antibiosis against *M. sacchari* (Wang et al 1990, Singh et al. 2004). *Digitaria* species accession P.I. 364357 showed sufficient antibiosis to *S. flava* that prevented reductions in dry-matter yield, percent dry matter, and percent protein in plants (Ratcliff and Oakes 1982).

Webster (1990) also concluded that antibiosis and tolerance were the main categories of resistance against *S. flava* in sorghum. Fungal endophyte-infected perennial ryegrass, *Lolium perenne* L., genotypes also exhibited significant feeding deterrence and antibiosis to both *S. graminum* and *S. flava* (Breen 1993). Cotton, *Gossypium hirsutum* L., cultivars did not differ in their effects on developmental duration and survivorship of the cotton aphid, *Aphis gossypii* Glover, but feeding on cultivars with high gossypol content resulted in lower adult longevity and fecundity (Du et al. 2004). Such negative effects of host plant on biology of sucking insects like aphids can result in less infestation, and may indirectly slow the spread of viruses and reduce the need of insecticides for aphid control (Hesler et al. 1999).

2.12.4. Tolerance

A tolerant plant is able to grow and reproduce or repair injury in spite of supporting a population of pest approximately equal to one damaging a susceptible host (Painter 1951). Tolerance was the main category of resistance in different wheat cultivars against *S. graminum* biotype 1 (Flinn et al. 2001). In tolerance tests with *S. flava* vs. sorghum lines, Webster (1990) recorded 36% and 77% reductions in growth of two lines when compared to the uninfested lines. However, it is possible that cultivars with strong tolerance can recover from injury if infestations are controlled (Dahms and Wood 1957). Among the three components of resistance, tolerance is considered most useful because the risk of biotype development is reduced on such plants (Robinson et al. 1990). In addition, the natural levels of aphids and their biocontrol agents are not disturbed (Schuster and Starks 1973, Teetes et al. 1974). Thus, HPR with tolerance being a major component of resistance is often a compatible tactic in an IPM program.

2.13. Effect of Amino Acids on Aphid Performance and Feeding Behavior

Nitrogen is critical for the growth of every living organism because of its centrality to metabolic processes, cellular structure and genetic coding, and thus is potentially limiting to the

development and reproduction (Mattson 1980). The availability of amino acids in host plants is critical to the population growth of many insect herbivores (McNeil and Southwood 1978), especially aphids that feed on sugar-rich but amino acid-poor phloem sap (Febvay et al. 1988, Prosser and Douglas 1992, Douglas 1998). However, one advantage of feeding on phloem sap is the availability of nutrients in soluble, readily assimilable, and renewable forms (Risebrow and Dixon 1987). Based on their role in insect biology, amino acids are categorized as essential or nonessential. The essential ones are those that insects cannot synthesize by themselves and their absence can prevent growth (Chapman 1998). Nonessential amino acids are the ones that insects can synthesize in their body and need not be provided in the food (Chapman 1998).

Several studies depicting associations between concentration and composition of amino acids in phloem sap and aphid biology suggest that host plant nutritional quality has a role in mediating aphid feeding behavior and performance (Auclair 1963, Prosser and Douglas 1992, Douglas 1998, Karley et al. 2002). Black bean aphids, for example, spent more time ingesting phloem sap from susceptible broad bean, *Vicia fabae* L., cultivars than on less susceptible lines, and susceptibility was associated with relatively high concentrations of free essential and nonessential amino acids (Cichocka et al. 2002). Aphids not only select particular host plants that are nutritionally advantageous, but even feed on the most nutritious parts within these plants (Risebrow and Dixon 1987). Wilkinson and Douglas (2003) assessed the phloem amino acid composition of 16 host plant species of the polyphagous *A. fabae* and concluded that differences in dietary amino acid requirements of aphid clones contribute to intraspecific variation in plant utilization patterns.

Composition or balance of amino acids is a major factor in the development and reproduction of several aphid species (Febvay et al. 1988, Prosser and Douglas 1992, Sandström and Petterson 1994). Weibull (1987) documented that relative growth rates of bird cherry-oat

aphid, *Rhopalosiphum padi* L., were directly proportional to amino acid concentrations in the phloem sap of oat and barley. Cole (1997) determined the relative importance of glucosinolates and FAAs in phloem sap on performance of *B. brassicae* and reported a correlation between amino acid concentration and intrinsic rate of increase of *B. brassicae*. *Melanaphis sacchari* populations have been shown to increase quickly on sorghum genotypes with high nitrogen, sugar, and chlorophyll content (Singh et al. 2004).

2.14. Effects of Aphid Feeding on Plant Amino Acid Levels

Aphid feeding can induce multiple changes in plant physiology and different aphid species also affect the same host differently. *Schizaphis graminum* feeding, for example, caused a significant decrease in relative water and chlorophyll content of wheat seedlings; however, *D. noxia* feeding showed significant increase in amino N content (Gellner et al. 1990). Ciepiela (1989) reported an increase in the content of amino acids phenylalanine and tyrosine in the ears of winter wheat after infestation by the grain aphid, *Sitobion avenae* F. Although *D. noxia* resistant and susceptible wheat cultivars showed similar amino acid levels in non-infested samples, comparison of phloem sap from a damaged and undamaged susceptible wheat cultivar revealed changes in amino acid composition and increases in levels of essential amino acids, indicating nutritional enhancement of phloem sap (Telang et al. 1999). However, this effect on phloem sap amino acid composition was not recorded on the resistant cultivar. Sandstrom et al. (2000) also documented that aphid feeding can result in elevated levels of phloem amino acids.

2.15. Endosymbionts in Aphid Hemolymph

The phloem sap of vascular plants has a low concentration of nitrogenous compounds, particularly essential amino acids (Dadd 1985); and differences in composition of phloem sap of resistant and susceptible host plants have been revealed in several studies (Febvay et al. 1988, Sandström and Petterson 1994, Cichocka et al. 2002). The nutritional deficiency in phloem feeding insect's diet can be compensated for by biosynthetic contribution of endosymbionts of

the genus *Buchnera* located in mycetocytes within the hemolymph. In this symbiotic association, the bacteria have a place to survive and reproduce, whereby producing limiting essential amino acids for the aphid (Prosser and Douglas 1992, Douglas 1998).

2.16. Honeydew

Plant sucking insects like aphids excrete honeydew, which can lead to the development of black sooty mold and associated problems. However, one advantage of honeydew is that it can provide insights into the role of endosymbionts or aphid ability to enhance the nutritional quality of a host plant phloem sap (Douglas 1998, 2004, Telang et al. 1999). The amount of honeydew excreted by aphids can be another indicator of phloem sap composition. *Aphis fabae* feeding on resistant bean cultivars characterized by low amino acid concentrations, for example, excreted less honeydew than those feeding on susceptible ones (Cichocka et al. 2002).

2.17. Effect of Plant Secondary Compounds on Host Plant Resistance to Aphids

Plants contain secondary compounds, called allelochemicals, which are generally considered to have role in plant defense against herbivores (Fraenkel 1969). If toxic to insects, these compounds have the potential to be used as alternatives to conventional insecticides. Application of polygodial in a field trial against high populations of *R. padi* and barley yellow dwarf virus resulted in yields equivalent to that achieved by application of synthetic chemicals (Pickett et al. 1992). Both aphid behavior and performance have been shown to be affected not only by primary nutrients but by secondary compounds as well (Risebrow and Dixon 1987, Pickett et al. 1992). Aphids feeding on wheat cultivars with high levels of hydroxamic acid, a DIMBOA glucoside, showed a reduction in mean relative growth rate and body size (Fuentes-Contreras and Niemeyer 1998).

Various classes of allelochemicals present in different groups of plants include alkaloids, terpenoids, phenolics, tannins, and proteinase inhibitors. Among these, phenolics are the most widely distributed in plants and are predominant especially in the family Poaceae. Phenolics are

toxic to insect herbivores in several cases (Kessler and Baldwin 2002). Although aphid stylets penetrate epidermal and mesophyll tissues intercellularly, avoiding contact with vacuoles and other organelles that can be high in phenolics (Dreyer and Campbell 1987), plants with higher concentrations of phenolics have been shown to impair growth, development, and fecundity of aphids (Leszczynski et al. 1995, Kessler and Baldwin 2002). Urbanska et al. (2002) concluded that phenolics can be an antifeedant to the grain aphid, *Sitobion avenae* F., in cereal crops. *Melanaphis sacchari* populations increase slowly on sorghum genotypes with relatively high concentrations of polyphenols (Singh et al. 2004). Many phenolics are known from sugarcane and sugarcane products (Godshall and Legendre 1988). Changes in sugarcane phenolic levels due to insect herbivory are possible. Sugarcane whitegrub, *Antitrogus parvulus* Britton, feeding on the roots of sugarcane significantly changed both the amount and type of phenolics in all 15 clones tested, which included both grub resistant and susceptible genotypes (Nutt et al. 2004). Concentrations of some phenolics decreased while other's increased. Silva et al. (2005) also reported a significant increase in phenolics not only in sugarcane roots but also in the leaves after attack by root sucking froghopper, *Mahanarva fimbriolata* Stal. The susceptible sugarcane cultivar was even more responsive in terms of increase in phenolics in roots. These changes in phenolic levels can affect aphids either positively or negatively because of their stimulant or repellent effects on aphid colonization (Niemeyer 1990). Fecundity and the intrinsic rate of increase of the grey pine aphid, *Schizolachnus pinetti* F., for example, were negatively correlated with total phenolic concentration in Scots pine, *Pinus sylvestris* L., seedlings that were damaged by aphids (Holopainen and Kainulainen 2004).

2.18. Louisiana Sugarcane Industry

Sugarcane was the leading agricultural row crop in Louisiana with a total value of \$601.7 million in 2008 (Salassi et al. 2009). Sugarcane was grown in 22 parishes in 2008 on 401,435

acres by 526 producers. The average yield of cane produced per harvested acre was 32.7 tons with a sugar production of 224 pounds per ton of cane or 7,325 pounds per acre, which contributed to 42% of total cane production and 19% of total sugar production in the United States (Salassi et al. 2009).

Sugarcane is a vegetatively propagated crop and is planted during August and September in Louisiana. Fields are bedded into rows with 1.8-m row spacing. Whole stalks of seed cane or billets (small pieces of sugarcane stalk) are placed in a furrow within the bed, and the furrows are covered with soil to avoid freeze damage. The lowest temperature at which growth of cane occurs is 11 to 13 °C. However, for optimal growth, temperatures should be above 21°C, and preferably in the range of 27 to 38 °C. Harvest of sugarcane in Louisiana occurs from late September through mid-January.

The main cultivars currently available to sugarcane growers in Louisiana include HoCP 85-845 (Legendre et al. 1994), LCP 85-384 (Milligan et al. 1994), HoCP 91-555 (Legendre et al. 2000), Ho 95-988 (Tew et al. 2005), HoCP 96-540 (Tew et al. 2005), L 97-128 (Gravois et al. 2008), L 99-226 (Bischoff et al. 2009), L 99-233 (Gravois et al. 2009), and HoCP 00-950 (Tew et al. 2009). Since its release in 1993, the Louisiana sugarcane industry has relied extensively on the early-maturing cultivar LCP 85-384 with 91% acreage in 2004 (Legendre and Gravois 2009), because of its desirable agronomic characteristics, including high populations of small-diameter stalks, stubbling ability, and relatively high sugar and cane yields (Milligan et al. 1994, LaBorde et al. 2008). It has been credited for saving Louisiana's sugar industry from collapse (Gravois and Bischoff 2001). The annual impact since the release of LCP 85-384 has been estimated at about \$100 million into Louisiana's economy through the sugar industry (Gravois and Bischoff 2001). However, concerns associated with the spread of common brown rust, *Puccinia melanocephala* Syd., have been instrumental in a shift in cultivar composition across the state in

recent years. A survey in 2008 indicated a substantial change in cultivar composition of the industry with 22, 2, 5, 44, and 17 % acreage under LCP 85-384, HoCP 91-555, Ho 95-988, HoCP 96-540, L 97-128, respectively (Legendre and Gravois 2009), which is likely to affect insect pest pressure on area wide bases.

Sugarcane is damaged by a wide range of insect groups in many parts of the world; however, Louisiana sugarcane is infested mainly by a stalk borer and several sap feeders. The major insect pest is the stalk-boring *D. saccharalis*, which is responsible for more than 90% of the insect related damage on sugarcane (Reagan 2001). Common sap feeders include aphids, West Indian canefly, *Saccharosydne saccharivora* Westwood, pink sugarcane mealybug, *Saccharococcus sacchari* Cockerell, sugarcane delphacid, *Perkinsiella saccharicida* Kirkaldy, and sugarcane tingid, *Leptodictya tabida* Herrich-Schaeffer (White et al. 1995, Woolwine 1998, Setamou et al. 2005).

2.19. Aphids on Sugarcane

There are at least 10 species of aphids recorded as colonizing sugarcane in different parts of the world (Blackman and Eastop 2000). These include *M. sacchari*, *S. flava*, the sugarcane wooly aphid, *Ceratovacuna lanigera* Zehntner, the sugarcane root aphid, *Geoica lucifuga* Zehntner, the rusty plum aphid, *Hysteroneura setariae* Thomas, *R. maidis*, the grain aphid, *Sitobion miscanthi* Takahashi, *Forda orientalis* George, *Tetraneura javensis* Goot, and *T. nigriabdominalis* Sasaki. The only two aphid species identified colonizing Louisiana sugarcane are *M. sacchari* and *S. flava*.

2.20. Host Plant Resistance Studies with Sugarcane in Louisiana

Diatraea saccharalis has been the focus of most IPM strategies, mainly insecticides and resistant cultivars, in Louisiana (White and Irvin 1987, Reagan and Martin 1989, Bessin et al. 1990, Bessin et al. 1991, White 1993, Reagan 2001, Posey et al. 2006). These studies have

shown that cultivars differ in their resistance levels and that physical factors such as tissue toughness might play a dominant role in resistance. However, insecticide selection and the use of resistant cultivars against major insect pests might shift the distribution and population levels of secondary pests (Setamou et al. 2005) like aphids. There has been an increase in *M. sacchari* populations and incidence of ScYLV in various sugarcane-growing areas in recent years.

Over the past 50 years, mostly small grains and cereal crops have been studied and developed for aphid resistance (Painter and Peters 1956, Dahms and Wood 1957, Chada 1959, Wood 1961, Schuster and Starks 1973, Starks et al. 1983, Webster et al. 1987, Dixon et al. 1990, Flinn et al. 2001, Zhu et al. 2005), and little attention has been given to other crops such as sugarcane (Hall 1987, Sosa 1990, White 1990, Hall 2001). Type of inheritance of resistance characters and nature of the crop *i.e.*, self-pollinated or cross-pollinated, has affected such efforts (Webster 1990). Due to the incidence of ScYLV in Louisiana, effective management programs are needed to reduce the area wide populations of *M. sacchari*; however, literature is sparse on several aspects of this insect's biology and ecology. This project was directed at the evaluation of several commercial sugarcane cultivars for resistance to *M. sacchari* and *S. flava* under greenhouse as well as field conditions. The possible role of amino acids and phenolics along with *M. sacchari* feeding behavior were also studied. Identification of aphid resistant germplasms and understanding the bases of aphid-sugarcane interactions can assist in future sugarcane breeding programs.

CHAPTER 3: CATEGORIZING SUGARCANE CULTIVAR RESISTANCE TO THE SUGARCANE APHID AND YELLOW SUGARCANE APHID (HEMIPTERA: APHIDIDAE)¹

3.1. Introduction

Sugarcane, interspecific hybrids of *Saccharum* spp., in Louisiana is colonized by two aphid species, the yellow sugarcane aphid, *Sipha flava* (Forbes), and the sugarcane aphid, *Melanaphis sacchari* (Zehntner). *Sipha flava* is yellow in color, its body length ranges from 1.3 to 2.0 mm, and it has numerous long bristle-like hairs with dusky transverse markings on the dorsum. The species has been found in North, Central, and South America and on various Caribbean islands, and it can feed on numerous genera of Gramineae including *Digitaria*, *Hordeum*, *Panicum*, *Paspalum*, *Pennisetum*, *Saccharum*, *Sorghum*, and *Triticum* (Blackman and Eastop 2000). This aphid has been an important pest of sugarcane in the United States and elsewhere (Hall and Bennett 1994), causing reddish leaf discoloration from the injection of a toxin that leads to chlorosis and necrosis (Breen and Teetes 1986, Webster 1990). In addition to direct feeding damage, another concern associated with this aphid is the possibility of transmitting non-persistent sugarcane mosaic potyvirus (Hall and Bennett 1994, Blackman and Eastop 2000).

Melanaphis sacchari is generally whitish in color under Louisiana conditions, with a body length ranging from 1.1 to 2.0 mm. This species is distributed throughout tropical and subtropical regions of the world on hosts in the genera *Echinochloa*, *Oryza*, *Panicum*, *Pennisetum*, *Saccharum*, and *Sorghum* (Blackman and Eastop 2000). In Louisiana sugarcane, *M. sacchari* has become the most abundant aphid species in recent years. Feeding by *M. sacchari* on sugarcane causes a fading of leaf greenness, and heavily infested leaves turn black from sooty mold developing on honeydew deposits (Hall and Bennet 1994). A major problem associated with *M. sacchari* is transmission of the persistent sugarcane yellow leaf virus (ScYLV), millet

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red leaf virus, and certain strains of the non-persistent sugarcane mosaic potyvirus (Blackman and Eastop 2000). ScYLV is a serious problem in Hawaii (Schenck and Lehrer 2000), and in Louisiana where absence of ScYLV has been added to certification standards for micropropagated seedcane to minimize spread of the virus (McAllister et al. 2008). Field surveys have shown that sugarcane yellow leaf virus infections occur at varying levels in all sugarcane-growing areas, with some fields having up to 25% infected plants (McAllister et al. 2005). Sugar yield losses of 11 and 14% resulting from ScYLV have been documented in first and second Louisiana ratoon crops, respectively (Grisham et al. 2001). The spread and incidence of ScYLV in sugarcane can be reduced by use of aphid-resistant cultivars (Smith 2005). However, little is known about sugarcane cultivar resistance to aphids. The objective of this study was to assess selected commercial sugarcane cultivars for their ability to tolerate aphid injury and to express antixenotic or antibiotic effects on *M. sacchari* and *S. flava*.

3.2. Materials and Methods

3.2.1. Aphids and Plants

Melanaphis sacchari and *S. flava* greenhouse colonies were based on aphids collected from sugarcane fields at the Louisiana State University Agricultural Center Sugar Research Station located at St. Gabriel, LA. The colonies were maintained on sorghum plants under natural light:dark conditions at temperatures ranging from 30-35 °C. The five commercial sugarcane cultivars used for *M. sacchari* assays were LCP 85-384 (Milligan et al. 1994), HoCP 91-555 (Legendre et al. 2000), Ho 95-988 (Tew et al. 2005), HoCP 96-540 (Tew et al. 2005), and L 97-128 (Gravois et al. 2008), which represented 90% of Louisiana sugarcane acreage in 2008 (Legendre and Gravois 2009). Based on results of these assays, experiments with *S. flava* were conducted only on LCP 85-384, HoCP 91-555, and L 97-128. Stalks used for planting were derived from seed-cane fields at the Sugar Research Station that had been heat-treated in water

(50 °C for two h) for the control of ratoon stunting disease (Comstock 2002). Billets (small pieces of sugarcane stalk) with at least one vegetative bud were planted in 9.46-L pots containing sterilized greenhouse soil (1:1:1 parts by volume of soil, sand, and peat moss) with 1.2 g of 19:6:12 (N-P-K) controlled release fertilizer (Osmocote, Scotts Miracle-Gro, Marysville, OH). The numbers of replications (pots) in *M. sacchari* experiments were seven for antixenosis determinations and five for both antibiosis and tolerance determinations. In separate experiments with *S. flava*, there were seven replications of each cultivar to determine antixenosis, antibiosis and tolerance. Sugarcane plants at 6-8 leaf stage (80-90 cm height from base of plant to the bottom of the whorl leaf) were used in assays.

3.2.2. Antixenosis

Pots of each cultivar were placed around a rectangular wooden platform raised to a level even with the tops of the pots, and were arranged such that at least one healthy, intact leaf of each cultivar touched the wooden platform. Fifty nymphs of mixed ages were released at the center of the wooden platform providing an equal chance for each cultivar to be selected. After 24 h, the numbers of aphids on each cultivar were recorded.

3.2.3. Antibiosis

Two nymphs from sorghum were removed with a camel hair brush and confined within a 2 × 0.6 cm double-sided adhesive cage (Scotch Mounting Tape, 3M, St. Paul, MN) on the abaxial surface of a leaf on each cultivar. The open top of the cage was covered with organdy cloth. The aphids were allowed to develop on the sugarcane leaf surface until after reproduction occurred to avoid possible effects of host plant conditioning on subsequent generations (Robinson 1993). After reproduction, adults were removed and two nymphs were allowed to mature on each cultivar. When these aphids became adults, one aphid was removed from each cage, leaving one per cage. This aphid served as the parent aphid (P1) and data on its life history

parameters such as prereproductive period (birth to onset of reproduction), reproductive period (days in reproduction), fecundity (total number of nymphs produced), fecundity per day, and longevity were recorded. When the first F1 was produced, it was moved to another cage on a different leaf of the same plant and allowed to mature. When this F1 nymph produced its first offspring, the number of days for the F1 to reach reproductive maturity was recorded and the aphid was removed from the plant. Antibiosis was assessed by computing aphid demographic statistics such as the intrinsic rate of natural increase (r_m), generation time (T), finite daily rate of increase (λ), and doubling time (DT). The female progeny per female per day is r_m , and was estimated by using the formula $r_m = 0.738(\log_e M_d)/d$, where “d” is the prereproductive period of F1 in days, M_d is number of nymphs produced by P1 in “d” days, and 0.738 (a correction constant) is the slope of M_d over d for four aphid species (Wyatt and White 1977). The mean time required for a given population to complete one generation is T and was calculated using the formula $T_d = d/0.738$ (Wyatt and White 1977). Lambda (λ) is a function of r_m and was estimated using the formula $\lambda = \text{antilog of } r_m$ (DeLoach 1974). Whereas, DT is the time required by a population to double its numbers and is also a function of r_m . It was calculated using the formula $DT = [\log_e (2)]/r_m$ (DeLoach 1974).

3.2.4. Tolerance

Retention of chlorophyll content was used as a measure of tolerance (Girma et al. 1998). Five plants served as treatment plants and five others of the same cultivar were controls. Treatment plants were infested with 15 *M. sacchari* nymphs confined in a 3 × 0.6 cm double-sided adhesive cage (Scotch Mounting Tape, 3M, St. Paul, MN) on the underside of a uniformly green leaf for one week. The cage was covered with a 4 × 2 cm organdy cloth. Newborn nymphs, if any, were removed from the cages to keep constant insect pressure on all cultivars, thus ensuring separation of tolerance from antibiosis (Reese et al. 1994b). After one week of

infestation, data on chlorophyll content was recorded using a Soil and Plant Analysis Development chlorophyll meter (SPAD-502, Minolta, Tokyo, Japan). Five representative chlorophyll measurements were taken from the infested caged region and five from a non-infested comparable leaf on the control plant. These readings were averaged and a SPAD chlorophyll-loss index was calculated using mean SPAD readings in the following formula: $SPAD\ index = (C-T)/C$ (Deol et al. 1997) where C = SPAD value for the control leaf, and T = SPAD value for the infested, caged, area of the leaf. The SPAD index values range from 0 (no loss) to 1.0 (total loss), and were used to calculate percent chlorophyll loss. Tolerant cultivars were expected to show less loss of chlorophyll, indicated by higher chlorophyll content readings and lower SPAD index values, as compared to susceptible cultivars (Flinn et al. 2001).

Chlorophyll content loss in connection with *S. flava* feeding was recorded as described for *M. sacchari*. Because *S. flava* feeding changes leaf coloration (Breen and Teetes 1986, Hall and Bennett 1994), degree of reddish discoloration within the caged area was used as an additional sign of tolerance. When aphids were removed after a week, the degree of discoloration in the caged area was estimated visually and rated on a scale of 1 (0-20% discolored) to 5 (81-100% discolored) (White 1990). Readings on chlorophyll content and leaf discoloration were also taken from the same spot one week after aphid removal to determine if recovery was occurring.

3.2.5. Data Analyses

The descriptive statistics in each experiment were obtained using Proc Means in SAS (SAS Institute 2006). Differences in aphid numbers recorded on each cultivar in antixenosis test were analyzed using one-way analysis of variance (ANOVA; Proc Mixed SAS Institute 2006). Differences in life history parameters and demographic statistics were also detected by subjecting computed values to one-way ANOVA (Proc GLM, SAS Institute 2006), and means

separated by the Tukey's HSD test at $P = 0.05$. Data on percent chlorophyll loss were first arcsin-square root-transformed and then analyzed using one-way ANOVA (Proc GLM, SAS Institute 2006) followed by Tukey's HSD mean separation at $P = 0.05$. Data on *S. flava* discoloration rating was also subjected to one-way ANOVA (Proc GLM, SAS Institute 2006).

3.3. Results

3.3.1. Antixenosis

Treatment differences were not detected in the number of *M. sacchari* present on different cultivars 24 h after release. Mean numbers (\pm SEM) of aphids on each cultivar were 9.1 ± 0.7 for LCP 85-384, 9.0 ± 0.7 for HoCP 91-555, 9.3 ± 0.7 for Ho 95-988, 8.6 ± 0.5 for HoCP 96-540, and 10.1 ± 0.6 for L 97-128. In the *S. flava* test, no cultivar preference was found, with treatment means of 15.3 ± 1.3 on LCP 85-384, 15.0 ± 0.8 on HoCP 91-555, and 18.0 ± 1.9 on L 97-128.

3.3.2. Antibiosis

The prereproductive period of *M. sacchari* was not influenced by any of these cultivars. However, differences among cultivars were detected in the reproductive period ($F = 5.98$; $df = 4, 20$; $P = 0.0025$) with ≈ 11 fewer days on HoCP 91-555 than on L 97-128 (Table 3.1). The mean number of nymphs per adult on L 97-128 was ≈ 6 -fold greater than on HoCP 91-555 ($F = 5.82$; $df = 4, 20$; $P = 0.0028$). Nymphs per day were 3.5-, 2.9-, 2.6-, and 2.3-fold greater on LCP 85-384, L 97-128, HoCP 96-540, and Ho 95-988, respectively, than on HoCP 91-555 ($F = 5.79$; $df = 4, 20$; $P = 0.0029$). Longevity of *M. sacchari* was 6.6 and 7.6 d shorter on LCP 85-384 and HoCP 91-555, respectively, than on L 97-128 ($F = 3.49$; $df = 4, 20$; $P = 0.0257$) (Table 3.1).

Among the cultivars tested, the lowest r_m value for *M. sacchari* was computed on HoCP 91-555, which was 50-64% less than that of other cultivars ($F = 12.19$; $df = 4, 20$; $P < 0.0001$) (Table 3.2). *Melanaphis sacchari* λ was $\geq 9.6\%$ lower on HoCP 91-555 than on other cultivars (F

= 11.47; df = 4, 20; $P < 0.0001$) (Table 3.2). Differences were not detected in T for aphids on different cultivars, but DT on HoCP 91-555 was 2.1 – 3.1-fold greater than on the other cultivars ($F = 7.05$; df = 4, 20; $P < 0.0001$) (Table 3.2).

Table 3.1. Life history parameters with mean (\pm SE) of *M. sacchari* reared on sugarcane cultivars- antibiosis test.

Cultivar	Prereproductive period (days)	Reproductive period (days)	Fecundity	Fecundity/day	Longevity (days)
LCP 85-384	8.0 \pm 0.4a	14.4 \pm 2.2b	15.8 \pm 2.9a	1.12 \pm 0.2a	25.0 \pm 1.9ab
HoCP 91-555	10.2 \pm 1.5a	10.6 \pm 0.9b	3.4 \pm 0.7b	0.32 \pm 0.1b	24.0 \pm 1.2b
Ho 95-988	9.8 \pm 0.8a	15.6 \pm 1.1ab	11.8 \pm 3.4ab	0.72 \pm 0.2ab	28.6 \pm 1.1ab
HoCP 96-540	10.4 \pm 0.7a	14.6 \pm 1.6ab	11.8 \pm 1.2ab	0.82 \pm 0.0ab	28.4 \pm 1.8ab
L 97-128	7.6 \pm 1.1a	21.2 \pm 1.6a	19.6 \pm 2.2a	0.92 \pm 0.6a	31.6 \pm 2.0a

Means within columns followed by the same letter are not significantly different ($P > 0.05$, Tukey's HSD test).

Differences were not detected among cultivars in the prereproductive period of *S. flava*; however, the reproductive period was 1.4- and 1.6-fold longer on LCP 85-384 and L 97-128, respectively, than on HoCP 91-555 ($F = 5.31$; df = 2, 18; $P = 0.0154$) (Table 3.3). Fecundity of

Table 3.2. Demographic statistics with mean (\pm SE) of *M. sacchari* reared on sugarcane cultivars- antibiosis test.

Cultivar	r_m^a	λ^b	T ^c (days)	DT ^d (days)
LCP 85-384	0.132 \pm 0.01a	1.142 \pm 0.01a	19.512 \pm 0.92a	5.309 \pm 0.37b
HoCP 91-555	0.057 \pm 0.01b	1.059 \pm 0.01b	16.260 \pm 2.74a	13.859 \pm 3.04a
Ho 95-988	0.115 \pm 0.01a	1.123 \pm 0.02a	18.428 \pm 1.33a	6.483 \pm 0.86b
HoCP 96-540	0.128 \pm 0.01a	1.137 \pm 0.01a	17.615 \pm 0.86a	5.515 \pm 0.39b
L 97-128	0.158 \pm 0.01a	1.172 \pm 0.01a	13.821 \pm 1.08a	4.468 \pm 0.32b

Means within columns followed by the same letter are not significantly different ($P > 0.05$, Tukey's HSD test).

^a r_m = intrinsic rate of aphid increase; ^b λ = finite rate of increase; ^cT = generation time; ^dDT = doubling time.

S. flava was 1.5-fold greater on L 97-128 than on LCP 85-384 and was 2.1-fold higher on LCP 85-384 than on HoCP 91-555 ($F = 17.35$; $df = 2, 18$; $P < 0.0001$). The number of nymphs produced per day was 1.4-fold higher on L 97-128 than on LCP 85-384, and LCP 85-384 resulted in 1.6-fold more aphids produced per day than on HoCP 91-555 ($F = 18.74$; $df = 2, 18$; $P < 0.0001$) (Table 3.3).

Sipha flava r_m on L 97-128 was 1.3-fold greater than on LCP 85-384, and r_m on LCP 85-384 was 1.4-fold greater than on HoCP 91-555 ($F = 11.54$, $df = 2, 18$; $P = 0.0006$) (Table 3.4). The value of λ on L 97-128 was 1.05-fold greater than on LCP 85-384, and λ on LCP 85-384 was 1.04-fold more than on HoCP 91-555 ($F = 11.10$; $df = 2, 18$; $P = 0.0007$) (Table 3.4). Treatment differences were not detected in the T values for *S. flava*, but DT on HoCP 91-555 was 1.3-fold greater than on LCP 85-384, and DT for LCP 85-384 was 1.3-fold greater than on L 97-128 ($F = 12.38$; $df = 2, 18$; $P = 0.0004$) (Table 3.4).

3.3.3. Tolerance

Differences in chlorophyll loss resulting from *M. sacchari* feeding were not detected among cultivars, although SPAD index values ranged from 17% (HoCP 96-540) to 30% (L 97-128). Treatment differences in chlorophyll loss were also not detected as a result of *S. flava* feeding (Table 3.5). Measurements taken after one week of aphid removal showed recovery of

Table 3.3. Life history parameters with mean (\pm SE) of *S. flava* reared on sugarcane.

Cultivar	Prereproductive period (days)	Reproductive period (days)	Fecundity	Fecundity/day	Longevity (days)
LCP 85-384	12.0 \pm 1.2a	16.6 \pm 1.0ab	13.4 \pm 1.5b	0.81 \pm 0.1c	31.3 \pm 1.6a
HoCP 91-555	11.3 \pm 1.3a	12.0 \pm 0.7b	6.4 \pm 0.8c	0.52 \pm 0.0b	26.4 \pm 1.8a
L 97-128	9.6 \pm 0.6a	18.7 \pm 2.3a	20.6 \pm 2.4a	1.12 \pm 0.1a	31.9 \pm 2.0a

Means within columns followed by the same letter are not significantly different ($P > 0.05$, Tukey's HSD test).

Table 3.4. Demographic statistics with mean (\pm SE) of *S. flava* reared on sugarcane cultivars antibiosis test.

Cultivar	r_m^a	λ^b	T ^c (days)	DT ^d (days)
LCP 85-384	0.153 \pm 0.01ab	1.165 \pm 0.02ab	15.292 \pm 1.01a	4.817 \pm 0.52b
HoCP 91-555	0.112 \pm 0.00b	1.118 \pm 0.01b	14.905 \pm 3.50a	6.258 \pm 0.25a
L 97-128	0.197 \pm 0.02a	1.219 \pm 0.02a	13.937 \pm 1.34a	3.640 \pm 0.28b

Means within columns followed by the same letter are not significantly different ($P > 0.05$, Tukey's HSD test).
^a r_m = intrinsic rate of aphid increase; ^b λ = finite rate of increase; ^cT = generation time, ^dDT = doubling time.

chlorophyll content in L 97-128; however, differences in chlorophyll loss still were not detected among cultivars. There were differences in ratings among cultivars because of variations in the degree of reddish stippling in the area caged with *S. flava* ($F = 4.76$; $df = 2, 18$; $P = 0.0219$) (Table 3.5). The maximum discoloration (61-80%) was recorded on LCP 85-384 and the least (0-20%) on HoCP 91-555. Measurements of the discolored area after one week of aphid removal indicated rating differences among cultivars similar to those at the time of aphid removal ($F = 7.11$; $df = 2, 18$; $P = 0.0053$). There were no changes in ratings on LCP 85-384, and HoCP 91-555. However, a slight recovery of the discolored area was noticed on L 97-128, but this did not result in a significant difference from HoCP 91-555 (Table 3.5).

Table 3.5. Chlorophyll loss and leaf discoloration ratings with mean (\pm SE) due to *S. flava* feeding injury on selected sugarcane cultivars- tolerance test.

Cultivar	% Chlorophyll loss		% Recovery	Rating	
	0 ^a	1 ^b		0 ^a	1 ^b
LCP 85-384	43.5 \pm 7.2a	44.1 \pm 5.0a	-1.5	4.0 \pm 0.5a	4.0 \pm 0.5a
HoCP 91-555	24.1 \pm 4.3a	27.0 \pm 3.1a	-12.0	2.1 \pm 0.3b	2.1 \pm 0.3b
L 97-128	34.6 \pm 9.0a	26.6 \pm 11.7a	28.1	2.3 \pm 0.6b	1.6 \pm 0.6b

Means within columns followed by the same letter are not significantly different ($P > 0.05$, Tukey's HSD test).

^aReadings taken immediately after aphid removal.

^bReadings taken after one week of aphid removal.

3.4. Discussion

Use of resistant cultivars is an effective management technique for aphids involving compatibility with other control tactics and reduction in the use of insecticides (Smith 2005).

Improving the applicability of host plant resistance requires identification of bases of resistance.

Painter (1951) used the terms nonpreference, antibiosis, and tolerance as three possible bases of insect resistance in crop plants. Our study is the first to categorize sugarcane resistance to *M. sacchari*, and it augments previous work on *S. flava* (White 1990, Sosa 1991).

Nonpreference, later termed antixenosis (Kogan and Ortman 1978), involves plant characters that attract or repel insects from a plant for oviposition, shelter, or food (Painter 1951). Other components of antixenosis include excitant, suppressant, or deterrent effects of host plants (Kogan 1994). Antixenosis is important because it influences the initial stage of plant infestation, and it has been identified as being one of the main categories of cereal crop resistance to aphids (Dixon et al. 1990, Webster et al. 1994, Flinn et al. 2001, Andarge and Westhuizen 2004). In our study the aphids found their host within an hour and did not leave those plants for the duration of the assay indicating no strong antixenosis. Several studies have shown that morphological features such as leaf size and shape, leaf bending at the seedling stage, plant height, distance between leaves, and quantity of waxy lamina and epicuticular wax on lower leaf surfaces are associated with reduced susceptibility of sorghum to *M. sacchari* (Singh et al. 2004). In sugarcane, Sosa (1991) reported antixenosis to *S. flava* based on leaf pubescence. However, pubescence on the leaf blade, the actual site of aphid feeding, was not a distinctive characteristic of cultivars in our study (LaBorde et al. 2008), and was not considered as a potential factor in antixenosis.

Antibiosis refers to adverse effects on insect biology from feeding on resistant plants (Painter 1951). Some of these adverse effects on aphids include, but are not limited to, reduced

nymphal weight (Starks and Merkle 1977), reproductive rate (Dixon et al. 1990, Webster 1990, Robinson 1993), survivorship (Zeng et al. 1993), relative growth rate and body size (Fuentes-Contreras and Niemeyer 1998), adult longevity (Hill et al. 2004), and increased proportion of alates (Hesler et al. 1999), and prenymphipositional period (Andarge and Westhuizen 2004). Thus negative effects on insect biology can be evaluated by looking at one or several different parameters. The use of r_m has been adopted in aphid research as an improved measure of antibiosis (Smith 2005) because it includes additional parameters, such as the prereproductive period, fecundity and reproductive period of the parent aphid, as well as prereproductive period of the first F1 nymph. The concept of r_m was developed (Birch 1948, Wyatt and White 1977) for insects that have relatively short developmental and longer reproductive times, and was based on the findings that a small delay in reproduction of an organism with a high intrinsic rate of increase can reduce net reproduction more than proportionally (Lewontin 1965). When r_m is low, then fecundity becomes a critical factor in altering rate of population growth. In our nonchoice tests, fewer aphids were produced on HoCP 91-555, which translated into the lowest estimate of r_m for both aphid species on this cultivar. Longevity of both aphid species was also reduced on HoCP 91-555. Estimates of several demographic statistics computed in our study suggested that HoCP 91-555 is a more resistant and L 97-128 a relatively more susceptible cultivar to both aphid species.

Tolerance refers to a situation where a host plant shows an ability to grow, reproduce itself, or to repair injury to a marked degree in spite of supporting a population equal to that damaging a susceptible host (Painter 1951). Measurement of tolerance has always been challenging particularly with continuously reproducing insects like aphids (Reese et al. 1994b). Because *M. sacchari* feeding can cause loss of chlorophyll (Singh et al. 2004) and *S. flava* feeding also results in leaf discoloration with probable photosynthetic decline (White 1990),

measuring chlorophyll content was used to detect tolerance for both aphid species (Deol et al. 1997, Diaz-Montano et al. 2007b). Although *M. sacchari* feeding does not cause visual symptoms, our SPAD measurements indicated a 17-30% loss of chlorophyll on each cultivar, and chlorophyll loss from *S. flava* feeding ranged from 27-44%. Previous attempts to categorize sugarcane resistance to *S. flava* included rating leaf discoloration which differed among some cultivars (White 1990). In our study, ranking of leaf discoloration associated with *S. flava* feeding was accompanied by determining rate of recovery for characterizing tolerance. While we found the least discoloration on HoCP 91-555, and the maximum on LCP 85-384, indicating different levels of tolerance, recovery within a week was not apparent on either cultivar.

In nature, single or multiple mechanisms conferring resistance act together and can influence the population build-up of insect pests, and the injury they inflict (Dixon et al. 1990, Unger and Quisenberry 1997, Flinn et al. 2001, Hill et al. 2004). Although it is difficult to characterize the relative role of each mechanism when several occur in concert (Unger and Quisenberry 1997), such cultivars provide resistance that is more stable or reliable than those with single resistance factors (Smith 2005). Because both antixenosis and antibiosis involve plant interaction with insect behavior or physiology, distinction between them can be challenging (Smith 2005). Microcages on plants are often used in antibiosis studies, but repellency or deterrence (*i.e.*, antixenosis) cannot be ruled out. Aphid behavior and performance are generally dependent on host plant structural features (Roberts and Foster 1983) and nutritional quality (Douglas 1998, Karley et al. 2002). Several studies, for example, including some on sugarcane, have shown that leaf pubescence can be important in antixenosis to aphids (Roberts and Foster 1983, Sosa 1991). In our study, no cultivar preference observed within 24 h of release indicates that antixenosis is likely not playing a role in plant defense in these cultivars.

In a previous evaluation of antibiosis with sugarcane cultivars (White 1990), *S. flava* reproduction was greater on CP 72-356, CP 76-331 and CP 74-383 than on CP 72-370. The

reproductive period and fecundity of *S. flava* were about two and three times shorter, respectively, on susceptible cultivars when compared to a resistant cultivar. Our study indicates that HoCP 91-555 permits a suppressed level of reproduction which likely imposes a relatively moderate selection pressure on both aphid species. Coupled with the low acreage of HoCP 91-555 in Louisiana (2% in 2008, Legendre and Gravois 2009), selection pressure on *M. sacchari* and *S. flava* is expected not be sufficient to elicit biotype development, a risk from cultivars that affect the behavior and biology of aphids (Auclair 1989, Smith 2005). Several studies (Auclair 1963, Douglas 1998, Karley et al. 2002) elucidating association between concentration and composition of essential amino acids in the phloem sap and growth and development rates of aphids suggest that host plant nutritional quality has a role in mediating population dynamics. Differences in free amino acid profiles of sugarcane cultivars have been associated with oviposition preference of another important pest of sugarcane, the Mexican rice borer, *Eoreuma loftini* (Dyar) (Reay-Jones et al. 2007, Showler and Castro 2009). It is likely that variations in the nutritional quality of phloem sap might contribute toward our observed differences in aphid biotic potential.

The estimates of r_m values on all cultivars in our study indicate that aphid growth potential on all of these cultivars is low. However, it is important to note that r_m is merely a comparative figure estimated under optimum conditions, expected to be different from field conditions where several biotic and abiotic components of the environment interact simultaneously. Variables such as temperature have been shown to affect development, reproduction, lifespan, and intrinsic rate of increase of aphids (Girma et al. 1990, Xia et al. 1999). The fact that r_m values were obtained by confining an individual aphid in a cage, which is different from their common aggregating behavior in field conditions, is another factor likely contributing toward lower aphid growth potential assessed in our study. Accelerated nymphal

development is possible when developing nymphs feed as a group in continued association with their parent aphid whose feeding increases plant suitability for the subsequent development of progeny (Qureshi and Michaud 2005).

The sugarcane cultivars in our study indicated different levels of resistance in antibiosis tests. The 64% and 43% reduction in r_m values of *M. sacchari* and *S. flava*, respectively, on HoCP 91-555 as compared to L 97-128 shows that both aphid species have slower population growth rate on HoCP 91-555. In addition to the fewer numbers of aphids being produced on HoCP 91-555, increases in the development time for aphids will likely extend the time over which natural enemies and other adverse environmental conditions can exert controlling effects on aphid populations (Dreyer and Campbell 1987). In conclusion, based largely on differences in antibiosis, the cultivars from the most to least susceptible to *M. sacchari* are L 97-128 > LCP 85-384 > HoCP 96-540 > Ho 95-988 > HoCP 91-555, and for *S. flava* are L 97-128 > LCP 85-384 > HoCP 91-555. These greenhouse experiments demonstrate the potential for using HoCP 91-555 as an aphid management tool; however, firm recommendations about cultivar performance under commercial growing conditions can be made after field studies which are underway.

CHAPTER 4: SUGARCANE APHID (HEMIPTERA: APHIDIDAE) FEEDING BEHAVIOR ON RESISTANT AND SUSCEPTIBLE SUGARCANE CULTIVARS

4.1. Introduction

The sugarcane aphid, *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae), is distributed throughout the tropical and subtropical regions of the world on hosts in the genera *Echinochloa*, *Oryza*, *Panicum*, *Pennisetum*, *Saccharum*, and *Sorghum* (Blackman and Eastop 2000). In Louisiana sugarcane, *M. sacchari* has become the most abundant aphid species in recent years. Feeding by *M. sacchari* on sugarcane causes a fading of leaf greenness, and heavily infested leaves turn black from sooty mold developing on honeydew deposits (Hall and Bennet 1994). A major problem associated with *M. sacchari* is transmission of persistent sugarcane yellow leaf virus (ScYLV), millet red leaf virus, and certain strains of non-persistent sugarcane mosaic potyvirus (Blackman and Eastop 2000). ScYLV is a serious problem in Hawaii (Schenck and Lehrer 2000), and in Louisiana where absence of ScYLV has been added to certification standards for micropropagated seedcane to minimize its spread (McAllister et al. 2008). Surveys have shown ScYLV infections occurring at varying levels in all sugarcane growing areas of Louisiana, with some fields having up to 25% infected plants (McAllister et al. 2005). Sugar yield losses of 11 and 14% resulting from ScYLV were documented in first and second Louisiana ratoon crops, respectively (Grisham et al. 2001). The spread and incidence of ScYLV in sugarcane can be reduced by use of aphid-resistant cultivars (Smith 2005). Previous studies on the biotic potential of *M. sacchari* on various commercial sugarcane cultivars have shown varying cultivar effects on aphid biology, and HoCP 91-555 has been identified as resistant, and L 97-128 as susceptible cultivars (see Chapter 3).

Generally, host plant resistance or susceptibility to herbivores depends on herbivore's access to the appropriate host tissue and the tissue's nutritional status. Aphids feed from the phloem sieve elements, but chemical or physical factors within the leaf can impede access to the

sieve elements (Mayoral et al. 1996). The behavior of aphids within the leaf tissue can be visualized using electrical penetration graph (EPG) technique (Tjallingii 1988). The alternating current-(AC) based EPG was originally devised by McLean and Kinsey (1964). Later, further developments in this technique permitted a direct current-(DC) based system (Tjallingii 1978). In EPG, the aphid and plant become part of an electrical circuit with one electrode attached to the aphid body and the other inserted into the soil in which the host plant is being grown. As soon as the aphid inserts its piercing stylets into the leaf tissue, the electrical circuit is completed and different waveforms, depending on the stylet tip's location, are recorded. These waveforms are named A, B, C, E1, E2, F, G, and pd and represent three behavioral phases: stylet pathway phase (SPP; waveforms A, B, C), sieve element phase (SEP; waveforms E1, E2), and xylem phase (waveform G) (Reese et al. 2000). The waveform F represents the stylet penetration difficulties and is part of SPP. During SPP, the main activities include stylet contact with the plant tissues, salivary sheath formation, and other related stylet pathway activities. Stylets are in intercellular position during SPP except when they puncture a cell membrane and a drop in electrical potential called potential drop (pd) is observed. This drop typically lasts 5-10 sec because the aphid pulls its stylets out from the cell membrane and continues penetration in intercellular spaces until it reaches the sieve elements (Tjallingii and Hogen Esch 1993). During SEP, E1 refers to salivation and is followed by E2 which indicates continuous ingestion from sieve elements. A different waveform G appears when aphids contact and ingest from xylem vessels.

In this study, EPG was used to detect differences between feeding behavior of *M. sacchari* on resistant and susceptible sugarcane cultivars, particularly in the time required for *M. sacchari* to reach sieve elements, a measure of accessibility and recognition of the target feeding site (Reese et al. 1994a, Tjallingii 2006); relative incidence of successful probes (sustained ingestion of > 10 min), a measure of phloem acceptance (Tjallingii 1990, Davis et al. 2008a);

and length of time an aphid continuously ingests sap, a measure of phloem-based resistance (Lei et al. 2001, Zehnder et al. 2001, Klinger et al. 2005, Diaz-Montano et al. 2007a).

4.2. Materials and Methods

4.2.1. Aphids and Plants

Melanaphis sacchari greenhouse colonies were started with aphids collected from sugarcane fields at the Louisiana State University Agricultural Center Sugar Research Station located at St. Gabriel, LA. The colonies were maintained in the greenhouse on sorghum plants under natural light:dark conditions at 25-30 °C. Commercial sugarcane cultivars used in this study were LCP 85-384 (moderately resistant), HoCP 91-555 (resistant), and L 97-128 (susceptible) (see Chapter 3). Stalks of each cultivar were harvested from the Sugar Research Station. Billets (small pieces of sugarcane stalk) with at least one vegetative bud were planted in 1.9-liter pots containing sterilized greenhouse soil (1:1:1 parts by volume of soil, sand, and peat moss) with 0.5 g of 19:6:12 (N-P-K) controlled release fertilizer (Osmocote, Scotts Miracle-Gro, Marysville, OH). Sugarcane plants at 4-6 leaf stage (60-75 cm height from base of plant to the bottom of whorl leaf) were used for experiments in this study.

4.2.2. EPG Setup and Data Recording

EPG experiments were conducted in a Faraday cage using a Giga 8DC EPG amplifier with 1-gigaohm input resistance and an AD conversion rate of 100 Hz (Wageningen Agricultural University, Wageningen, The Netherlands). A DAS-800 Digital Acquisition Card (Keithley Instruments, Inc., Cleveland, OH) digitalized analog signals, which were displayed and recorded using WinDaq/Lite software (DATAQ Instruments, Inc., Akron, OH). A 4-cm gold wire (GoodFellow Metal Ltd., Cambridge, United Kingdom) of diameter 25- μ m was attached to the aphid dorsum with silver conductive paint (Pelco Colloidal Silver no. 16034, Ted Pella, Inc., Redding, CA). The other end of the gold wire was connected by the silver paint to one end of a

piece of flattened copper wire peg. The aphid was allowed to acclimate to walking with the wire for 1 h. After tethering, the flat pegs with wired aphids were connected at the loop end to the monitor input electrodes and held in place over the test plant by metal stands. One of the lower five leaves of the sugarcane plant, favored sites for *M. sacchari* feeding, was turned abaxial surface face upward. Then aphids were lowered to contact the abaxial surface and EPG monitoring began immediately. Feeding behavior was recorded for 4 h, based on preliminary tests indicating that this time frame was sufficient for *M. sacchari* to penetrate the leaf tissue to the sieve elements. There were three aphids per recording with a total of 32 aphids studied per cultivar using 6-7 different plants.

Measured parameters included the start and end of each probe, time to reach SPP (from start of experiment to first probe), time to reach xylem phase, SEP (from start of first probe to contact xylem or phloem vessels), start and end of each individual phase, numbers of pds to reach SEP, total numbers of pds during probing, numbers of SPPs, xylem phases, and SEPs. Based on these readings, computations included the total probe time (sum of all probing time within a 4-h period); non-probe time; total time in SPP, xylem, E1, and E2; mean duration of SPP, xylem phase, E1, E2 (sum of time spent in each individual phase/number of events for that phase).

4.2.3. Waveform Interpretation and Statistical Analyses

A probe was defined as all behaviors occurring from start of stylet penetration into plant tissue until stylet withdrawal (Backus 2000). Feeding behavior waveforms identifying specific aphid probing activities were identified using the characteristics listed in Tjallingii and Hogen Esch (1993). Waveform F, when observed, was included in the SPP (Diaz-Montano et al. 2007a). Because we were interested in time spent with stylet in the sieve elements from initial contact to the end of ingestion, waveforms E1 and E2 were combined and labeled as waveform E

in calculating total time spent and mean duration of each individual SEP (Diaz-Montano et al. 2007a). In instances, where some probing behaviors (G or SEP) were not recorded, data for time to reach, total time spent, and mean duration of each phase were entered without adjustment and unobserved probing behavior was treated as missing (Brewer and Webster 2001). Feeding behavior parameters were not normally distributed and were analyzed using the Kruskal-Wallis test at $P = 0.05$ (Proc NPAR1WAY, SAS Institute 2006).

4.3. Results

Melanaphis sacchari spent 25, 17, and 19% of the 4-h experimental period in non-probing on LCP 85-384, HoCP 91-555, and L 97-128, respectively, but cultivar differences were not detected (Table 4.1). However, both total probe time and mean probe duration differed among cultivars; with 1.13-fold longer total probe time on HoCP 91-555 than on LCP 85-384, and 1.54-fold longer probe duration on L 97-128 than on LCP 85-384 (Table 4.1). The numbers of SPP and SEP (SE1, SE2) were not influenced by cultivars, but the number of xylem phases was ≈ 2.9 -fold greater on L 97-128 and HoCP 91-555 than on LCP 85-384 (Table 4.1). Although E1 was consistently preceded by E2 in all three cultivars, only 53, 46, and 70% resulted in ingestion lasting more than 10 min on LCP 85-384, HoCP 91-555, and L 97-128, respectively (Table 4.1).

The total numbers of pds were not affected by cultivar, and proportions of aphids with at least one successful probe were $\geq 75\%$ among the three cultivars (Table 4.1). *Melanaphis sacchari* took an average of 22 min to reach the SPP (*i.e.*, to start probing) regardless of cultivar, and the time required for commencing contact with xylem and phloem vessels after onset of the first probe was also unaffected (Table 4.1). The proportion of aphids that made contact with xylem vessels was lowest on LCP 85-384 and greatest on L 97-128 (Table 4.1).

While *M. sacchari* probed, most time was in SPP without cultivar influence. The total time spent ingesting from xylem vessels was 2.4-fold longer on HoCP 91-555 than on L 97-128

Table 4.1. Feeding behavior parameters (mean \pm SE) of *M. sacchari* during a 4-h period on three different sugarcane cultivars.

Parameter ^{a,b}	Sugarcane cultivar			χ^2	df	<i>P</i>
	LCP 85-384	HoCP 91-555	L 97-128			
Non-probe time	62.6 \pm 10.5	43.0 \pm 12.5	47.6 \pm 10.0	1.82	2	0.4023
Probe time ^c	180.0 \pm 10.1b	203.7 \pm 12.5a	192.4 \pm 10.4ab	10.23	2	0.0059
Probe duration	62.3 \pm 10.4b	91.9 \pm 15.7ab	96.4 \pm 12.2a	8.43	2	0.0147
Time to SPP	21.7 \pm 7.3	27.3 \pm 10	18.2 \pm 4.5	1.27	2	0.5304
Time to xylem ^d	79.3 \pm 19.2	35.1 \pm 9.9	83.4 \pm 15.9	5.41	2	0.0668
Time to SEP	80.5 \pm 10.2	105.7 \pm 14.4	101.2 \pm 14	1.32	2	0.5180
No. SPP	6.16 \pm 0.62	5.96 \pm 0.61	4.73 \pm 0.46	2.89	2	0.2353
No. xylem phases	0.41 \pm 0.16b	1.17 \pm 0.33a	1.20 \pm 0.27a	7.81	2	0.0201
No. E1	1.69 \pm 0.28	1.39 \pm 0.25	1.13 \pm 0.16	1.53	2	0.4654
No. E2	1.69 \pm 0.28	1.39 \pm 0.25	1.13 \pm 0.16	1.53	2	0.4654
E2 < 10 min	0.75 \pm 0.19	0.75 \pm 0.19	0.33 \pm 0.12	4.68	2	0.0962
E2 > 10 min	0.91 \pm 0.22	0.64 \pm 0.16	0.80 \pm 0.11	1.69	2	0.4295
Total no. pds	30.6 \pm 3.6	43.2 \pm 5.8	36.6 \pm 3.6	2.83	2	0.2427
No. pds to SEP	16.4 \pm 2.1	22.8 \pm 2.7	22.4 \pm 2.4	5.77	2	0.0558
% successful probes ^e	81.2	75.0	76.6			

^aAbbreviations: SPP, stylet pathway phase; SEP, sieve element phase; E1, sieve element salivation; E2, sieve element ingestions; pds, potential drops.

^bTime in minutes. Means within rows followed by different letters differ significantly (Kruskal-Wallis test at $\alpha = 0.05$).

^cTotal probes on LCP 85-384 = 32, HoCP 91-555 = 28, L 97-128 = 30.

^dAphids that made contact with xylem on LCP 85-384 $n = 7$, HoCP 91-555 $n = 13$, L 97-128 $n = 17$.

^eAt least one ingestion event from sieve elements >10 min (LCP 85-384 $n = 26$, HoCP 91-555 $n = 21$, L 97-128 $n = 23$). Data were not statistically analyzed because there was no replication.

($\chi^2 = 8.55$, $df = 2$, $P = 0.0139$) (Fig. 4.1). In the sieve elements, the total time spent in E1 averaged 21 sec on all three cultivars, whereas the time in E2 was \approx 2-fold greater on L 97-128 than on HoCP 91-555 ($\chi^2 = 7.31$, $df = 2$, $P = 0.0258$) (Fig. 4.1).

Cultivar treatment effects were not found for mean duration of SPP, the xylem phases, and E1. However, the mean duration of E2 was 2-fold longer on L 97-128 than on LCP 85-384

($\chi^2 = 5.68$, $df = 1$, $P = 0.0171$), and 2.3-fold longer on L 97-128 than on HoCP 91-555 ($\chi^2 = 9.25$, $df = 1$, $P = 0.0023$) (Fig. 4.2).

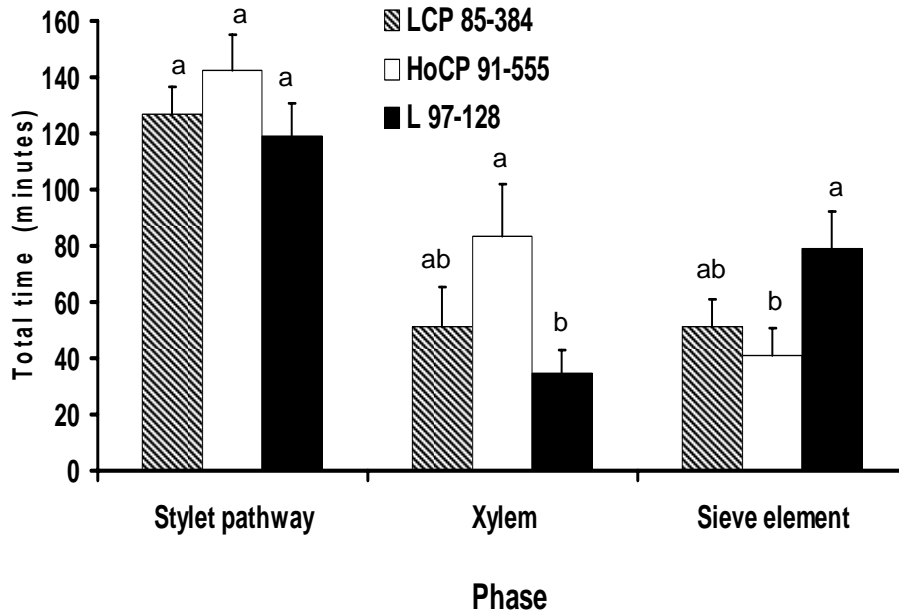


Figure 4.1. Mean (\pm SE) total time *M. sacchari* spent in each phase during probing on three sugarcane cultivars. Bars representing means within each phase followed by the same letter do not differ significantly (Kruskal-Wallis test, $\alpha = 0.05$).

4.4. Discussion

The host-selection process in phytophagous insects involves a succession of events. Five phases in this process include (1) host habitat finding, (2) host finding, (3) host recognition, (4) host acceptance, and (5) host suitability (Kogan 1994). On reaching a plant surface, an aphid uses its antennae and proboscis to assess host suitability (Dixon 1998). Subsequent feeding behavior and performance are mostly governed by host plant structural features (Roberts and Foster 1983) and nutritional quality (Douglas 1998, Karley et al. 2002, Wilkinson and Douglas 2003). Cuticular components and leaf pubescence in wheat, *Triticum aestivum* L., tobacco, *Nicotiana tabacum* L., and sugarcane, for example, can be deterrents to aphids (Roberts and Foster 1983, Jackson and Sisson 1990, Sosa 1990). In the greenhouse, differences were detected

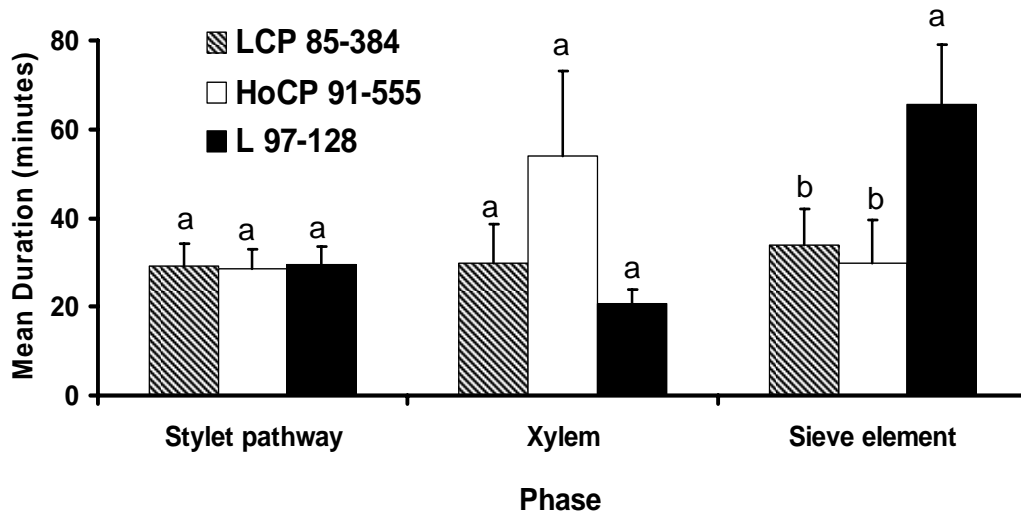


Figure 4.2. Mean (\pm SE) duration of individual events in each phase by *M. sacchari* on three sugarcane cultivars. Bars representing means within each phase followed by same letter do not differ significantly (Kruskal-Wallis test, $\alpha = 0.05$).

among cultivars in antibiosis no-choice assays, but not in antixenosis choice assays (see Chapter 3). Both the number of nymphs produced and number of reproductive days were reduced on HoCP 91-555 as compared to L 97-128. Although differences were detected in total probe time, mean probe duration, total time spent in xylem vessels, and total numbers of xylem phases, those measurements appear to be of little value in contrast with time required to reach the SEP and duration of time spent in E2 (Reese et al. 2000, Brewer and Webster 2001). These feeding behavior parameters are commonly used to differentiate between resistant and susceptible varieties (Kennedy et al. 1978, Campbell et al., 1982, Lei et al. 2001).

Sieve elements are the target site of aphid feeding; therefore reaching SEP is indispensable for host plant acceptance and colonization (Davis et al. 2008a). However, before accessing there, aphids might have to contend with physical or chemical barriers. High levels of 2, 4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), a hydroxamic acid in the leaves of wheat was attributed to both longer SEP access time and fewer aphids reaching SEP (Givovich and Niemeyer 1991). Gabryś and Pawluk (1999) showed that deterrent factors inside the leaf

differ in activity and can hinder stylet penetration of epidermal, parenchyma, and phloem cells by the cabbage aphid, *Brevicoryne brassicae* L. Leaf cells are held together by a layer of intercellular pectin called middle lamella. Duration of SPP has been correlated with the rate of pectin depolymerization by pectinase, an enzyme in aphid saliva that is injected into intercellular spaces as aphids probe (Dreyer and Campbell 1987). Increased time between stylet insertion into the epidermis and start of E1 indicates physiochemical resistance in the intercellular spaces (Morris and Foster 2008). In our study, the percentage of aphids reaching SEP was relatively high on all three cultivars and time required to reach SEP was not extended in one cultivar over the other, suggesting no resistance to locating sieve elements (Reese et al. 1994a). This was corroborated by lack of cultivar-associated differences in the total numbers of pds and numbers of pds to reach SEP. Other aphid antixenosis experiments also indicated a lack of cultivar-associated deterrent or repellent effects (see Chapter 3). Similarities in preingestion activities in our EPG studies support the contention that morphological or chemical factors outside sieve elements do not affect aphid recognition of and access to sieve elements on these cultivars.

More time spent ingesting phloem sap indicates host plant acceptance and suitability (Montllor and Tjallingii 1989, Lei et al. 2001). The total as well as mean duration of time spent by the cowpea aphid, *Aphis craccivora* Koch, in SEP was lower on resistant than on susceptible lupin, *Lupinus* spp. (Zehnder et al. 2001). In our study, once the sieve elements were reached, the number of aphids engaged in ingestion for >10 minutes was not influenced by the cultivars and indicated host acceptance (Davis et al. 2008a). Shorter duration of ingestion from sieve elements has been attributed to the relatively lower estimates of intrinsic rate of increase for the green peach aphid, *Myzus persicae* Sulzer, on barley, *Hordeum vulgare* L., and rye, *Secale cereale* L. (Davis et al. 2008a). Because of the relatively short time the foxglove aphid, *Aulacorthum solani* Kaltentbach, ingested from the sieve elements of resistant soybean, nutrient uptake was reduced

resulting in reduced aphid survival rate, reproduction, and development (Takahashi et al. 2002). Klinger et al. (2005) documented that *A. kondoi* spent less time ingesting phloem sap of an aphid-resistant legume than a susceptible variety, and concluded that the resistance mechanism occurred at the phloem sieve element level. In our study, more than two-fold differences in the mean duration of SEP and total time in SEP between resistant HoCP 91-555 and susceptible L 97- 128 might explain cultivar-related differences in the biotic potential of *M. sacchari* (see Chapter 3). The differences detected mainly in parameters associated with the sieve elements (*i.e.*, total time spent as well as mean duration of SEP on susceptible and resistant cultivars) suggest that the resistance occurs at the phloem sieve elements.

Aphid feeding from sieve elements triggers wound responses such as coagulation of p-proteins in the plant's phloem sieve elements and in the food canal of the aphid stylet (Tjallingii 2006). Aphids overcome coagulation responses by injecting watery saliva into the sieve elements during E1 and E2. However, each E1 may or may not be followed by E2 depending upon the difficulty of transitioning from E1 to E2 phases, and resistance can result in cessation of phloem phase after a single E1 (Tjallingii 2006). Apart from cultivar effects on numbers of E1 phases, the duration of E1 can be extended on resistant cultivars (Tjallingii 2006). In our study, no cultivar effects were detected for numbers and durations of E1, and numbers of E1 followed by E2, suggesting that *M. sacchari* had no difficulty recognizing sieve elements and initiating phloem sap ingestion regardless of cultivar. van Helden and Tjallingii (1993) also documented similar numbers and durations of E1 periods for the lettuce aphid, *Nasonovia ribisnigri* Mosley, on resistant and susceptible lines of lettuce, *Lactuca sativa* Compositae. Numbers of aphids showing phloem acceptance, indicated by E2 activities >10 minutes, were also similar among the cultivars in our study. Recently, Tjallingii (2006) hypothesized that prolonged E1 and shortened E2 on resistant plants result from the aphid's reduced ability to suppress phloem wound

responses. Our findings, however, indicate that the most likely basis of resistance in HoCP 91-555 is another phloem-associated mechanism.

Insect feeding behavior, total food consumption, and consumption rate can be affected by nutritional suitability of the host plant (Mattson 1980). Aphids feed on phloem sap mostly comprised of sucrose and limited quantities of nitrogenous compounds such as amino acids (Douglas 1998). Concentrations of amino acids essential to insect growth and development in the phloem sap of vascular plants are particularly low (Dadd 1985), but are available in soluble, readily assimilable and renewable forms (Risebrow and Dixon 1987). Other studies depicting associations between concentrations and compositions of essential amino acids in phloem sap and aphid biology suggest that host plant nutritional quality has a role in mediating aphid feeding behavior and performance (Auclair 1963, Prosser and Douglas 1992, Douglas 1998, Karley et al. 2002). Black bean aphids, *Aphis fabae* Scopoli, for example, spent more time ingesting phloem sap from susceptible broad beans than on less susceptible cultivars (Cichocka et al. 2002). Analysis of free amino acids (FAA) in phloem sap revealed that black bean aphids preferred bean cultivars with relatively high concentrations of free essential and nonessential amino acids. Differences in FAA profiles of several sugarcane cultivars have been associated with oviposition preference of and levels of infestations by the Mexican rice borer, *Eoreuma loftini* (Dyar) (Reay-Jones et al. 2007, Showler and Castro 2009), and populations of stunt nematodes, *Tylenchorhynchus annulatus* (Casidy) Golden (Showler et al. 1990). It is likely that variations in FAA concentrations at the phloem sap level contributed toward observed differences in aphid feeding behavior on L 97-128 and HoCP 91-555, leading to reduced population growth on HoCP 91-555 (see Chapter 3). Other possibilities for the differences observed in aphid feeding behavior on sugarcane cultivars include the presence of a feeding deterrent or lack of a feeding stimulant in the sap of HoCP 91-555, stimulating early withdrawal of the stylet from the phloem (Zehnder

et al. 2001), but lack of differences in numbers of pds in our study suggest that these possibilities are unlikely. Effects of sugarcane cultivars on two principle feeding behavior parameters of *M. sacchari* were revealed in this study. *Melanaphis sacchari* accesses and accepts sieve elements with relative ease regardless of the host cultivar. The total times and durations of individual phloem sap ingestion events were shortest on the resistant cultivar HoCP 91-555, indicating a phloem-based resistance factor.

CHAPTER 5: IDENTIFICATION OF FREE AMINO ACIDS IMPLICATED IN SUGARCANE RESISTANCE TO THE SUGARCANE APHID

5.1. Introduction

Aphids find and utilize host plants through a sequence of steps that include (1) orientation to the prospective host plant, (2) external examination, (3) probing into plant tissues, (4) tapping into sieve elements, and (5) ingestion (Pollard 1973, Klingauf 1987, Montllor 1991). After landing on the surface of a plant, aphid probing behavior and performance can depend on several factors. Cuticular components and leaf pubescence in wheat, *Triticum aestivum* L., and sugarcane, *Saccharum* spp., for example, can be important physical barriers for deterring aphids (Roberts and Foster 1983, Jackson and Sisson 1990, Sosa 1990). Another obstacle might be plant secondary compounds, such as phenolics, in leaf tissues (Fraenkel 1969, Todd et al. 1971, Risebrow and Dixon 1987). Aphid feeding occurs primarily on phloem sap within sieve elements (Douglas 1998). Phloem sap is partially comprised of sugars and small amounts of amino acids, which, because of their scarcity, are considered limiting factors for aphid growth, development, and survival (Douglas 1998, Karley et al. 2002, Wilkinson and Douglas 2003).

In Louisiana sugarcane the sugarcane aphid, *Melanaphis sacchari* Zehntner, has become the most abundant aphid species in recent years. A major problem associated with *M. sacchari* is transmission of persistent sugarcane yellow leaf virus (ScYLV) (Blackman and Eastop 2000), which is particularly serious in Hawaii (Schenck and Lehrer 2000) and in Louisiana where absence of ScYLV has been added to certification standards for micropropagated seedcane (McAllister et al. 2008). Studies on the biotic potential of *M. sacchari* on various commercial sugarcane cultivars have revealed cultivar effects: HoCP 91-555 was identified as resistant and L 97-128 as susceptible (see Chapter 3). Electrical penetration graph studies showed that cultivars did not influence time for *M. sacchari* to access phloem sieve elements, but both total time and duration of individual event associated with phloem sap ingestion were diminished on HoCP 91-

555 as compared to L 97-128, suggesting that a resistance factor occurs in the phloem sieve elements (see Chapter 4). The purpose of this study was to assess the composition of free amino acids (FAAs) in the phloem sap of these two cultivars, and to quantify concentrations of total phenolics, total available carbohydrates (TACs), water potential, and FAAs in whole leaf tissues.

5.2. Materials and Methods

5.2.1. Aphids and Plants

Melanaphis sacchari greenhouse colonies were founded with aphids collected from sugarcane fields at the Louisiana State University Agricultural Center Sugar Research Station located at St. Gabriel, LA. The colonies were maintained on sorghum plants under natural light:dark conditions at temperatures ranging from 30 to 35 °C. Commercial sugarcane cultivars used in these assays were the resistant HoCP 91-555 and the susceptible L 97-128 (see Chapter 3). Stalks used for planting were obtained from seed-cane fields at the Sugar Research Station that had been heat-treated in water (50 °C for 2 h) to protect against ratoon stunting disease (Comstock 2002). Billets (small pieces of sugarcane stalk) with at least one vegetative bud were planted in 7.6-L pots containing sterilized greenhouse soil (equal parts, by volume, soil:sand:peat) and 1.2 g of 19:6:12 (N-P-K) controlled release fertilizer (Osmocote, Scotts Miracle-Gro, Marysville, OH). There were 30 replications (1 pot = 1 replicate) of each cultivar at the 6-8 leaf stage (80-90 cm height from soil surface to bottom of the whorl leaf). Fifteen plants of each cultivar were used for honeydew collection, and one of the five lowest leaves (favored site of feeding, see Chapter 6) of each of the remaining 15 plants was used for measurements of water potential and extractions of total phenolics, TACs, total FAAs in whole tissue and phloem sap.

5.2.2. Plant Biochemical Extractions and Analyses

For total phenolic extraction, excised leaf from each of 15 plants of both cultivars was cut into small pieces, weighed, and submerged in 5 ml of 50% methanol. These samples were

incubated at room temperature for one week. The total phenolic content of each sample was quantified using the Folin-Ciocalteu reagent assay (Stout et al. 1998). A 100- μ L aliquot of the methanol extract was diluted to 2.75 ml with distilled water in test tube and vortexed for five min. Folin-Ciocalteu reagent (0.5 ml of 1 N solution; Sigma-Aldrich, St. Louis, MO) was then added to the diluted plant extract. After 5 min, 0.5 ml of 20% sodium carbonate was added, the solution vortexed for five min, and allowed to sit for 90 min at room temperature. The absorbance of samples as measured at 720 nm with a Shimadzu UV-1601 Spectrophotometer (Shimadzu Scientific Instruments, Columbia, MD). Total phenolic concentration in each sample was calculated based on a standard curve constructed with ferulic acid.

For determining TAC contents, one leaf from each of 12 plants of both cultivars was excised and freeze-dried for 24 h. Leaves were then cut into small pieces and ground using a Wiley Mini Mill (Thomas Scientific, Swedesboro, NJ). TACs were extracted from 30 mg of lyophilized tissue with 1 ml deionized water, stirred for 30 min at 25 °C, incubated at 4 °C for 16 h, and centrifuged at 13,000 rpm for 15 min. Fifty microliters of extract was mixed with 1,500 μ l anthrone-sulfuric acid reagent (12.7 M H₂SO₄ in water containing 0.1% [wt:vol] anthrone and 0.1% [wt:vol] thiourea) and incubated at 60 °C for 20 min, 0 °C for 3 min, and 25 °C for 20 min. Reactions were quantified at 625 nm. Glucose was used as a standard to calculate TAC content in milligrams per gram dry weight. A linear regression of dry weight on fresh weight (fresh weight = dry weight [3.61348] + 0.07665; R² = 0.99) was used to convert TAC values to milligram per gram fresh weight (Moran and Showler 2005).

For water potential measurement, one leaf from each of 15 plants of both cultivars was excised and water potential was measured with a Model 610TM pressure bomb (PMS Instrument Co., Corvallis, Oregon). For whole leaf tissue FAA extractions, 1-g sample of another leaf from the same plant was homogenized with 10 ml 0.1 N HCl using a Virtishear homogenizer (Virtis,

Gardiner, New York). A 4 ml homogenate from each sample was placed in separate 10-ml tubes and centrifuged at 10,000 rpm for 30 min. Samples were stored at -80 °C until FAA concentrations were measured using high-performance liquid chromatograph (HPLC) (Showler and Castro 2009).

Phloem sap was obtained using the ethylenediaminetetraacetic acid (EDTA)-exudation technique (King and Zeevaart 1974). One leaf from each of 15 plants of both cultivars was excised at a ligule with clean sharp scissors, and the cut end was immediately immersed in a 1.5-ml solution of 5-mM EDTA at pH 7 in 15-ml vial. The gap between the leaf and vial opening was sealed with parafilm to avoid evaporation loss. The vials were immediately taken to the laboratory and placed in a dark incubator at 25 °C and more than 90 % RH for one hour. Then the leaves were discarded and EDTA with the exudate was pipetted into 1.5-ml Eppendorf tubes and stored at -80 °C until the samples were prepared for FAA analysis using an HPLC.

Because *M. sacchari* are small (typically <2 mm long) and excrete smaller honeydew droplets, it was not possible to determine the composition of honeydew excreted by individual aphids. Instead each sample consisted of honeydew collected from 10 nymphs confined within a 2 × 0.6-cm double-sided adhesive cage (Scotch Mounting Tape, 3M, St. Paul, MN) on the abaxial surface of a leaf. The cage was covered with Parafilm and aphids were allowed to deposit honeydew on it for three days. The aphids were then removed from the plant, and the Parafilm with the honeydew drops was weighed. Initial attempts to analyze composition of honeydew failed because of undetectable levels of most amino acids; therefore, honeydew from aphids feeding on three plants of the same cultivar was pooled and five such samples per cultivar were obtained. Honeydew was washed off from the Parafilm with 1-ml distilled water and stored in 1.5-ml Eppendorf tubes. The Parafilm was allowed to dry, and was weighed again to determine amount of honeydew dissolved in 1-ml of distilled water. These samples were immediately stored at -80 °C until analyzed in the HPLC.

For measuring FAA concentrations, 1-ml of supernatant from each of whole leaf tissue, phloem sap, and honeydew samples was filtered through a 0.5- μ l filter fitted to a 5-ml plastic syringe. Samples were placed in the autosampler of an Agilent 1100 Series (Agilent Technologies, Atlanta, Georgia) reversed-phase HPLC with a binary pump delivering solvent A [1.36 g sodium acetate trihydrate + 500 ml purified HPLC grade water + 90 μ l triethylamine (TEA) + sufficient acetic acid to bring the pH to 7.2 ± 0.05 (95% C.I.)] and solvent B [1.36 g sodium acetate trihydrate + 100 ml purified HPLC grade water (acetic acid added to this mixture to bring the pH to 7.2 ± 0.05 [95% C.I.] + 200 ml acetonitrile + 200 ml methanol] at 100 and 1.0 ml/min on a Zorbax Eclipse AAA 4.6 \times 150 mm 3.5 μ column (Agilent Technologies). Absorbances at 262 and 338 nm were monitored on a variable wavelength detector for 48 min per sample. The autosampler measured and mixed 6 μ l sodium borate buffer (0.4 N, pH 10.2 in water), 1 μ l 9-fluorenylmethylchloroformate (FMOC), and 1 μ l ophthalaldehyde (OPA) derivitizing agents, and 2 μ l of sample, then injected 2 μ l for chromatographic separation of FAAs. Identification and quantification of 17 derivitized FAAs, alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, and valine were achieved by calibrating with a standard mixture of amino acids. Peak integration accuracy was enhanced by manual establishment of peak baselines using Agilent software.

5.2.4. Concentration Calculations and Statistical Analyses

The concentrations of total FAAs in each sample of whole leaf tissue (pmoles/ μ l extract), phloem sap (pmoles/ μ l phloem sap exudate), and honeydew (pmoles/mg honeydew) were calculated by combining individual concentrations of all detectable FAAs in that sample. The total concentration of essential amino acids was comprised of arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine (Gilmour 1961, Dadd 1985).

Tryptophan was the only free essential amino acid that was not detectable using our system. The total concentration of nonessential amino acids was the sum concentration of alanine, aspartic acid, cystine, glutamic acid, glycine, proline, serine, and tyrosine. The percentage concentration of individual FAAs were calculated by using the formula (arginine is selected for illustrative purposes): $(\text{pmole arginine}/\text{total pmoles FAAs}) \times 100$. Because the amount of honeydew dissolved in each sample varied, the concentrations of FAAs in honeydew samples were adjusted for weight of honeydew in each sample by dividing total concentration by respective sample weight. Treatment differences in terms of total FAA concentrations in whole leaf tissue, phloem sap, and honeydew; concentrations of TACs and phenolics; and measurements of water potential were detected using the Student's *t*-test (SAS Institute 2006). The percentage concentrations of individual FAAs were arcsin-square root-transformed before using the Student's *t*-test (SAS Institute 2006).

5.3. Results

Cultivar effects were not detected in levels of TAC and total phenolics, and water potential between *M. sacchari*-susceptible L 97-128 and -resistant HoCP 91-555 (Table 5.1).

5.3.1. FAAs in Whole Leaf Tissue of L 97-128 and HoCP 91-555

Total FAAs, total free essential amino acids, and total free nonessential amino acids were 2.2-fold ($t = 6.13$; $df = 1, 22$; $P < 0.0001$), 5.5-fold ($t = 7.55$; $df = 1, 22$; $P < 0.0001$), and 2.0-fold ($t = 5.13$; $df = 1, 22$; $P < 0.0001$) higher, respectively, in L 97-128 than in HoCP 91-555 (Table 5.1).

FAAs in whole leaf tissue of both cultivars were predominantly free nonessential amino acids, accounting for 78% and 91% in the leaves of L 97-128 and HoCP 91-555, respectively. Alanine was the most abundant free amino acid in both cultivars accounting for 27% and 37% of total FAAs in L 97-128 and HoCP 91-555, respectively (Fig. 5.1). Cystine was not detected in either cultivar. Among the free nonessential amino acids, cultivar differences were not detected

Table 5.1. Mean (\pm SE) measurements of TAC, water potential, total phenolics, and total FAAs in whole leaf tissue and phloem sap of *M. sacchari* susceptible (L 97-128) and resistant (HoCP 91-555) sugarcane cultivars.

Measurement	<i>n</i>	Sugarcane Cultivar		<i>P</i>
		L 97-128	HoCP 91-555	
Whole leaf tissue				
TAC ^a	12	214.5 \pm 24.6	250.0 \pm 12.2	0.2110
Total phenolics ^b	15	15.9 \pm 0.7	14.7 \pm 0.9	0.3145
Water potential ^c	15	5.4 \pm 0.6	3.9 \pm 0.5	0.0735
Total FAAs ^d	12	22,996 \pm 1,288	10,274 \pm 1,625	< 0.0001
Total free essential amino acids ^d	12	5,124 \pm 507	941 \pm 223	< 0.0001
Total free nonessential amino acids ^d	12	17,872 \pm 816	9,334 \pm 1449	< 0.0001
Phloem sap				
Total FAAs ^d	11,14 ^e	688 \pm 73	781 \pm 117	0.5361
Total free essential amino acids ^d	11,14 ^e	152 \pm 56	39 \pm 20	0.0480
Total free nonessential amino acids ^d	11,14 ^e	536 \pm 51	742 \pm 102	0.1104

^aExpressed as mg/g fresh weight

^bExpressed as μ moles/g fresh weight

^cExpressed as barr

^dExpressed as pmol/ μ l

^eFirst number for L 97-128, the second for HoCP 91-555.

for alanine and glutamic acid (Fig. 5.1). However, concentrations of aspartic acid (1.8-fold; $t = 6.47$; $df = 1, 22$; $P < 0.0001$), glycine (1.4-fold; $t = 2.15$; $df = 1, 22$; $P = 0.0430$), serine (1.3-fold; $t = 3.95$; $df = 1, 22$; $P = 0.0007$), and tyrosine (12.5-fold; $t = 6.95$; $df = 1, 22$; $P < 0.0001$) were greater in L 97-128 than in HoCP 91-555. Free proline was 4.2-fold ($t = 5.00$; $df = 1, 22$; $P < 0.0001$) more abundant in HoCP 91-555 than in L 97-128 (Fig. 5.1).

Among the free essential amino acids, cultivar differences were not detected for concentrations of methionine, threonine, and valine, but higher concentrations of free arginine

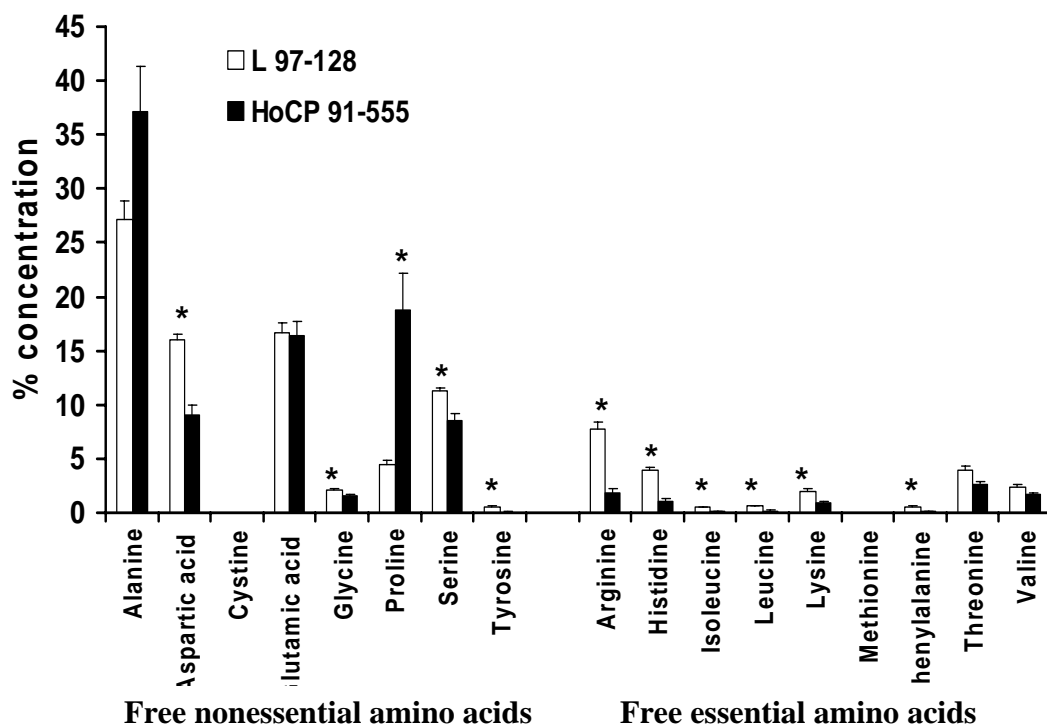


Fig. 5.1. Percentages of free nonessential and essential amino acids in whole leaf tissue of *M. sacchari* susceptible (L 97-128) and resistant (HoCP 91-555) sugarcane cultivars (*, $P \leq 0.05$).

(4.2-fold; $t = 6.92$; $df = 1, 22$; $P < 0.0001$), histidine (3.9-fold; $t = 5.68$; $df = 1, 22$; $P < 0.0001$), isoleucine (5.6-fold; $t = 6.74$; $df = 1, 22$; $P < 0.0001$), leucine (5-fold; $t = 6.51$; $df = 1, 22$; $P < 0.0001$), lysine (2.2-fold; $t = 3.59$; $df = 1, 22$; $P < 0.0001$), and phenylalanine (5.1-fold; $t = 5.90$; $df = 1, 22$; $P < 0.0001$) were detected in L 97-128 (Fig. 5.1).

5.3.2. FAAs in Phloem Sap of L 97-128 and HoCP 91-555

In phloem sap, free essential amino acids comprised 22% and 5% of the total FAAs in L 97-128 and HoCP 91-555, respectively ($t = 2.09$; $df = 1, 23$; $P = 0.0480$) (Table 5.1). The full spectrum of detectable FAAs was not found in any of the phloem sap samples, and the arrays of FAAs also varied. Eight FAAs were detected in the phloem sap of L 97-128, whereas seven were found in HoCP 91-555 (Fig. 5.2). The FAA profile of phloem sap of both cultivars was predominantly comprised of nonessential amino acids, the most abundant of which were alanine, accounting for 26% and 35% of total FAAs in L 97-128 and HoCP 91-555, respectively; and

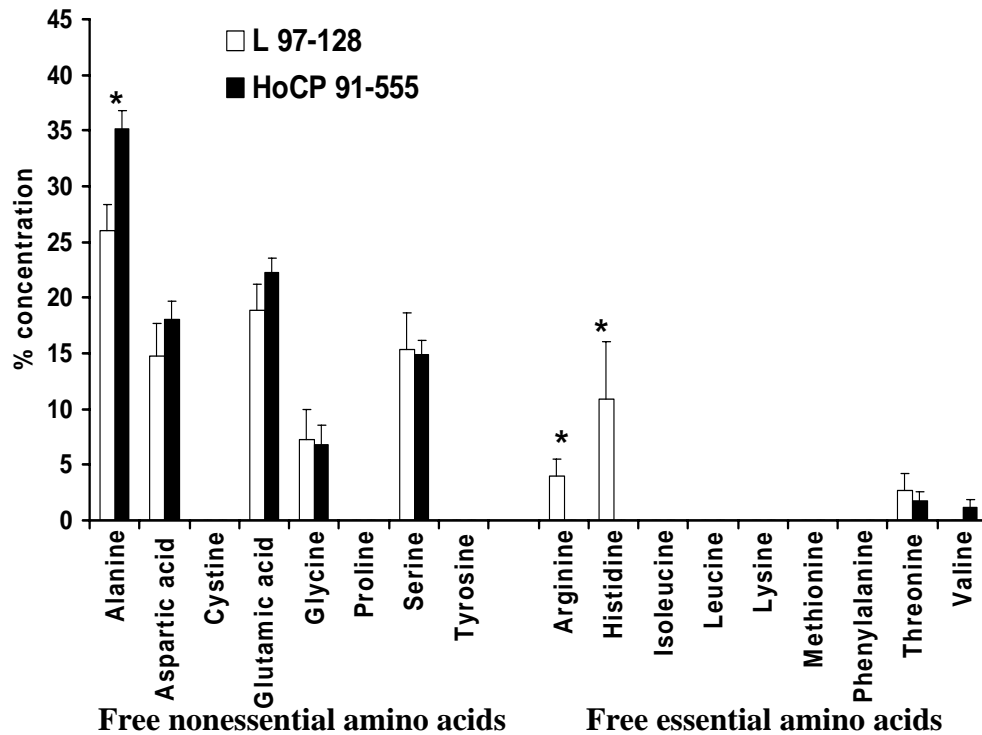


Figure 5.2. Percentages of free nonessential and essential amino acids in the phloem sap exudates of *M. sacchari* susceptible (L 97-128) and resistant (HoCP 91-555) sugarcane cultivars (*, $P \leq 0.05$).

glutamic acid, accounting for 19% and 22% of total FAAs in L 97-128 and HoCP 91-555, respectively. Alanine and glutamic acid were also the only two FAAs detected in every sample, and alanine was 1.3-fold more concentrated in HoCP 91-555 than in L 97-128 ($t = 3.24$; $df = 1, 23$; $P = 0.0036$) (Fig. 5.2). Aspartic acid and serine, though not ubiquitous, were commonly found, but cystine, proline, and tyrosine were not detected in either cultivar. Among free essential amino acids, histidine ($t = 2.87$; $df = 1, 23$; $P = 0.0086$) and arginine ($t = 3.18$; $df = 1, 23$; $P = 0.0042$) (Fig. 5.2) were found only in the phloem sap of L 97-128, whereas valine was detected only in the phloem sap of HoCP 91-555 plants (Fig. 5.2), but not in every sample.

5.3.3. FAAs in Whole Leaf Tissue versus Phloem Sap

The general pattern of free nonessential amino acid composition was similar between phloem sap and whole leaf tissue of L 97-128, excluding free proline ($t = 24.06$; $df = 1, 21$; $P <$

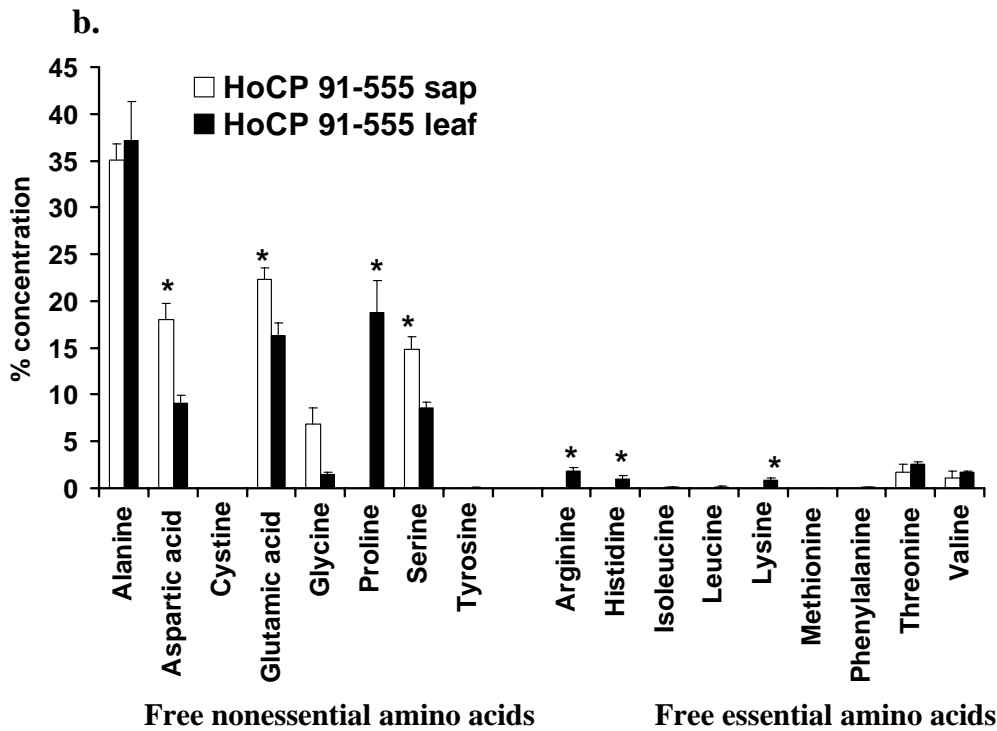
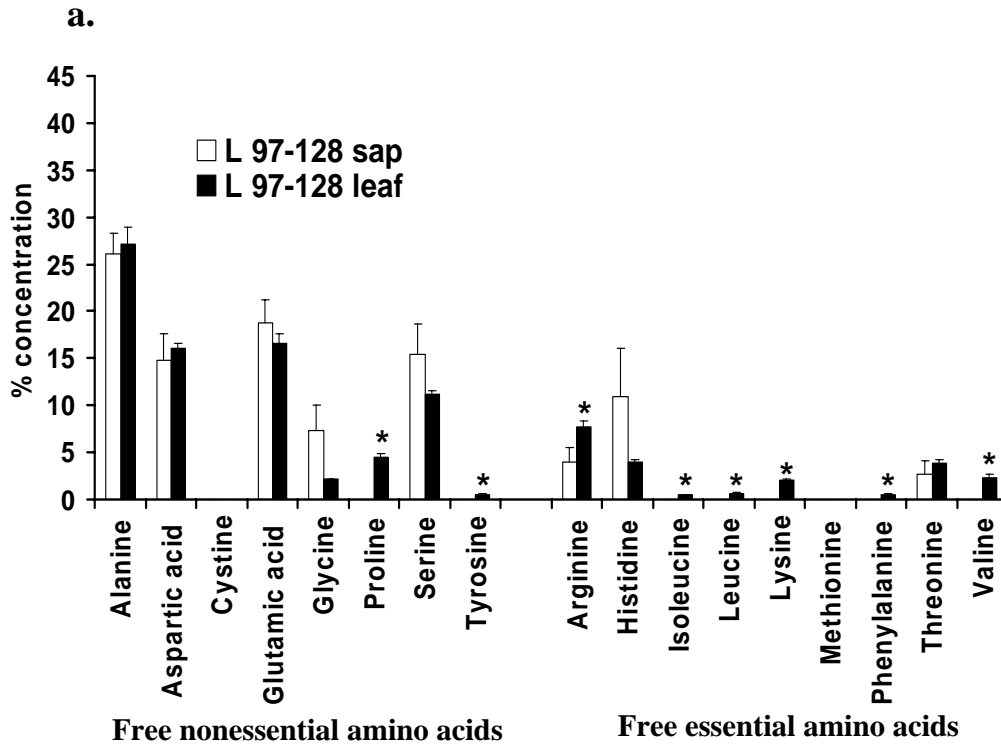


Fig. 5.3. Percentages of free nonessential and essential amino acids in whole leaf tissue and phloem sap exudates of (a) *M. sacchari*-susceptible and (b) –resistant sugarcane cultivars (*, $P \leq 0.05$).

0.0001) and tyrosine ($t = 9.62$; $df = 1, 21$; $P < 0.0001$), both of which were detected in whole leaf tissue, but not in the sap (Fig. 5.3a). Among free essential amino acids, arginine had higher concentrations in whole leaf tissue than in phloem sap (1.9-fold; $t = 2.97$; $df = 1, 21$; $P = 0.0073$), whereas isoleucine ($t = 27.66$; $df = 1, 21$; $P < 0.0001$), leucine ($t = 38.08$; $df = 1, 21$; $P < 0.0001$), lysine ($t = 16.32$; $df = 1, 21$; $P < 0.0001$), phenylalanine ($t = 16.66$; $df = 1, 21$, $P < 0.0001$), and valine ($t = 12.23$; $df = 1, 21$, $P < 0.0001$) were detected in whole leaf tissue of L 97-128, but not in the sap (Fig. 5.3a).

Free nonessential amino acids in whole leaf tissue were also found in the sap of HoCP 91-555, excluding proline ($t = 10.82$; $df = 1, 24$; $P < 0.0001$) (Fig. 5.3b). Relative concentrations of aspartic acid ($t = 2.97$; $df = 1, 24$; $P < 0.0001$), glutamic acid ($t = 3.47$; $df = 1, 24$; $P = 0.0020$), and serine ($t = 12.23$; $df = 1, 21$, $P = 0.0231$) were 2-fold, 1.4-fold, and 1.7-fold higher, respectively, in phloem sap than in whole leaf tissue. Free essential amino acids arginine ($t = 7.32$; $df = 1, 21$; $P < 0.0001$), histidine ($t = 3.47$; $df = 1, 21$; $P = 0.0020$), and lysine ($t = 7.52$; $df = 1, 24$; $P < 0.0001$) were detected only in whole leaf tissue of HoCP 91-555, but not in phloem sap (Fig. 5.3b).

5.3.4. FAAs in Phloem Sap versus Excreted Honeydew

Comparison of FAAs in phloem sap and honeydew associated with each cultivar revealed shifts in composition and concentration (Fig. 5.4). Alanine was the most abundant FAA in phloem sap, while glutamic acid and aspartic acid were the predominant FAAs in honeydew regardless of host cultivar (Figs. 5.4). Free arginine and histidine were the most abundant free essential amino acids detected in L 97-128 phloem sap and honeydew of aphids feeding on that cultivar (5.4a). There were seven FAAs detected in the honeydew of aphids feeding on L 97-128 that were not found in phloem sap, five of which were essential: isoleucine ($t = 3.76$; $df = 1, 14$; $P = 0.0021$), leucine ($t = 3.50$; $df = 1, 14$; $P = 0.0035$), lysine ($t = 3.59$; $df = 1, 14$; $P = 0.0029$),

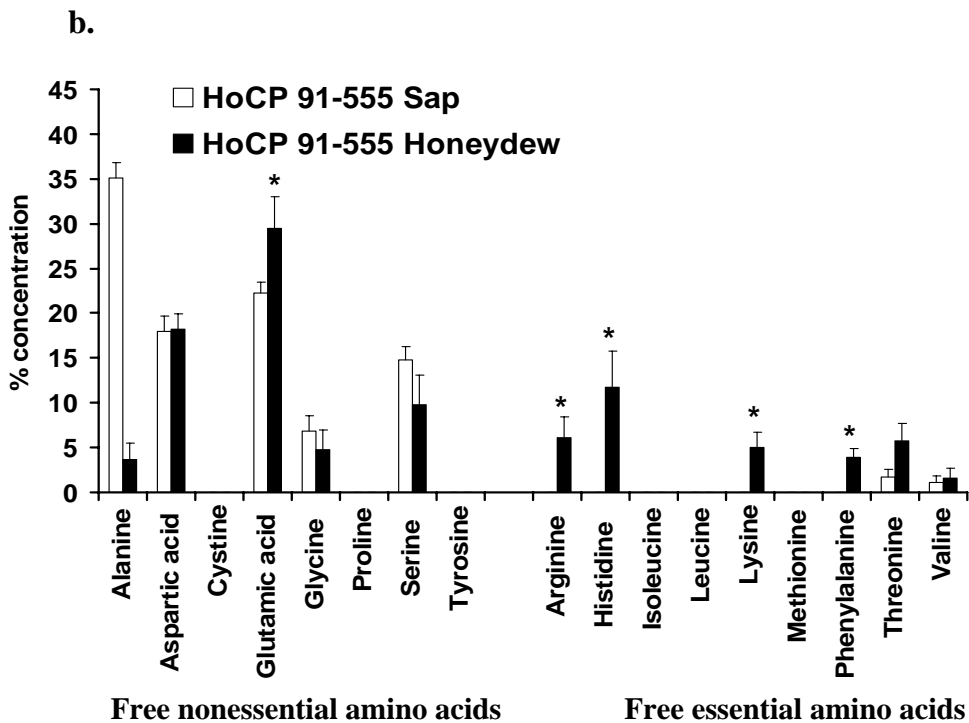
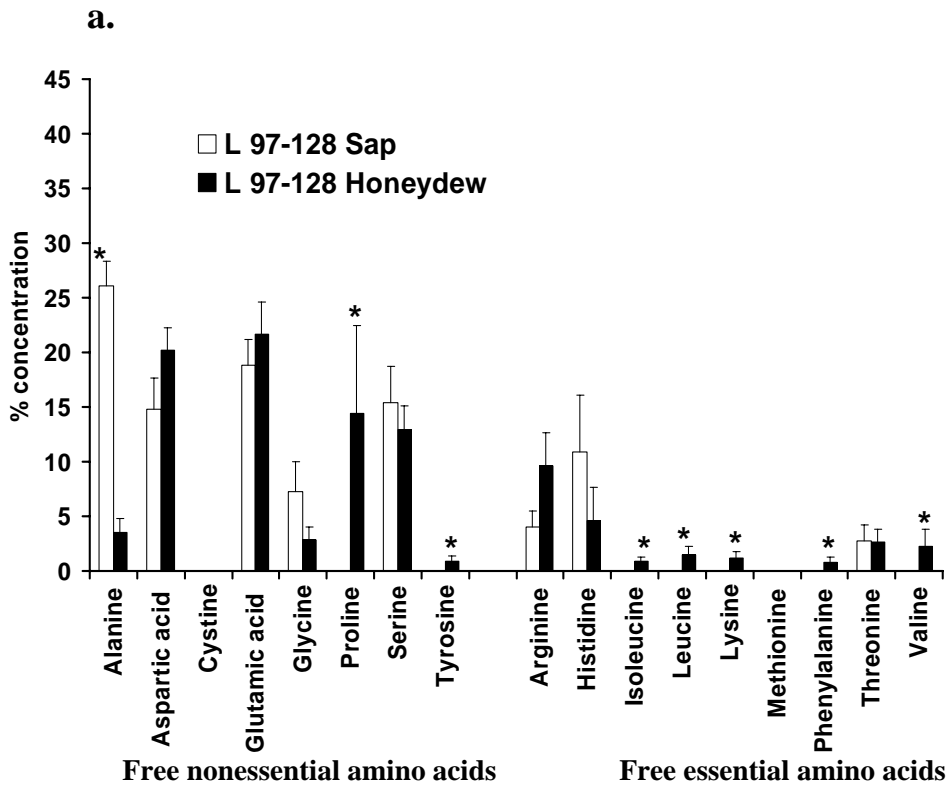


Fig. 5.4. Percentages of free essential and nonessential amino acids in phloem sap exudates and in excreted honeydew of *M. sacchari* feeding on (a) susceptible and (b) resistant sugarcane cultivars (*, $P \leq 0.05$).

phenylalanine ($t = 2.50$; $df = 1, 14$; $P = 0.0253$), valine ($t = 2.45$; $df = 1, 14$; $P = 0.0279$) (Fig. 5.4a). Free nonessential amino acids tyrosine ($t = 3.69$; $df = 1, 14$; $P = 0.0024$) and proline ($t = 3.73$; $df = 1, 14$; $P = 0.0022$) were also present in honeydew but not in phloem sap (Fig. 5.4a).

For aphids feeding on HoCP 91-555, the four FAAs detected in honeydew that were not present in phloem sap were all essential: arginine ($t = 5.99$; $df = 1, 17$; $P < 0.0001$), histidine ($t = 6.10$; $df = 1, 17$; $P < 0.0001$), lysine ($t = 6.37$; $df = 1, 17$; $P < 0.0001$), and phenylalanine ($t = 6.92$; $df = 1, 17$; $P < 0.0001$) (Fig. 5.4b).

5.3.5. FAAs in Honeydew of *M. sacchari* Feeding on L 97-128 and HoCP 91-555

Differences between the two cultivars in terms of total free essential amino acids in honeydew excreted by *M. sacchari* were not detected. However, honeydew from aphids feeding on L 97-128 had 4.1-fold ($t = 4.37$; $df = 1, 8$; $P = 0.0024$) and 5.1-fold ($t = 3.77$; $df = 1, 8$; $P = 0.0054$) greater abundances of total FAAs and total nonessential amino acids, respectively, than HoCP 91-555 (Fig. 5.5). There were 15 FAAs detected in the honeydew of aphids feeding on L 97-128 as compared to 11 from aphids feeding on HoCP 91-555 (Fig. 5.6). The four FAAs detected only in the honeydew of aphids feeding on susceptible L 97-128 were comprised of two free essential amino acids isoleucine ($t = 2.42$; $df = 1, 8$; $P = 0.0416$) and leucine ($t = 2.26$; $df = 1, 8$; $P = 0.0539$), and the free nonessential amino acids tyrosine ($t = 2.38$; $df = 1, 8$; $P = 0.0447$) and proline ($t = 2.41$; $df = 1, 8$; $P = 0.0428$) (Fig. 5.6).

5.4. Discussion

This study was the first to quantify selected primary and secondary metabolites in association with sugarcane resistance to aphids, providing new insights on bases of aphid-sugarcane interactions. Although some nutritional components, such as water and TAC, are important to insect feeding and survival (Chapman 2003), lack of observed cultivar differences suggest their roles in governing *M. sacchari* population growth are negligible for these two

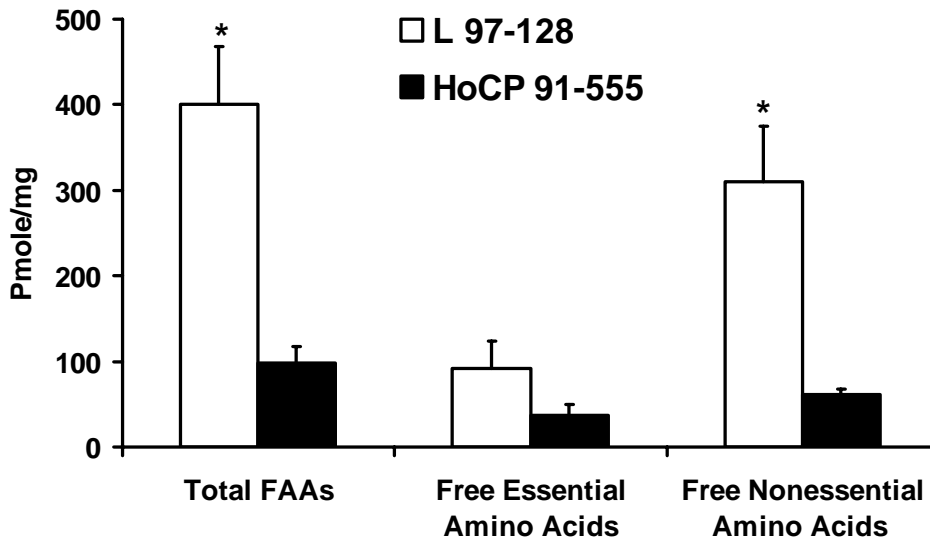


Fig. 5.5. Concentration (pmol/mg) of free total, essential, and nonessential amino acids in honeydew of *M. sacchari* feeding on susceptible (L 97-128) and resistant (HoCP 91-555) sugarcane cultivars (*, $P \leq 0.05$).

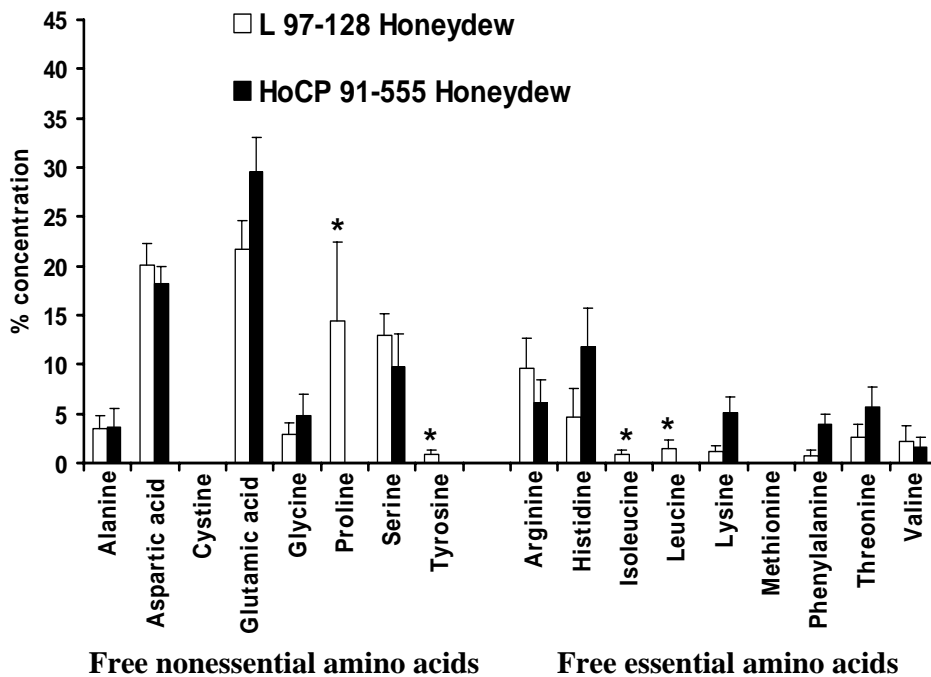


Fig. 5.6. Percentages of free essential and nonessential amino acids in honeydew of *M. sacchari* feeding on susceptible (L 97-128) and resistant (HoCP 91-555) sugarcane cultivars (*, $P \leq 0.05$).

sugarcane cultivars. Sugarcane cultivar differences in terms of water potential and Mexican rice borer, *Eoreuma loftini* (Dyar), cultivar preferences were also not observed (Reay-Jones et al. 2005, Showler and Castro 2009). Among secondary metabolites, phenolic compounds are widely distributed in plants and are particularly common in members of Poaceae. Although aphid stylets penetrate epidermal and mesophyll tissues intercellularly, avoiding contact with vacuoles and other organelles that can be high in phenolics (Dreyer and Campbell 1987), plants with relatively higher concentrations of phenolics have been shown to impair growth, development, and fecundity of aphids (Leszczynski et al. 1995, Kessler and Baldwin 2002, Urbanska et al. 2002, Sing et al. 2004). Many phenolics occur in sugarcane (Godshall and Legendre 1988), but the lack of cultivar differences in our study suggest that levels of these secondary metabolites might not play role in resistance to aphids.

At a more fundamental level, nitrogen is critical for growth because of its centrality to metabolic processes, cellular structure, and genetic coding; therefore it is potentially limiting to development and reproduction (Mattson 1980). After carbohydrates, nitrogen is the most important nutrient affecting performance of aphids (Prosser and Douglas 1992). *Melanaphis sacchari* populations, for example, increase quickly on sorghum genotypes high in nitrogen, sugar, and chlorophyll (Singh et al. 2004). Aphids primarily target phloem sieve elements where nutrients are available in soluble, readily assimilable, and renewable forms, such as sucrose and FAAs (Risebrow and Dixon 1987, Febvay et al. 1988), which can affect aphid performance (Auclair 1963, Douglas 1998, Karley et al. 2002). In our study, free nonessential amino acid compositions in L 97-128 and HoCP 91-555 phloem sap were not different, but variation was observed in the free essential amino acids histidine and arginine. One possible reason for these differences might be cultivar variations in sieve elements (Weibull et al. 1990, Sandström and Petterson 1994) that might cause aphids to discriminate between those sieve elements for sustained feeding (Tjallingii 1994). *Melanaphis sacchari* total feeding time and mean duration of

sustained individual ingestion events were lower on HoCP 91-555 than on L 97-128 (see Chapter 4). The commonality in these two cultivars' phloem sap was the prevalence of free nonessential amino acids, also reported by others in cereals (Weibull et al. 1990, Telang et al. 1999). Although alanine was most prevalent in sugarcane phloem sap, it was followed by aspartic acid, glutamic acid, and serine, all three of which have been shown to be most abundant in the phloem sap of wheat, *Triticum aestivum* L.; oats, *Avena sativa* L.; and barley, *Hordeum vulgare* L. (Hayashi and Chino 1986, Weibull et al. 1990). Wilkinson and Douglas (2003) also found nonessential amino acids to be prevalent, especially asparagine and glutamine, in the sap of 16 host plant species of the black bean aphid, *Aphis fabae* Scopoli. In our study, analysis of FAAs in whole leaf tissue corroborated the composition in phloem sap, especially with regard to most nonessential amino acids. However, detection of several different free essential amino acids in the whole leaf tissue but not in phloem sap indicates either undetectable or nil concentrations of these amino acids in phloem sap.

Although the chemical composition of honeydew might not be conclusive evidence of phloem sap chemical composition (Molyneux et al. 1990), it indicates the role of aphid endosymbionts or aphid's metabolic processes to alter nutritional constituents of plant phloem sap (Douglas 1998, Telang et al. 1999). In our study, the analysis of honeydew indicated shifts in FAA composition from that of phloem sap, particularly aspartic and glutamic acids that were greater in honeydew than in phloem sap of either cultivar. Asparagine and glutamine, amide forms of aspartic acid and glutamic acid, respectively, are also commonly found in honeydew of different aphid species (Douglas 1992, Sandström and Moran 2001, Fisher et al. 2002). Perhaps the most important differences between FAAs in phloem sap and honeydew involved essential amino acids. The presence of free arginine, histidine, lysine, and phenylalanine in the honeydew of aphids feeding on HoCP 91-555, each of which were absent in the phloem sap, suggests that

M. sacchari or its endosymbionts derived these FAAs, ruling out their role in sugarcane resistance to *M. sacchari*. Free leucine, isoleucine, tyrosine, and proline, however, were absent only in the honeydew of aphids feeding on HoCP 91-555, which indicates their possible roles in this cultivar's resistance. Dadd and Krieger (1968) found that free isoleucine was an essential amino acid for normal development of the green peach aphid, *Myzus persicae* Sulzer. Cole (1997) showed a positive correlation between rate of cabbage aphid, *Brevicoryne brassicae* L., population increase and four FAAs, including leucine and tyrosine. Tyrosine, an important amino acid needed for sclerotization of insect cuticle after molting (Urich 1994), is derived from phenylalanine (Sandström and Moran 1999). Both phenylalanine and tyrosine were detected in the honeydew of aphids feeding on L 97-128, but only phenylalanine was found in the honeydew of HoCP 91-555, suggesting the aphid's inability to derive tyrosine on that cultivar. Biotic and abiotic stresses on sugarcane can result in increased accumulation of proline (Showler et al. 1990, Singh et al. 1993, Reay-Jones et al. 2005b, Showler and Castro 2009). Proline detection only in the honeydew of aphids feeding on aphid-susceptible L 97-128 might have occurred because *M. sacchari* were confined in a small cage for three days, possibly causing enough localized stress to elicit accumulation of more proline in L 97-128 phloem sap.

Insect feeding behavior, total food consumption, and consumption rate are affected by nutritional suitability of host plants (Mattson 1980). Black bean aphids, for example, spent more time ingesting phloem sap from susceptible broad bean, *Vicia fabae* L., cultivars than on less susceptible lines, and susceptibility was associated with relatively high concentrations of free essential and nonessential amino acids (Cichocka et al. 2002). Composition of amino acids is a major factor in the development and reproduction of several species of aphids (Febvay et al. 1988, Prosser and Douglas 1992, Sandström and Petterson 1994), including the bird cherry-oat aphid, *Rhopalosiphum padi* L., which had a growth rate directly proportional to amino acid

concentrations in the phloem saps of oat and barley (Weibull 1987). Differences observed in *M. sacchari* performance (see Chapter 3) and feeding behavior (see Chapter 4) on two sugarcane cultivars can as well be attributed to dissimilarities in FAA profiles or the ability of aphids to derive specific essential and nonessential amino acids, or from other biochemicals not measured in this study.

CHAPTER 6: FIELD EVALUATION OF LOUISIANA SUGARCANE CULTIVARS FOR RESISTANCE TO THE SUGARCANE APHID AND YELLOW SUGARCANE APHID

6.1. Introduction

Sugarcane, interspecific hybrids of *Saccharum* spp., in Louisiana is colonized by two aphid species, the yellow sugarcane aphid, *Sipha flava* (Forbes), and the sugarcane aphid, *Melanaphis sacchari* (Zehntner). *Sipha flava* is yellow, 1.3-2.0 mm long, and has numerous bristle-like hairs with dusky transverse markings on the dorsum. The species has been found in North, Central, and South America and on various Caribbean islands, and it can feed on numerous genera of Gramineae including *Digitaria*, *Hordeum*, *Panicum*, *Paspalum*, *Pennisetum*, *Saccharum*, *Sorghum*, and *Triticum* (Blackman and Eastop 2000). *Sipha flava* has been an important pest of sugarcane in the United States and elsewhere in its range (Hall and Bennett 1994), causing reddish leaf discoloration from injection of a toxin leading to tissue chlorosis and necrosis (Breen and Teetes 1986, Webster 1990). In addition to direct feeding damage, another concern is transmission of non-persistent sugarcane mosaic potyvirus (Hall and Bennett 1994, Blackman and Eastop 2000).

Melanaphis sacchari was first discovered in Louisiana in September 1999 on the USDA-ARS Ardoyne Research Farm near Houma and a subsequent survey showed that 8 of 21 sugarcane-producing parishes were infested (White et al. 2001). This species is generally whitish under Louisiana conditions, and 1.1-2.0 mm long. *Melanaphis sacchari* is distributed throughout tropical and subtropical regions of the world on hosts of the genera *Echinochloa*, *Oryza*, *Panicum*, *Pennisetum*, *Saccharum*, and *Sorghum* (Blackman and Eastop 2000). In recent years in Louisiana, *M. sacchari* has become the most abundant species on sugarcane. A major problem associated with *M. sacchari* is transmission of the persistent sugarcane yellow leaf virus (ScYLV), and in Louisiana absence of ScYLV has been added to certification standards for micropropagated seedcane for minimizing spread of the virus (Schenck and Lehrer 2000,

McAllister et al. 2008). The spread and incidence of ScYLV in sugarcane can be reduced by use of aphid-resistant cultivars (Smith 2005). Greenhouses studies on predominant Louisiana sugarcane cultivars have shown differences with regard to resistance/susceptibility to *M. sacchari* and *S. flava* on L 97-128 (susceptible) and HoCP 91-555 (resistant) (see Chapter 3). The objective of this study was to assess several sugarcane cultivars under field conditions to corroborate greenhouse results, and also to determine peak population times for aphid infestations to assist with better management decisions.

6.2. Materials and Methods

Five commercial sugarcane cultivars; LCP 85-384 (Milligan et al. 1994), HoCP 91-555 (Legendre et al. 2000), Ho 95-988 (Tew et al. 2005), HoCP 96-540 (Tew et al. 2005), and L 97-128 (Gravois et al. 2008) were planted using whole stalks in Youngsville, Louisiana on 15 August 2006. Plots were comprised of single 7.3-m long sections of row with a 1.2-m gap at the end of each plot. Treatments were arranged in a randomized complete block design with five replications. Conventional agronomic and cultural practices were used in the field, but foliar insecticides were not applied at any time. Sampling for natural populations of aphids began 4 April and continued until 29 August 2007, and 4 April through 26 August 2008. Aphids of each species were counted on ten randomly selected sugarcane plants in each plot during the first and third weeks of every month (\approx 15 days apart).

Aphid count data were $\log(x+1)$ transformed before analysis to normalize. Season-long cultivar effects were compared using repeated measures ANOVA (Proc Mixed, SAS Institute 2006). Replication and replication x cultivar were entered into the model as random effects, and replication x cultivar was entered as the within-subject (repeated) effect. Separate analyses were performed for each sampling date to compare cultivar effects on total aphid numbers on each date (Proc Mixed, SAS Institute 2006). Similar analysis was performed to compare aphid species

numbers on each cultivar during peak population times (June and July). Effects of cultivar and crop year on averaged aphid numbers during peak population times (June and July) were determined using two-way ANOVA (Proc Mixed, SAS Institute 2006). Comparisons among cultivar means were made using the Tukey's honestly significant difference test at $\alpha = 0.05$ (Tukey 1953).

6.3. Results

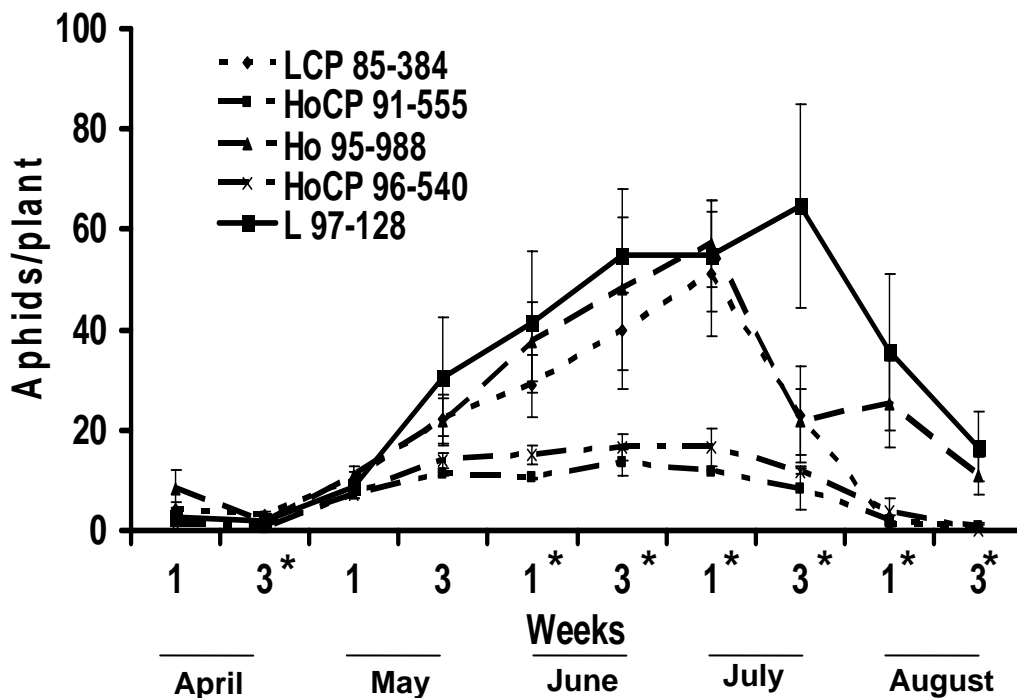
Repeated measure ANOVA showed that both cultivar and sampling date influenced aphid numbers, and the two factors interacted for both plant (cultivar $F = 32.01$; $df = 4, 16$; $P < 0.0001$; sampling date $F = 63.74$; $df = 9, 180$; $P < 0.0001$; cultivar \times sampling date $F = 2.98$; $df = 36, 180$; $P < 0.0001$) and ratoon cane (cultivar $F = 55.93$; $df = 4, 16$, $P < 0.0001$; sampling date $F = 9.23$; $df = 9, 180$; $P < 0.0001$; cultivar \times sampling date $F = 1.59$; $df = 36, 180$; $P < 0.0255$) (Fig. 6.1).

Peak populations on all cultivars occurred during the third week of June or in July (Fig. 6.1). Plant cane (2007) differences between cultivars were found as early as the last week of April ($F = 19.41$; $df = 4, 16$; $P < 0.0001$), when LCP 85-384 had 10-fold and 4-fold more aphids than HoCP 91-555 and HoCP 96-540, respectively (Fig. 6.1a). Although differences among cultivars were not detected during May, aphid numbers on all cultivars increased by 2.1-fold on LCP 85-384, 1.6-fold on HoCP 91-555, 2-fold on Ho 95-988, 1.9-fold on HoCP 96-540, and 4-fold on L 97-128. In early and late June, L 97-128 had the highest numbers of aphids that were, respectively, 4- ($F = 7.70$; $df = 4, 16$; $P = 0.0012$) and 4-fold ($F = 8.72$; $df = 4, 16$; $P = 0.0006$) more than on HoCP 91-555. In early July, Ho 95-988 had the highest numbers of aphids that were 4.8- and 3.4-fold more than on HoCP 91-555 and HoCP 96-540, respectively ($F = 11.0$; $df = 4, 16$; $P = 0.0002$). In late July, again L 97-128 had the highest numbers of aphids that were 8.0- and 5.5-fold more than on HoCP 91-555, HoCP 96-540, respectively ($F = 6.12$; $df = 4, 16$; P

= 0.0035). In early August, L 97-128 had 27.3-, 18.0-, and 9.1-fold more aphids than on LCP 85-384, HoCP 91-555, and HoCP 96-540, respectively ($F = 9.65$; $df = 4, 16$; $P = 0.0004$). By late August, these differences increased to 28-, 18.4-, and 17-fold more aphids on L 97-128 than on LCP 85-384, HoCP 91-555, and HoCP 96-540, respectively ($F = 12.76$; $df = 4, 16$; $P < 0.0001$) (Fig. 6.1a).

In ratoon cane, 2008, cultivar effects were detected in late April when a steep increase in aphid numbers occurred on Ho 95-988 with 28.5-, 28.5-, 30.6-, and 5.3-fold more aphids on this cultivar than on LCP 85-384, HoCP 91-555, HoCP 96-540, and L 97-128, respectively ($F = 18.32$; $df = 4, 16$; $P < 0.0001$) (Fig. 6.1b). Differences were not detected between Ho 95-988 and L 97-128 after late April. The highest numbers of aphids that were recorded on Ho 95-988 on all sampling dates, excluding early July when L 97-128 had the highest numbers of aphids. HoCP 91-555 and HoCP 96-540 had the fewest aphids season-long (Fig. 6.1b). In early May, Ho 95-988 had 11.5-, 12.7-, and 8.9-fold more aphids than on LCP 85-384, HoCP 91-555, and HoCP 96-540, respectively ($F = 8.20$; $df = 4, 16$; $P = 0.0009$). In late May, these differences increased to 12.9-, 18.1-, and 21.1-fold ($F = 9.65$; $df = 4, 16$; $P = 0.0004$). At this time, L 97-128 also had 4.6- and 5.4-fold more aphids than on HoCP 91-555 and HoCP 96-540, respectively. In early June, there were 15- and 12.9-fold more aphids on Ho 95-988 than on HoCP 91-555 and HoCP 96-540, respectively, whereas L 97-128 had 5.2-fold more aphids than on HoCP 91-555 at this time ($F = 9.17$; $df = 4, 16$; $P = 0.0005$). Populations were highest on all cultivars in late June, excluding L 97-128. At this time, aphid numbers on Ho 95-988 were 3.8-, 8-, and 4.1-fold higher than LCP 85-384, HoCP 91-555, and HoCP 96-540, respectively, while L 97-128 had 4.1-fold more aphids than on HoCP 91-555 ($F = 7.47$; $df = 4, 16$; $P = 0.0014$). Aphid populations peaked in L 97-128 in early July, and there were 17.5- and 5.4-fold more aphids on this cultivar than on HoCP 91-555 and HoCP 96-540, respectively, whereas Ho 95-988 had 12- and 3.6-fold more

a. Plant cane, 2007



b. First ratoon cane, 2008

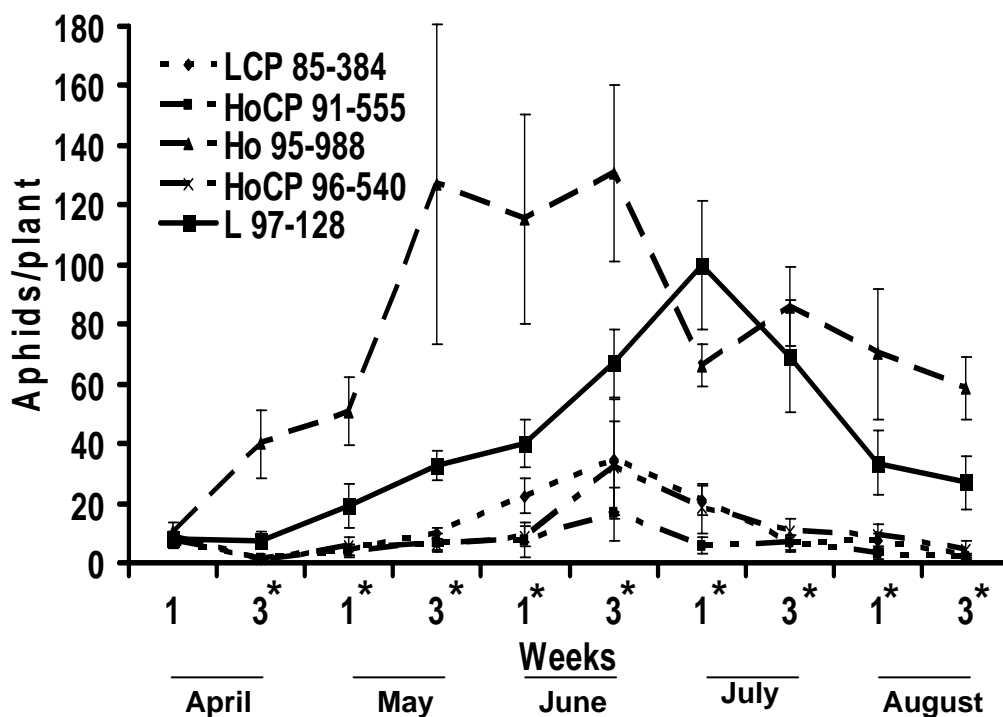


Fig. 6.1. Aphid populations per plant (mean \pm SE) on a) plant sugarcane, 2007, and b) ratoon sugarcane, 2008 during the first and third weeks of each of five months, Youngsville, Louisiana, one-way ANOVA for each sampling time, $n = 10$ (*), $P < 0.05$).

aphids than these two cultivars, respectively ($F = 11.57$; $df = 4, 16$; $P = 0.0001$). By late July, there were 13.0-, 13.2-, and 8.1-fold more aphids on Ho 95-988 than LCP 85-384, HoCP 91-555, and HoCP 96-540, respectively, while, L 97-128 had 10.5-, 10.7-, and 6.5-fold more aphids than on these three cultivars, respectively ($F = 14.68$; $df = 4, 16$; $P = 0.0001$). In early August, the only difference detected was between Ho 95-988 and HoCP 91-555 with 24-fold more aphids on Ho 95-988 ($F = 6.18$; $df = 4, 16$; $P = 0.0033$). By late August, Ho 95-988 had 24.4-, 34.5-, and 13.6-fold more aphids than on LCP 85-384, HoCP 91-555, and HoCP 96-540, respectively, whereas L 97-128 had 11.2-, 15.8-, and 6.3-fold more aphids than on these three cultivars, respectively ($F = 18.47$; $df = 4, 16$; $P < 0.0001$) (Fig. 6.1b).

Melanaphis sacchari was more abundant than *S. flava* on almost all cultivars and on all sampling dates for both plant and ratoon cane. In plant cane, 2007, in early June, *M. sacchari* were 3.2-, 15-, 3.8-, and 9.3-fold more than *S. flava* on LCP 85-384, Ho 95-988, HoCP 96-540, and L 97-128, respectively ($F = 42.37$; $df = 1, 36$; $P < 0.0001$) (Table 6.1). In late June, 3.2-, 5.3-, and 9.5-fold more *M. sacchari* than *S. flava* were recorded on HoCP 96-540, Ho 95-988, and L 97-128, respectively ($F = 18.06$; $df = 1, 36$; $P = 0.0001$). In early July, differences were not detected between aphid species numbers on HoCP 96-540 and HoCP 91-555, but 2.1-, 10.0-, and 5.2-fold more *M. sacchari* than *S. flava* were found on LCP 85-384, Ho 95-988, and L 97-128, respectively ($F = 28.54$; $df = 1, 36$; $P < 0.0001$). *Melanaphis sacchari* were 6.7-, 5.0-, 7.6-, and 37.0-fold more numerous than *S. flava* by late July, respectively, on LCP 85-384, Ho 95-988, HoCP 96-540, and L 97-128 ($F = 64.10$; $df = 1, 36$; $P < 0.0001$) (Table 6.1).

In ratoon cane, 2008, *S. flava* were not found on Ho 95-988 from early June onward, and on LCP 85-384 from early July onward. No *S. flava* were recorded on HoCP 96-540 in early June, and *M. sacchari* were 2.7- and 14.3-fold more abundant than *S. flava* at this time on LCP 85-384 and L 97-128, respectively ($F = 80.61$; $df = 1, 36$; $P < 0.0001$) (Table 6.1). Differences

Table 6.1. Mean (\pm SE) total aphid populations per plant, during 2007 plant cane and 2008 first ratoon cane, on selected sugarcane cultivars, Youngsville, Louisiana^a.

Cultivar	Sampling time ^b							
	Early June		Late June		Early July		Late July	
	<i>M. sacchari</i>	<i>S. flava</i>	<i>M. sacchari</i>	<i>S. flava</i>	<i>M. sacchari</i>	<i>S. flava</i>	<i>M. sacchari</i>	<i>S. flava</i>
Plant cane, 2007								
LCP 85-384	22.1 \pm 7.2a	6.7 \pm 2.2b	18.0 \pm 1.7a	21.8 \pm 8.3a	34.5 \pm 12.5a	16.7 \pm 15.0b	20.2 \pm 7.8a	3.0 \pm 2.1b
HoCP 91-555	4.4 \pm 1.7a	6.0 \pm 1.6a	4.1 \pm 1.4a	9.4 \pm 3.4a	6.5 \pm 1.7a	5.4 \pm 1.6a	5.5 \pm 3.6a	2.9 \pm 0.9a
Ho 95-988	35.2 \pm 8.1a	2.4 \pm 0.5b	40.7 \pm 15.8a	7.6 \pm 4.4b	51.9 \pm 5.1a	5.2 \pm 3.9b	18.0 \pm 4.2a	3.6 \pm 2.6b
HoCP 96-540	11.8 \pm 2.1a	3.1 \pm 1.9b	12.6 \pm 3.8a	4.0 \pm 1.9b	11.2 \pm 4.7a	5.5 \pm 1.6a	10.6 \pm 3.5a	1.3 \pm 0.5b
L 97-128	37.4 \pm 15.1a	4.0 \pm 1.8b	49.7 \pm 8.2a	5.2 \pm 2.4b	46.0 \pm 8.2a	8.8 \pm 4.6b	63.0 \pm 20.0a	1.7 \pm 0.7b
Ratoon cane, 2008								
LCP 85-384	16.2 \pm 5.7a	6.1 \pm 4.3b	34.4 \pm 20.2a	0.4 \pm 0.4b	20.8 \pm 4.8a	0.0 \pm 0.0b	6.6 \pm 2.5a	0.0 \pm 0.0b
HoCP 91-555	7.1 \pm 5.7a	0.4 \pm 0.2a	15.2 \pm 8.9a	1.2 \pm 0.6b	4.4 \pm 2.9a	1.3 \pm 0.8a	4.8 \pm 2.6a	1.7 \pm 0.8a
Ho 95-988	115.3 \pm 34.9a	0.0 \pm 0.0b	130.9 \pm 29.7a	0.0 \pm 0.0b	66.2 \pm 7.1a	0.0 \pm 0.0b	85.9 \pm 13.1a	0.0 \pm 0.0b
HoCP 96-540	8.8 \pm 3.1a	0.0 \pm 0.0b	28.7 \pm 15.7a	3.1 \pm 2.0b	17.8 \pm 8.3a	0.6 \pm 0.4b	9.1 \pm 4.0a	1.5 \pm 0.9b
L 97-128	37.3 \pm 7.2a	2.6 \pm 1.8b	64.0 \pm 12.2a	2.9 \pm 1.3b	97.1 \pm 20.7a	2.5 \pm 1.6b	48.8 \pm 7.9a	20.5 \pm 15.4b

^aMeans in the same rows within the same sampling time followed by similar letter are not significantly different ($P > 0.05$; Tukey's [1953] HSD).

^bEarly June, 7 June 2007, 9 June 2008; Late June, 22 June 2007, 27 June 2008; Early July, 6 July 2007, 9 July 2008; Late July, 24 July 2007, 28 July 2008.

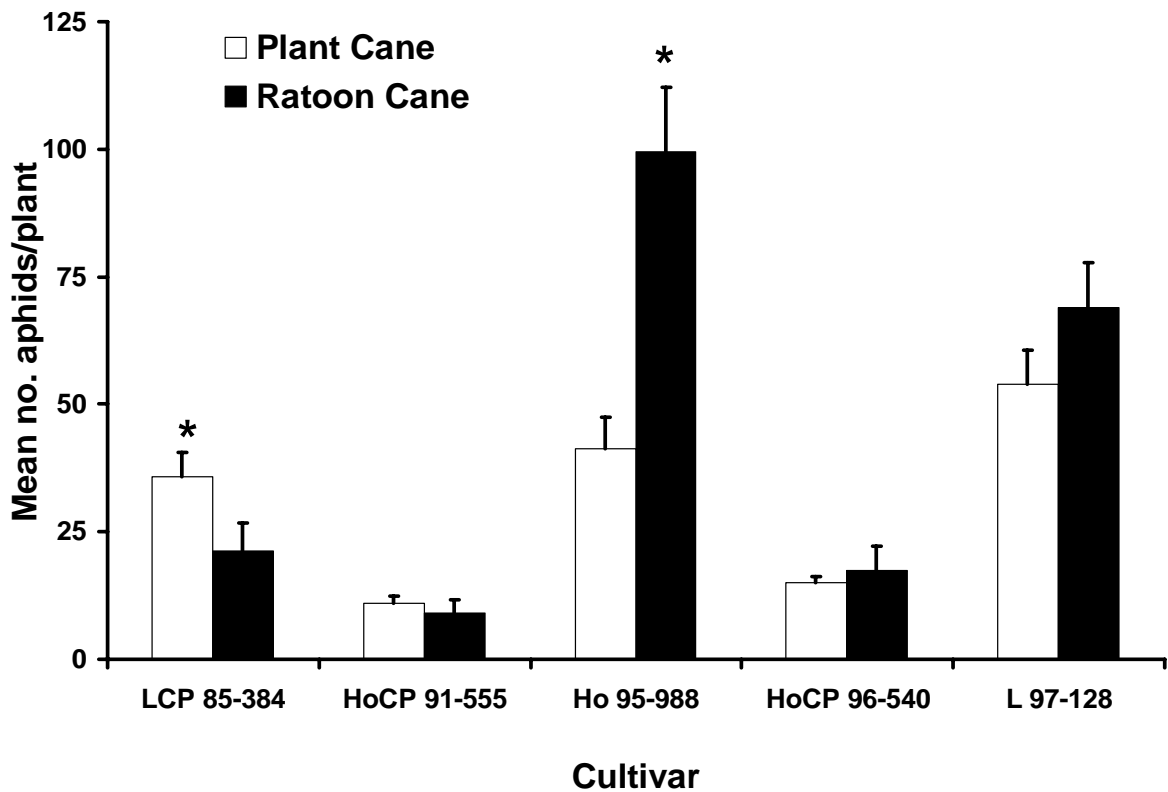


Fig. 6.2. Mean (\pm SE) aphid populations per plant during June and July on plant (2007) and ratoon sugarcane (2008) of five sugarcane cultivars, Youngsville, LA (*, $P \leq 0.05$).

between aphid numbers increased to 86-, 12.7-, 9.3-, and 22-fold more *M. sacchari* than *S. flava* on LCP 85-384, HoCP 91-555, HoCP 96-540, and L 97-128, respectively, by the end of June ($F = 92.79$; $df = 1, 36$; $P < 0.0001$). In early July, there were 29.6- and 38.8-fold more *M. sacchari* than *S. flava* on HoCP 96-540 and L 97-128, respectively ($F = 161.71$; $df = 1, 36$; $P < 0.0001$). By late July, *M. sacchari* was 6.0- and 2.3-fold more numerous than *S. flava* on HoCP 96-540 and L 97-128, respectively ($F = 59.69$; $df = 1, 36$; $P < 0.0001$) (Table 6.1).

Averaged peak population numbers of aphids were not influenced by year, but significant cultivar effects ($F = 35.75$, $df = 4, 32$, $P < 0.0001$) and cultivar \times year interactions ($F = 6.15$, $df = 4, 32$, $P = 0.0009$) were recorded. In first ratoon cane, there was a 1.69-fold ($F = 6.58$; $df = 1, 32$; $P = 0.0152$) decrease in number of aphids than on plant cane on LCP 85-384, but a 2.41-fold ($F = 8.39$, $df = 1, 32$; $P = 0.0068$) increase on Ho 95-988 (Fig. 6.2).

6.4. Discussion

Host plant resistance to insect pests is a major component of integrated pest management for Louisiana sugarcane. Currently available cultivars for use in Louisiana include HoCP 85-845, LCP 85-384, HoCP 91-555, Ho 95-988, HoCP 96-540, L 97-128, L 99-226, L 99-233, and HoCP 00-950 (Legendre and Gravois 2009). Because sugarcane is perennial and three to five crops are typically harvested from each planting, cultivar selection can be crucial to long-term production (Posey et al. 2006). The major insect problem in Louisiana sugarcane is the sugarcane borer, *Diatraea saccharalis* F., the focus of most varietal resistance efforts (Bessin et al. 1990, Reagan 2001). Most studies aimed at determining host plant resistance mechanisms of *M. sacchari* are on sorghum, *Sorghum bicolor* (L.) Moench (Setokuchi 1988, Kawada 1995, Teetes et al. 1995). While White (1990) conducted a greenhouse evaluation of *S. flava* resistance in selected sugarcane cultivars, our study is the first such assessment under field conditions. Our study confirms McAllister et al. (2005) findings that *M. sacchari* infestations are low in the spring, but build over May and June, with peak populations in July, followed by population crashes. Similar trends in *M. sacchari* population patterns have also been reported in Florida sugarcane (Hall and Bennett 1994). The observed cultivar effects on aphid populations in our study indicated that some cultivars are more resistant than others. Resistance we documented in HoCP 91-555 in the greenhouse (see Chapter 3) was operating under field conditions against both aphid species season-long. However, levels of resistance in sugarcane to insects can vary depending on insect pressure and environmental conditions (Reay-Jones et al. 2003, Showler and Castro 2009); and multi-location data under heavier aphid pressure are needed to fully validate HoCP 91-555's resistance to *M. sacchari* and *S. flava*.

The reasons for abundance of *M. sacchari* compared to *S. flava* are not clear, but cursory observations of differences in amount of honeydew excreted by these aphid species and ant

attendance suggest a possible role of fire ants in protecting more *M. sacchari* from predators as compared to *S. flava* (Flatt and Weisser 2000, Yao and Akimoto 2001, Woodring et al. 2004). In addition, ratoon cane had higher infestations than plant cane in susceptible cultivars Ho 95-988 and L 97-128, which can be likely attributed to a longer establishment of fire ants in the ratoon crop (White 1980), as their activity was more noticeable on ratoon cane. In contrast, ratoon LCP 85-384 had fewer aphids compared to plant cane, which we believe was due to early appearance of common brown rust, *Puccinia melanocephala* Syd., that likely affected aphid feeding on this cultivar. However, detailed characterization of such species-specific and temporal interactions requires additional investigations.

Rapid population buildup of *M. sacchari* on L 97-128 and Ho 95-988 indicates enhanced colonization, reproduction potential, and substantial survival in contrast with the other three cultivars. Comparison of phloem sap composition of *M. sacchari* susceptible L 97-128 and resistant HoCP 91-555 shows that these cultivars do not differ in their nonessential amino acids, but differences were detected in essential amino acids in the sap and aphid ability to derive additional amino acids while feeding on these cultivars (see Chapter 5). Therefore, differences observed in aphid densities in this field study can be attributed to variations in availability of limiting required nutrients that influence aphid host preference and survival. HoCP 91-555 has also been found to have relatively low infestations of another hemipteran, the sugarcane tinger, *Leptodictya tabida* Herrich Schaeffer, in field surveys of the Lower Rio Grande Valley of Texas (Setamou et al. 2005), suggesting similar nutritionally-based resistance mechanism. On the other hand, where LCP 85-384, HoCP 91-555, and HoCP 96-540 were comparatively resistant to both aphid species, they are relatively susceptible to lepidopteran stalk borers (Reay-Jones et al. 2003, Posey et al. 2006), suggesting the need for variation in management tactics for different insect groups in Louisiana sugarcane.

Since its release in 1993, the Louisiana sugarcane industry has relied extensively on the early-maturing cultivar LCP 85-384, with 91% acreage in 2004 (Legendre and Gravois 2009), because of its desirable agronomic characteristics, including high stalk populations, stubbling ability, and relatively high sugar and cane yields (Milligan et al. 1994, LaBorde et al. 2008). It has been credited for saving Louisiana's sugar industry from collapse (Gravois and Bischoff 2001). In our study, LCP 85-384 showed moderate resistance to *M. sacchari*. McAllister et al. (2008) also reported moderate levels of resistance to *M. sacchari* in association with low incidence of ScYLV (McAllister 2008). High susceptibility to common brown rust in LCP 85-384 is forcing farmers to adopt different cultivars (Hoy et al 2000). A survey in 2008 indicated a substantial shift in cultivar composition from 2004 such that 22, 2, 5, 44, and 17% of Louisiana sugarcane production land was planted to LCP 85-384, HoCP 91-555, Ho 95-988, HoCP 96-540, and L 97-128, respectively (Legendre and Gravois 2009). Both HoCP 91-555 and HoCP 96-540 are resistant to several diseases, but are susceptible to the sugarcane borer, and therefore, are not recommended where insecticides cannot be applied (Legendre et al. 2000, Tew et al. 2005). Our study has shown that these two cultivars are resistant to both aphid species season-long, and might be good choice in areas with aphid problems. Although biotype development, aphid ability to overcome host plant resistance factors, is a risk when relying on insect-resistant cultivars (Auclair 1989, Smith 2005), low acreage of HoCP 91-555 in Louisiana is unlikely to exert sufficient selection pressure on *M. sacchari* and *S. flava* to elicit biotype development.

CHAPTER 7: LIFE CYCLE AND LARVAL MORPHOLOGY OF *DIOMUS TERMINATUS* (SAY) (COLEOPTERA: COCCINELLIDAE) AND ITS POTENTIAL AS A BIOLOGICAL CONTROL AGENT OF THE SUGARCANE APHID, *MELANAPHIS SACCHARI* ZEHNTNER²

7.1. Introduction

The sugarcane aphid, *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae), a small ant-tended hemipteran with various body colors, is distributed throughout the tropical and subtropical regions of the world (Blackman and Eastop 1984). The first finding in Louisiana was reported on 9 September 1999, on the USDA-ARS Ardoyne Research Farm near Houma. A subsequent survey showed that eight out of 21 parishes where sugarcane is planted were infested (White et al. 2001). *Melanaphis sacchari* is a key pest of sorghum (*Sorghum* spp.) and of sugarcane in many parts of Africa, Asia, Australia, the Far East and in Central and South America (Singh et al. 2004). Other hosts include rice (*Oryza sativa*), maize (*Zea mays*), millet (*Setaria* spp.), barnyard grass (*Panicum colonum*), bermuda grass (*Cynodon dactylon*) and several additional grasses. Feeding by *M. sacchari* on sugarcane leaves causes a slight loss of leaf greenness, and heavily infested leaves turn black from sooty mold developing on honeydew deposits (Hall and Bennett 1994). *Melanaphis sacchari* is also an important vector of sugarcane yellow leaf virus especially in Hawaii where the infection level in several commercial cultivars reached up to 95% (Schenck and Lehrer 2000). Recent studies in Louisiana indicated that *M. sacchari* was the most abundant aphid species recorded in bi-weekly surveys, and up to 25% of the area within fields in several locations was infected with yellow leaf virus disease (McAllister et al. 2005). Sugar yield losses up to 11 and 14% have been reported in first and second ratoon crops, respectively, in Louisiana because of the sugarcane yellow leaf virus (Grisham et al. 2001). In order to minimize the spread of virus, yellow leaf has been added to the certification standards for micropropagated seedcane.

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Singh et al. (2004) presented a comprehensive review of *M. sacchari* biology and listed more than 47 natural enemies in different countries. These included pathogens (*Verticillium lecanii*), parasitoids (Hymenoptera) and predators (Diptera, Neuroptera, Coleoptera, and Hemiptera). Among these groups, ladybeetles (Coccinellidae), lacewings (Chrysopidae), and hover flies (Syrphidae) seemed more important because they cause greatest mortality to the *M. sacchari* populations (Singh et al. 2004).

Diomus terminatus (Say) (Coleoptera: Coccinellidae) is a generalist aphid predator native to the Eastern and Midwestern United States (Gordon 1976). It has been successfully reared under laboratory conditions on a number of aphids including the yellow sugarcane aphid, *Sipha flava* (Hall 2001, Hentz and Nuessly 2002), corn leaf aphid, *Rhopalosiphum maidis*, cotton aphid, *Aphis gossypii*, and green peach aphid, *Myzus persicae* (Hallborg 2003). This species was observed feeding on *M. sacchari* in Louisiana (White et al. 2001), but studies have not been conducted on its life cycle using this aphid as prey. Larvae of this beetle were noticed feeding on *M. sacchari* in a variety field test near Youngsville, LA, on July 10, 2007. The larvae were collected, reared in the laboratory, and studied for biological control potential. Hentz and Nuessly (2002) provided cursory descriptions of various life stages of *D. terminatus*, but details of taxonomically informative characters were not given. Ślipiński (2007) provided a generic larval description based on the Australian species *D. notescens* (Blackburn) and an unidentified *Diomus* sp., including illustrations of the latter. He also discussed issues related to the generic diagnosis of the genus involving adult characters. A detailed morphological description of first and fourth instars of *D. terminatus* is also provided here. These descriptions will allow integration of characters into phylogenetic analyses of coccinellids and other cucujoid beetle taxa, and provide a more comprehensive basis for distinguishing larvae of this species from those of other coccinellids.

7.2. Materials and Methods

7.2.1. Life Cycle Studies

The *D. terminatus* colony was initiated in July 2007 with 25 late instars collected from a small-plot sugarcane variety test near Youngsville (Lafayette Parish, LA). *Melanaphis sacchari* feeding on small cut pieces of sugarcane leaves were used as prey in this study. This was important to mimic natural conditions and to avoid loss of any plant physical or chemical cues that might be helpful to *D. terminatus* in finding and utilizing its prey (Hallborg 2003). Beetles were provided with fresh aphids every 1-2 days that were collected either from an aphid colony in the greenhouse or directly from the field at the Louisiana State University Agricultural Center Sugar Research Station (Iberville Parish, Louisiana). The beetle colony was maintained in an incubator at 26 °C, 14:10 L:D photoperiod and 75±5% RH. The life stage studies were conducted in the same incubator. Beetle larvae along with *M. sacchari* on cut sugarcane leaves were brought into the laboratory and placed in a rearing chamber, a 42,875 cubic cm plexi glass box with a round opening of 10 cm diameter covered with perforated plastic to prevent beetle escape and ensure ventilation. Larvae were also provided with a 20% sugar solution as an additional food source inside the chamber, and wax paper and Kimwipes[®] as pupation and oviposition sites for larvae and adults, respectively.

Newly-hatched larvae were taken out of the chamber and placed individually in 15 ml scintillation vials to avoid cannibalism or reduced survivorship due to insufficient aphid supply (Hallborg 2003). Each first instar was provided with 10-15 aphid nymphs feeding on three to four approximately 5 cm pieces of sugarcane leaf. The later instars were provided with 15-20 nymphs. Vials were examined daily to record exuviae and number of aphids consumed. The old leaf and aphids were replaced with fresh leaf pieces and aphids until the last instar was seen stuck at its posterior end, an indication of initiation of pupation. Larvae were transferred into

clean vials when needed. The length and maximum width of pupae, their preferred location for pupation on the leaf piece, day of pupation for each surviving larva, and day of emergence for each surviving pupa were recorded. Adult beetles emerging from each vial were placed in a petri dish (8.5 cm diameter, at least four beetles/petri dish) with a moist cotton ball and several aphids on three or four approximately 5 cm sugarcane leaf pieces. The gender of the beetles was not determined at this stage. However, based on visual determination of differences in body size, the beetles were placed in petri dishes in a target male to female ratio of 1:1. The presence of females in petri dishes was confirmed by observing eggs on leaves or on the bottom of petri dishes the next day. Beetles were transferred into new petri dishes with fresh aphids and sugarcane leaves every day. The previously-used petri dishes were saved along with sugarcane leaf pieces and moist cotton ball to determine egg hatch. This assured similar age for the hatched larvae as well as determination of correct numbers of days for egg hatch. Newly-hatched larvae were used either for life cycle studies as mentioned above or preserved in 70% alcohol for morphological descriptions.

For the longevity test, adults were placed individually in petri dishes and provided every other day with 20 to 30 aphids of mixed ages on small sugarcane leaf pieces. A moist cotton ball was also put in each petri dish as a source of moisture and to delay the desiccation of sugarcane leaf pieces. There were 10 replicates (individual adults) in this experiment, and the experiment was terminated when all adults had died.

7.2.2. Description of *Diomus terminatus* Larvae

The following measurements were recorded from specimens preserved in alcohol: head length (clypeus to occipital foramen), head width at level of stemmata, maximum body width and length of normally extended specimens. Measurements were made using calibrations on drawing paper superimposed on specimens via a camera lucida mounted on an Olympus SZH10

stereomicroscope at 70X. Larvae of each of the four instars were measured and results are presented as means and ranges. First and fourth instars were also examined using an Olympus BX50 compound microscope. Fourth instars are described and illustrated in detail. Characters specific to first instars are described and illustrated with special attention to secondary setae. Observations were made at 200-400X and drawings were prepared using a camera lucida. Habitus illustrations were prepared by drawing lateral halves of specimens as a series of separate drawings. These were inked, scanned, and then reduced and assembled for bilateral symmetry using Adobe Photoshop[®]. Bilateral symmetry was achieved by duplicating bilaterally reversed images and splicing them at midlines. Specimens were prepared for microscopic examination by clearing in warm (50°C) 10% KOH aqueous solution, washing in alcohol, and slide mounting in glycerin. Larval terminology follows that of Ślipiński (2007). Voucher specimens are deposited in the Louisiana State Arthropod Museum. Abbreviations used include T1-T3 (thoracic segments 1-3) and A1-A9 (abdominal segments 1-9).

7.2.3. Assessment of *D. terminatus* as a Biological Control Agent

The potential of larval *D. terminatus* as a biological control agent of *M. sacchari* was assessed by dividing the total number of aphids consumed/killed by the number of days for larval development. Potential of adults was assessed through voracity tests. In this test, individual beetles were starved for at least 24 h, and then each beetle was provided 30 *M. sacchari* nymphs of mixed ages on three or four 5 cm pieces of sugarcane leaves from an aphid-susceptible variety (L 97-128) that was grown in the greenhouse. A small piece of moist cotton ball was also placed inside to avoid desiccation of leaf pieces. There were 15 replicates (individual adults) of this experiment including three controls with 30 nymphs on pieces of sugarcane leaves added without beetles to assess natural mortality. The numbers of aphids killed in the treatment or dead in the control were recorded after 24 h and voracity was calculated using the following formula from Soares et al. (2003):

$$V_o = (A - a_{24})ra_{24}$$

where V_o is the calculated number of aphids eaten in 24 hour (adjusted for aphid mortality in the controls), A is number of aphid available, a_{24} is number of aphids alive after 24 h, and ra_{24} is the ratio of aphids found alive after 24 h to the initial number in the control treatment.

7.2.4. Data Analysis

Data on size for each developmental stage, and days for egg hatch, larval and pupal development, adult longevity, and total aphids killed by the larvae or adults were subjected to Proc Means (SAS Institute 2005).

7.3. Results and Discussion

7.3.1. Life Cycle of *Diomus terminatus*

The field-collected larvae pupated inside the rearing chamber on pieces of sugarcane leaves rather than on wax paper or Kimwipes[®]. This is contrary to observations by Hall (2001), Hentz and Nuessly (2002), and Hallborg (2003) that wax paper or Kimwipes[®] were the preferred pupation sites. Beetles were provided with one of their natural preys (*i.e.*, *M. sacchari*) on sugarcane leaves in the current study, whereas in previous studies although the aphids were provided, sorghum leaves were not always provided, which might have affected the beetles' choice of a pupation site. *Diomus terminatus* laid eggs singly, primarily on the sugarcane leaf. Egg deposition on wax paper or Kimwipes[®] was rare, and the few deposited on the bottom of petri dishes failed to hatch. The eggs were usually deposited near the leaf midrib and on the underside of leaf pieces. Length of the convex and elongate eggs was 0.67 ± 0.03 mm (range 0.58-0.76 mm). Hentz and Nuessly (2002) and Hallborg (2003) reported similar measurements for *D. terminatus* eggs. In the current study, the egg stage lasted an average of 4.5 ± 0.09 days (range 4.3-4.7 days) (Table 7.1). Hallborg (2003) reported 6.3 and 6.2 days for the egg stage duration when beetles were fed on *A. gossypii* or *M. persicae*, respectively, and incubated at 22 °C (vs. 26 °C in this study). Hall (2001) observed about 3 days for egg stage duration at 27.7 °C

when beetles were fed *S. flava*. The differences in temperature and prey species might have caused these observed variations in egg stage duration. Although sugarcane leaf pieces had

Table 7.1. Number of days of *Diomus terminatus* at specific stages of development on *Melanaphis sacchari* nymphs feeding on sugarcane leaves.

Stage (no.)*	Days (\pm SE)
Egg (28)	4.50 \pm 0.09
Larvae 1 st instar (21)	1.66 \pm 0.10
2 nd instar (18)	1.61 \pm 0.12
3 rd instar (18)	1.77 \pm 0.10
4 th instar (17)	1.70 \pm 0.18
Total larval development (24)	6.79 \pm 0.55
Pupa (19)	4.89 \pm 0.18
Total larvae to adult (16)	12.12 \pm 0.59
Adult (10)	26.1 \pm 1.9

*Figures in parenthesis indicate the number of individuals as replicates.

desiccated by day 4, eggs were still able to hatch. Fecundity was not recorded in the current study; however, Hall (2001) determined that *D. terminatus* laid 3.0 eggs per day for 17.0 days, for a total mean of 42 eggs per female when fed *S. flava*.

The numbers of days for the other developmental stages are given in Table 7.1. On average, each of the four instars lasted less than 2 days. The last instar formed a prepupa, most of which were attached to the underside of the sugarcane leaf near the midrib. The larva attached itself to the leaf with a sticky substance released from the abdomen. The last instars sometimes were also seen attaching to the glass wall of vials, but those individuals were unable to pupate. On average, 6.79 \pm 0.55 days (range 5.65-7.93 days) in the larval stage were recorded. However, Hall (2001) reported a 10 day duration at 27.7 °C, while Hentz and Nuessly (2002) reported 4

days at 27.5 °C for the larval stage while feeding on *S. flava*. Hallborg (2003) reported 9.4 and 7.4 days duration in the larval stage for *D. terminatus* when either *A. gossypii* or *M. persicae*, respectively, were used as prey at 22 °C. In the current study, the pupal stage lasted an average of 4.89 ± 0.18 days (range 4.50-5.28 days). Hall (2001) and Hentz and Nuessly (2002) reported similar pupation time (4-5 days) when *S. flava* was used as prey. Hallborg (2003) reported 6.4 and 4.1 days in the pupal stage for *D. terminatus* when either *A. gossypii* or *M. persicae*, respectively, were used as prey. From larval hatch to adult emergence, the current study reports an average of 12.12 ± 0.59 days (range 10.86-13.38 days) at 26 °C. The differences in larval and pupal growth periods in various studies are most probably attributed to different prey species and/or incubation conditions.

In the adult longevity test, an average life span of 26.1 ± 1.9 days (range 21.9-30.3 days) for *D. terminatus* adults (Table 7.1) was recorded, but other studies have shown a survival of 143, 75, and 30 days when fed on *A. gossypii*, *M. persicae*, or *R. maidis*, respectively (Hallborg 2003), and 50 days (Hentz and Nuessly 2002) or 17 days (Hall 2001) when fed on *S. flava*. Hallborg (2003) also reported that adults could survive on as little as one *R. maidis* per day for 10 days. This variation in adult survival may be attributed to different prey and/or different incubation conditions such as temperature etc.

7.3.2. Description of *Diomus terminatus* Larvae

Size measurements of head and body for various life stages are given in Table 7.2.

First instar (Fig. 7.1, Table 7.2): **Body**- fusiform, gradually broadened from head to A2-A3 then tapering evenly to A8. Color mottled light gray to brown, with coarse asperites dorsally, fine asperites ventrally. Lateral lobes of body wall less prominent than on fourth instars. Dorsal secondary setae similar in size to homologous setae on fourth instar, so proportionally much larger relative to overall body size. Legs longer relative to body than on fourth instar. Primary setae apparently absent from thoracic nota.

Table 7.2. Size and range (in mm) of different stages of *Diomus terminatus* reared on *Melanaphis sacchari* feeding on sugarcane leaves. If ranges are not given, no variation was evident.

Stage (no.)*	Head Length	Head Width	Body Length	Body Width
Egg (10)	-	-	0.67 (0.58-0.76)	-
Larvae 1 st Instar (15)	0.14 (0.12-0.15)	0.20 (0.16-0.20)	1.07 (0.70-1.40)	0.37 (0.20-0.50)
2 nd Instar (2)	0.18 (0.16-0.20)	0.29 (0.28-0.30)	2.15 (2.00-2.30)	0.75 (0.70-0.80)
3 rd Instar (2)	0.23	0.39 (0.35-0.42)	2.80	0.80
4 th Instar (3)	0.23 (0.20-0.25)	0.39 (0.38-0.40)	3.00 (2.70-3.50)	1.28 (1.20-1.35)
Pupae (19)	-	-	1.41 (1.25-1.56)	0.76 (0.65-0.87)
Adult (15)	-	-	1.73 (1.59-1.87)	-

*Figures in parenthesis indicate the number of individuals as replicates.

- Data not recorded.

Head- occiput bearing a pair of large medially curved frayed and serrate secondary setae (possibly egg bursters). Two pairs of frayed, jagged, secondary setae present in postfrontal area, and a single pair of jagged goblet shaped secondary setae present just medial to stemmata.

Thorax- pronotum with three pairs of blunt, jagged secondary setae in a submedian row.

Postmedian area of pronotal disc with a pair of large goblet shaped secondary setae, each borne on a low, sclerotized chalaza. Two pairs of smaller, jagged, goblet setae present, one near middle of disc, the other near anterior lateral margin. Lateral margin with six pairs of jagged secondary setae of varying sizes and shapes.

Mesonotum and metanotum similar with a median raised area bearing a pair of large goblet setae as on prothorax, and a row of four jagged setae along lateral margin of raised area. Lateral margins each with three pairs of jagged setae, the first two approximate, curved and serrate, the third goblet shaped.

Pro-, meso-, and metaventrites each with a single submedian pair of primary setae.

Abdomen- abdominal segments A1-A8 similar, with four pairs of small fan shaped secondary setae, the median two pairs in a transverse line, the lateral two pair in a longitudinal

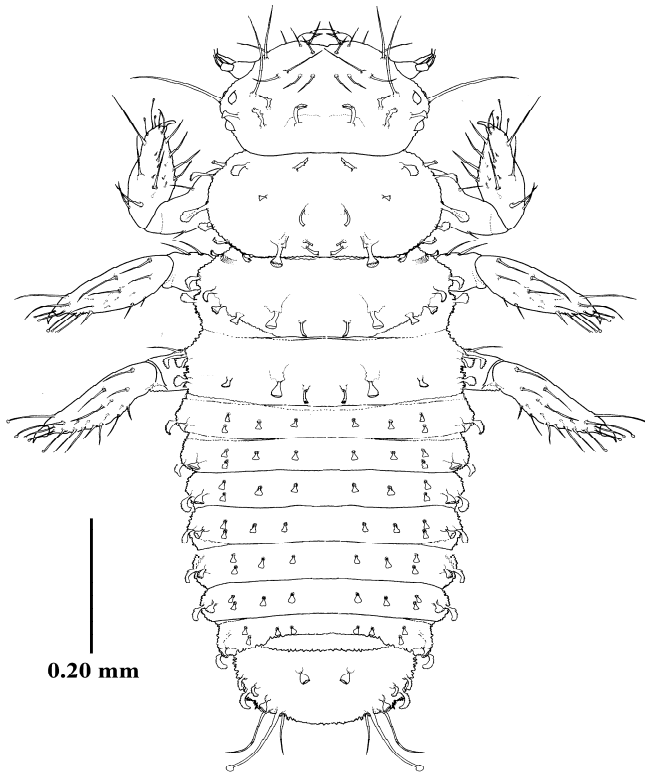


Fig. 7.1. *Diomus terminatus*, first instar larva, dorsal habitus. Integumental asperites omitted.

line. Each lateral lobe with a goblet seta borne on a low tubercle and a jagged curved seta ventral to it. Openings of repugnatorial glands not visible. A9 circular in dorsal view, bearing a postmedian pair of fan shaped secondary setae, four pairs of jagged setae on lateral and posterior aspect of disc, and three pairs of long primary setae along posterior margin, the longest pair distinctly clubbed apically.

Abdominal ventrites each with three pairs of primary setae in transverse rows, the middle pair shorter than either the median or lateral pair. Each segment with a single primary seta located along lateral margin ventral to lobe.

Fourth instar (Fig. 7.2, Table 7.2): **Body-** fusiform, gradually broadened from head to A2-A3 then tapering evenly to A8, live larvae not covered by waxy exudate. Color of head,

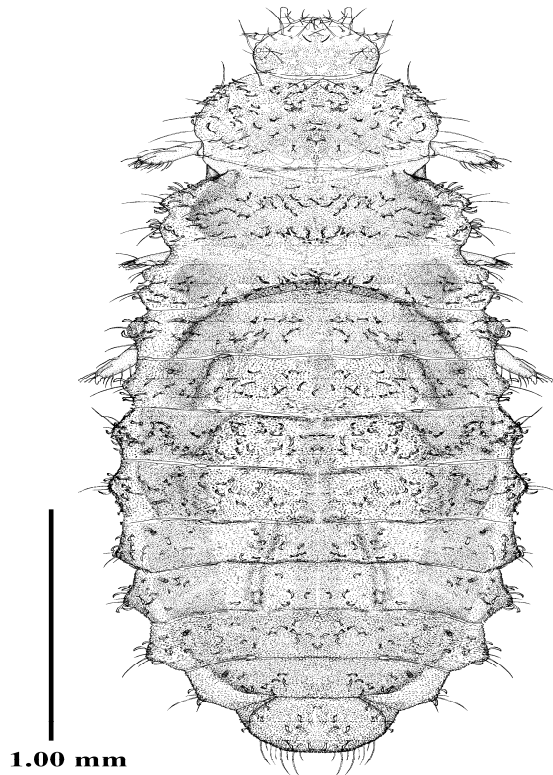


Fig. 7.2. *Diomus terminatus*, mature fourth instar larva, dorsal habitus.

mouthparts, legs, and pale brown, dorsal surface of integument brownish gray with darker granulations, imparting a medium gray to gray-brown color overall, lateral lobes of all segments lighter in color than discs. T1 evenly light grayish brown; T2-T3 darker brown, especially in median two-thirds; A1-A5 gray-brown with vaguely defined darker brown areas laterally. A6-8 evenly medium gray-brown. Ventrally light gray. Thoracic nota lacking sclerotized plates. Dorsal integument covered with fine spiny asperites. Dorsal secondary setae of body stout, blunt, ragged along shaft and often with jagged apices, not borne on tubercles or other specialized processes. Distributed evenly or in irregular groups throughout dorsal integument. Secondary setae absent ventrally and from legs and mouthparts. Primary setae normally aciculate on body and mouthparts, tarsugular setae clubbed. Ventral integument with granulate asperites that are much finer than dorsal asperites.

Head- weakly hypognathus, broader than long, arcuate across anterior face to stemmata,

then straight and weakly convergent to occiput. Surface microgranulate, dull. Epicranial stem absent. Three stemmata on each side, arranged in a close triangle. Antennae (Fig. 7.3a) 3-segmented with relative antennomere lengths from base to apex 0.5, 1.0, 0.5. Antennal base broad, membranous. Segment 1 simple. Segment 2 with three subapical and three apical setae and a conical sensorium that extends 2X length of segment 3. Segment 3 bearing one long seta and three shorter setae. Labrum triangular, anterior margin straight, posterior margin convergent to angular apex. Mandible (Fig. 7.3b) simple, apically acute, with shallow incisor groove and flat, straight mola. Scrobe with a single short seta. Hypostomal ridge strong and distinct. Maxilla (Fig. 7.3c) with rounded, simple mala bearing three sublateral and one distal setae. Maxillary

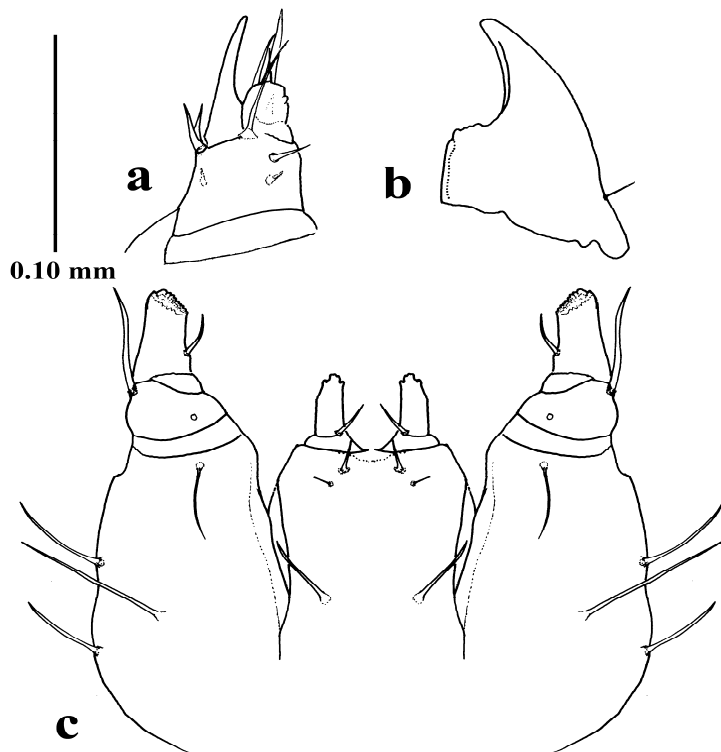


Fig. 7.3. *Diomus terminatus*, mature fourth instar larva, details of head. (a) Antenna. (b) Mandible. (c) Ventral mouthparts.

palpi 2-segmented, segment 1 broadly triangular, with a single strong seta at apicolateral angle; segment 2 narrower and 1.5X longer than 1, with a single small seta along medial margin and a clump of sensory papillae apically. Mentum/submentum quadrate, with basal and distal pairs of setae, palpifer distinct. Labial palpi simple, 1-segmented with single basal seta and terminal cluster of sensillae.

Thorax- prothorax with a row of three transverse pairs of primary setae near anterior margin, and four pairs along lateral margin. Meso- and metathorax similar in length and width, with low, transverse oval elevated area in middle two-thirds and two broad lobes laterally on each segment, anterior lobe bearing two primary setae, posterior lobe bearing one seta.

Legs well-developed, five segmented, widely separated, each with five to seven clubbed setae arising near apex of tibiotarsus in addition to typical primary setae.

Abdomen- abdominal segments A1-A8 similar, lacking elevated median areas, lateral lobes single, each bearing a pair of primary and numerous secondary setae. Paired gland openings present along anterior margins of A1-A8. A9 circular in dorsal view, bearing four long primary setae along posterior margin and additional four pairs of shorter setae along margin and deflexed ventral submarginal aspect.

Spiracles annular, simple, borne laterally on T2 and dorsolaterally on A1-A8.

Primary setae of ventrites smaller and more slender than dorsal setae, each segment bearing a submedian pair.

7.3.3. Potential of *Diomus terminatus* as Biological Control Agent

Although 10-15 aphids were provided for each first instar, only an average of 7.71 ± 0.38 aphids (range 6.17-9.25 aphids) were consumed. The larvae on average consumed a total of 29.88 ± 1.81 aphid nymphs (range 26.04-33.72 nymphs) for complete development with a consumption rate of 4.65 ± 0.38 aphids per day (range 3.85-5.45/day). The aphids killed by the

larvae were almost always lying upside down and either all of their ventral body parts were consumed or just body fluid was sucked up.

In the adult voracity test, there was no mortality in the control. The adults killed a maximum of 23 aphid nymphs but the average for 12 beetles was 19.08 ± 0.89 aphid nymphs/day (range 17.10-21.06). The consumption rates of *D. terminatus* vary when other species were used as prey. Hall (2001) observed *D. terminatus* consuming 5-10 *S. flava* per day, whereas Hallborg (2003) cited average daily consumption rates of 13.5 *A. gossypii* and 8.7 *M. persicae*. But the specific stage (*i.e.*, nymph or adult) of the prey aphid was not mentioned in those reports. The size of the prey also affects the numbers consumed by the coccinellids (Hodek 1996). Only nymphs were used in studying larval development and adult voracity in the current study. A few cursory observations of the feeding behavior of the beetle indicated that one adult beetle took approximately three minutes to devour the whole aphid body. Mostly, the adults consumed the whole aphid but sometimes just sucked up the aphid body fluids and left the exoskeleton. A common observation was that beetles moved around randomly for several minutes before attacking the next aphid.

The food consumption rate of coccinellids is affected by several environmental factors including temperature. Isikber and Copland (2001) reported an increase in the consumption rate of *Scymnus levaillanti* and *Cycloneda sanguinea* on *A. gossypii*, with increase in temperature from 25 to 30 °C. The current studies were conducted at 26 °C, which might have undermined the daily consumption rate of this beetle because temperature generally stays above 30 °C during summer days in Louisiana. However, data are not available for comparisons of *D. terminatus* consumption rate at different temperatures or to other coccinellids feeding on *M. sacchari*. Furthermore, the size of the predatory coccinellids also affects the number of aphids consumed (Hodek 1996, Isikber and Copland 2001). The extremely small size of larvae as well as adults of *D. terminatus* is a possible explanation for the small number of aphids consumed.

The current commercial cultivars of sugarcane in Louisiana sustain very low populations of *M. sacchari*. Greenhouse studies have shown an r_m (intrinsic rate of aphid increase) value as low as 0.05 on the resistant variety HoCP 91-555 or as high as 0.15 on the susceptible variety L 97-128 (see Chapter 3). Predation of *D. terminatus* larvae on *M. sacchari* was first noticed in a small plot variety test on July 10 although aphids were monitored biweekly starting in early April. The abundance of *D. terminatus* seemed to coincide with the peak population time for *M. sacchari* in Louisiana sugarcane, late June through July (McAllister et al. 2005, see Chapter 6). With low numbers of aphids and effectiveness of *D. terminatus*, chemical insecticides might not be needed for *M. sacchari* control. However, careful consideration of beneficials such as *D. terminatus* is important in the development of any new chemistry for managing major insect pest problems in Louisiana sugarcane.

CHAPTER 8: SUMMARY

Because aphids are becoming more serious pests of sugarcane in Louisiana, probably due to the increasing dominance of the red imported fire ant, *Solenopsis invicta* Buren, in this habitat, greenhouse, field, biochemical, and biocontrol studies were initiated to better understand certain insect-plant interactions and population dynamics relationships. Greenhouse experiments were conducted to categorize sugarcane resistance to *M. sacchari* in sugarcane cultivars LCP 85-384, HoCP 91-555, Ho 95-988, HoCP 96-540, and L 97-128 representing > 90% of Louisiana sugarcane acreage in 2008. Similar experiments were also conducted with *S. flava* in cultivars LCP 85-384, HoCP 91-555, and L 97-128. These studies demonstrated that antibiosis is important to sugarcane resistance against both aphid species. Field experiments revealed that cultivars HoCP 91-555 and HoCP 96-540 were relatively resistant, whereas L 97-128 and Ho 95-988 were more susceptible. Differential responses of aphids on different cultivars in this study pointed out the value of host plant resistance as a potential management tactic for aphids in sugarcane. HoCP 91-555 was shown to be useful in areas with aphid problems, and could also provide germplasm for developing new aphid resistant cultivars. L 97-128 and Ho 95-988 are likely to support relatively high aphid populations, contributing to serious plant injury, extensive sooty mold build up, and the spread of sugarcane yellow leaf virus. *Sipha flava* was of lesser concern because it occurred in relatively low numbers regardless of cultivar, whereas *M. sacchari* populations showed greater variability. This study suggests that the most appropriate scouting time for managing aphid infestation in South Louisiana is June and early July, and that aphids congregate on the underside of lower, senescing leaves. *Melanaphis sacchari* infestations were greatest in ratoon sugarcane, especially on L 97-128 and Ho 95-988. Activity of *Diomus terminatus* (Coccinellidae: Coleoptera) coincided with peak populations of aphids, and laboratory studies indicated that these beetles could have an additional role in managing *M. sacchari*.

Use of the electrical penetration graph technique assisted identification of differential feeding behavior among cultivars, and facilitated identification of sugarcane tissues that influence resistance to *M. sacchari*. Differences were not detected in the time required to reach sieve elements of L 97-128 or HoCP 91-555, suggesting that these cultivars did not affect *M. sacchari* access to and acceptance of sieve elements. However, the duration of time spent ingesting substances from sieve elements was twice as long on L 97-128 than on HoCP 91-555, suggesting a biochemical basis of resistance in the phloem sap of HoCP 91-555.

Differences were not detected in levels of total phenolics, available carbohydrates, and water potential between L 97-128 and HoCP 91-555, suggesting negligible roles for these metabolites affecting *M. sacchari* populations. However, analysis of phloem sap showed differences in the free amino acid composition between these cultivars, including arginine and histidine, two essential amino acids for insect growth and development, found only in the phloem sap of L 97-128. A novel method was developed to collect sufficient amounts of honeydew excreted by *M. sacchari* while feeding on L 97-128 and HoCP 91-555 sugarcane plants. Significant shifts in free amino acid composition from that of phloem sap were observed in the honeydew. Two free essential amino acids (leucine, isoleucine) and two free nonessential amino acids (proline, tyrosine) were absent in the honeydew of *M. sacchari* feeding on HoCP 91-555. These results suggest that absence of arginine and histidine in the phloem sap of HoCP 91-555 and aphid inability to derive leucine, isoleucine, tyrosine, and proline are the underlying causal factors for shorter duration of ingestion from sieve elements and reduced aphid performance on this cultivar.

This work has provided the basis for a potential role of resistant cultivars in an IPM program for aphids of sugarcane. Information on timing of *M. sacchari* infestations can be helpful in making judicious management decisions. Discovery of *D. terminatus* at peak population times of *M. sacchari* asserts the need for integration of a biological control

component in developing comprehensive management strategies for Louisiana sugarcane insect pests. In addition, feeding behavior and amino acid studies have added to our understanding of the underlying mechanisms of aphid-sugarcane interactions.

Future studies involving the use of chemically defined diets lacking individual amino acids may better reveal cause and effect relationships between particular amino acid and *M. sacchari* behavior and performance. Studies may also include the use of aposymbiotic *M. sacchari* *i.e.*, aphids deprived of symbiotic bacteria, to determine their role in upgrading the nutritional status of aphid diet. The tri-trophic interactions among sugarcane cultivar, *M. sacchari*, and coccinellid beetles or fire ants may also be investigated. Studying inter-specific competition between *M. sacchari* and *S. flava* can help understand reasons for the prevalence of *M. sacchari* in Louisiana sugarcane.

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APPENDIX A: SAS CODES FOR CHAPTER 3

```
dm'log;clear;output;clear';
```

```
Title Chapter 3 Number of sugarcane aphid found on different cultivars after 24 hours of realesae-Ghouse data 2005;
```

```
options nodate nonumber ps=55 ls=78;
```

```
data SA;
```

```
input variety$ day rep number;
```

```
cards;
```

```
128 1 1 9  
384 1 1 12  
540 1 1 8  
555 1 1 10  
988 1 1 8  
128 1 2 10  
384 1 2 7  
540 1 2 7  
555 1 2 12  
988 1 2 11  
128 1 3 13  
384 1 3 10  
540 1 3 10  
555 1 3 8  
988 1 3 7  
128 2 4 11  
384 2 4 10  
540 2 4 7  
555 2 4 7  
988 2 4 11  
128 2 5 8  
384 2 5 8  
540 2 5 9  
555 2 5 7  
988 2 5 12  
128 3 6 10  
384 3 6 7  
540 3 6 10  
555 3 6 10  
988 3 6 8  
128 3 7 10  
384 3 7 10  
540 3 7 9  
555 3 7 9  
988 3 7 8
```

```
;
```

```
run;
```

```
Proc sort;
```

```
by variety;
```

```

run;
Proc means mean n stderr clm;
var number;
by variety;
run;
proc mixed data=SA;
class DAY REP variety;
model number = variety variety*day / htype=3;
random day;
random rep;
lsmeans variety variety*day / diff cl adjust=tukey;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'c:\Documents and Settings\wakbar\Desktop\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;

```

```
dm'log;clear;output;clear';
```

Title Chapter 3 Number of yellow sugarcane aphid found on different cultivars after 24 hours of realesae-Ghouse data 2007;

```
options nodate nonumber ps=55 ls=78;
```

```
data YSA;
```

```
input variety$ day rep number;
```

```
cards;
```

128	1	1	20
128	1	2	22
128	1	3	25
384	1	1	15
384	1	2	13
384	1	3	10
555	1	1	12
555	1	2	15
555	1	3	13
128	2	4	20
128	2	5	12
384	2	4	14
384	2	5	18
555	2	4	15
555	2	5	17
128	3	6	14
128	3	7	13
384	3	6	20
384	3	7	17
555	3	6	15
555	3	7	18

```
;
```



```

run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var number;
by variety;
run;
proc mixed data=YSA;
class DAY REP variety;
model number = variety variety*day / htype=3;
random day;
random rep;
lsmeans variety variety*day / diff cl adjust=tukey;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'c:\Documents and Settings\wakbar\Desktop\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
quit;

```

```
dm'log;clear;output;clear';
```

Title Chapter 3 Sugarcane Aphid Life History Parameters-Ghouse data 2005;

```
options nodate nonumber ps=55 ls=78;
```

```
data SA;
```

```
input variety$ rep$ dm dr ddays longevity totny nyperday;
```

```
/* dm= prereproductive days, dr= reproductive days, ddays= dull days, totny= fecundity,
nyperday= fecundity per day*/
```

```
cards;
```

128	1	9	23	3	35	20	0.869565217
128	2	5	25	2	32	24	0.96
128	3	6	16	2	24	15	0.9375
128	4	11	19	4	34	14	0.736842105
128	5	7	23	3	33	25	1.086956522
384	1	9	7	2	18	7	1
384	2	7	18	3	28	11	0.611111111
384	3	9	12	4	25	19	1.583333333
384	4	8	19	2	29	20	1.052631579
384	5	7	16	2	25	22	1.375
540	1	11	9	3	23	8	0.888888889
540	2	9	13	4	26	12	0.923076923
540	3	10	18	3	31	15	0.833333333
540	4	13	17	3	33	13	0.764705882
540	5	9	16	4	29	11	0.6875
555	1	11	8	4	23	2	0.25
555	2	7	13	5	25	3	0.230769231
555	3	11	12	2	25	5	0.416666667

555	4	7	10	3	20	5	0.5
555	5	15	10	2	27	2	0.2
988	1	7	15	4	26	4	0.266666667
988	2	11	16	4	31	7	0.4375
988	3	9	19	3	31	25	1.315789474
988	4	11	16	2	29	17	1.0625
988	5	11	12	3	26	6	0.5

```

;
run;
Proc sort data=SA;
by variety;
run;
Proc means data= SA mean n stderr std var clm alpha=0.01;
var dm dr ddays longevity totny nyperday;
by variety;
run;
proc glm data=SA order=data;
class variety;
model dm = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=SA order=data;
class variety;
model dr = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=SA order=data;
class variety;
model ddays = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=SA order=data;
class variety;
model longevity = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=SA order=data;
class variety;
model totny = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=SA order=data;
class variety;
model nyperday = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
quit;

```

```

dm'log;clear;output;clear';
Title Chapter 3 Sugarcane Aphid Demographic Statistics-Ghouse data 2005;
options nodate nonumber ps=55 ls=78;
data SA;
input variety$ rep rm lambda T DT;
cards;
128 1 0.153462786 1.1658644 13.5501355 4.516711834
128 2 0.202693967 1.224697613 10.8401084 3.419673461
128 3 0.127885655 1.136423051 16.2601626 5.420054201
128 4 0.146924276 1.158266252 12.19512195 4.717717161
128 5 0.162302026 1.176215435 16.2601626 4.270724147
384 1 0.119673474 1.127128755 16.2601626 5.791986782
384 2 0.106206737 1.112051755 21.6802168 6.52639557
384 3 0.144866398 1.155885131 20.32520325 4.784734012
384 4 0.146155034 1.157375607 18.9701897 4.742547425
384 5 0.147390028 1.158805841 20.32520325 4.702809211
540 1 0.109616276 1.115849809 18.9701897 6.323396567
540 2 0.135129312 1.144684796 16.2601626 5.129510192
540 3 0.166714646 1.181417095 14.90514905 4.157686182
540 4 0.115825124 1.122799504 18.9701897 5.984428557
540 5 0.115825124 1.122799504 18.9701897 5.984428557
555 1 0.063942827 1.066031449 10.8401084 10.8401084
555 2 0.057912562 1.05962234 18.9701897 11.96885711
555 3 0.081077587 1.084455033 13.5501355 8.549183653
555 4 0.056838069 1.058484395 12.19512195 12.19512195
555 5 0.026923296 1.027289002 25.74525745 25.74525745
988 1 0.073077517 1.075813928 18.9701897 9.485094851
988 2 0.102577264 1.09905566 18.9701897 7.33867114
988 3 0.138178151 1.148180082 21.6802168 5.016329825
988 4 0.146155034 1.157375607 18.9701897 4.742547425
988 5 0.118776518 1.126118223 13.5501355 5.835725719
;
run;
Proc sort data=SA;
by variety;
run;
Proc means data= SA mean n stderr std var clm alpha=0.01;
var rm lambda T DT;
by variety;
run;
proc glm data=SA order=data;
class variety;
model rm = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=SA order=data;
class variety;
model lambda = variety / ss3;

```

```

means variety /alpha=0.05 tukey lsd;
run;
proc glm data=SA order=data;
class variety;
model T = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=SA order=data;
class variety;
model DT = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;

dm'log;clear;output;clear';
Title Chapter 3 Yellow Sugarcane Aphid Life History Parameters-Ghouse data 2007;
options nodate nonumber ps=55 ls=78;
data YSA;
input variety$ rep dm dr ddays longevity totny nyperday;
/* dm= prereproductive days, dr= reproductive days, ddays= dull days, totny= fecundity,
nyperday= fecundity per day*/
cards;
128 1 9 17 2 28 19 1.117647059
128 2 11 12 4 27 17 1.416666667
128 3 11 16 5 32 21 1.3125
128 4 7 26 2 35 27 1.038461538
128 5 11 28 3 42 31 1.107142857
128 7 11 14 5 30 15 1.071428571
128 9 9 18 2 29 14 0.777777778
384 1 7 16 4 27 15 0.9375
384 3 12 22 3 37 19 0.863636364
384 4 15 16 3 34 10 0.625
384 5 14 15 2 31 7 0.466666667
384 6 9 14 2 25 16 1.142857143
384 8 12 18 3 33 15 0.833333333
384 9 15 15 2 32 12 0.8
555 1 7 10 3 20 4 0.4
555 3 13 13 4 30 8 0.615384615
555 4 8 12 4 24 7 0.583333333
555 6 12 14 3 29 9 0.642857143
555 8 9 10 2 21 4 0.4
555 9 17 11 3 31 5 0.454545455
555 10 13 14 3 30 8 0.571428571
;
run;
Proc sort data=YSA;
by variety;
run;
Proc means data=YSA mean n stderr std var clm alpha=0.01;

```

```

var dm dr ddays longevity totny nyperday;
by variety;
run;
proc glm data=YSA order=data;
class variety;
model dm = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=YSA order=data;
class variety;
model dr = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=YSA order=data;
class variety;
model ddays = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=YSA order=data;
class variety;
model longevity = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=YSA order=data;
class variety;
model totny = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=YSA order=data;
class variety;
model nyperday = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
quit;

```

```
dm'log;clear;output;clear';
```

Title Chapter 3 Yellow Sugarcane Aphid Demographic Statistics-Ghouse data 2007;

```
options nodate nonumber ps=55 ls=78;
```

```
data YSA;
```

```
input variety$ rep rm lambda T DT;
```

```
cards;
```

128	1	0.194762431	1.2150223	13.5501355	3.558936789
128	2	0.204617048	1.227055071	13.5501355	3.387533875
128	3	0.140428597	1.150766909	21.6802168	4.935940351
128	4	0.209091145	1.232557335	13.5501355	3.315047996
128	5	0.276356302	1.3183175	10.8401084	2.508164912
128	7	0.176964671	1.193588924	13.5501355	3.916867565
128	9	0.179510211	1.196631123	10.8401084	3.861324521

384	1	0.212413475	1.236659107	10.8401084	3.263197785
384	3	0.153733927	1.166180556	17.61517615	4.508745694
384	4	0.127885655	1.136423051	16.2601626	5.420054201
384	5	0.091366552	1.095670552	17.61517615	7.586443435
384	6	0.153733927	1.166180556	17.61517615	4.508745694
384	8	0.176964671	1.193588924	13.5501355	3.916867565
384	9	0.153462786	1.1658644	13.5501355	4.516711834
555	1	0.101346984	1.106660568	10.8401084	6.839346922
555	3	0.127885655	1.136423051	16.2601626	5.420054201
555	4	0.132231849	1.141372915	13.5501355	5.241907957
555	6	0.110467822	1.116800412	17.61517615	6.274652347
555	8	0.101346984	1.106660568	10.8401084	6.839346922
555	9	0.107978653	1.114023964	14.90514905	6.419298291
555	10	0.102308524	1.107725179	20.32520325	6.775067751

```

;
run;
Proc sort data=YSA;
by variety;
run;
Proc means data= YSA mean n stderr std var clm alpha=0.01;
var rm lambda T DT;
by variety;
run;
proc glm data=YSA order=data;
class variety;
model rm = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=YSA order=data;
class variety;
model lambda = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=YSA order=data;
class variety;
model T = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=YSA order=data;
class variety;
model DT = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
QUIT;

```

```
dm'log;clear;output;clear';
```

```

Title 'Effect of aphid feeding on chlorophyll contents tolerance test feb. 2006';
options nodate nonumber ps=55 ls=78;

```

```

data ChlorophyllContents;
input variety$ rep spad spadarcisin;
cards;
128 3 27.38693467 31.55557007
128 6 19.1318328 25.93807675
128 7 40.72948328 39.65747164
128 9 19.52380952 26.22246037
128 1 40.86021505 39.73367805
384 1 8.403361345 16.85110227
384 4 21.63461538 27.71865224
384 8 63.92694064 53.08650792
384 12 33.7398374 35.5110553
384 13 14.9321267 22.7319923
540 1 15.04178273 22.82000115
540 4 7.039337474 15.38581943
540 5 4.545454545 12.30998866
540 6 12.90322581 21.05172444
540 7 46.45892351 42.96941274
555 4 8.529411765 16.98082032
555 5 16.66666667 24.09484255
555 6 35.98014888 36.85804917
555 9 13.39285714 21.46683029
555 11 63.1443299 52.62070592
988 2 8.14479638 16.58221576
988 3 27.27272727 31.48215411
988 4 20.14563107 26.66920996
988 1 11.74377224 20.04096287
988 5 34.24657534 35.81752564
;
run;
Proc sort data=ChlorophyllContents;
by variety;
run;
Proc means data=ChlorophyllContents mean n stderr std var clm alpha=0.01;
var spad;
by variety;
run;
proc glm data=ChlorophyllContents order=data;
class variety;
model spadarcisin = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;

dm'log;clear;output;clear';
Title 'Effect of yellow aphid feeding on chlorophyll contents tolerance test 2007';
options nodate nonumber ps=55 ls=78;
data ChlorophyllContents;
input variety$ rep spad spadarcisin;

```

```

cards;
128 2 49.78723404 44.87809372
128 3 2.864259029 9.743704881
128 4 18.32460733 25.3452988
128 5 59.13312693 50.26244186
128 7 66.07515658 54.37692614
128 9 25.83941606 30.55231361
128 10 19.92512479 26.51138803
384 1 18.15087918 25.21641393
384 4 38.10289389 38.11744274
384 5 53.18390219 46.82547668
384 6 44.25373134 41.70033965
384 7 20.92020129 27.21856329
384 8 65.60350219 54.09201265
384 9 64.51612903 53.43863472
555 1 32.80287474 34.94137214
555 2 32.70117888 34.8792942
555 3 27.64116576 31.71865662
555 5 8.597046414 17.05006453
555 6 8.90052356 17.35777401
555 7 22.41555783 28.258524
555 8 35.82860093 36.76754782
;
run;
Proc sort data=ChlorophyllContents;
by variety;
run;
Proc means data=ChlorophyllContents mean n stderr std var clm alpha=0.01;
var spad;
by variety;
run;
proc glm data=ChlorophyllContents order=data;
class variety;
model spadarcsin = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;

```

```

dm'log;clear;output;clear';
Title 'Effect of yellow aphid feeding on chlorophyll contents after one week of removal tolerance test 2007';
options nodate nonumber ps=55 ls=78;
data ChlorophyllContents;
input variety$ rep spad spadarcsin;
cards;
128 2 22.94429708 28.62024506
128 3 59.6397087 50.55794705
128 4 9.555125725 18.00582389
128 5 1.394101877 6.780850525

```



```

128 6 64.58333333 53.47888165
128 9 1.232114467 6.373001956
384 1 32.9476584 35.02966722
384 4 60.19955654 50.88522359
384 5 41.8297456 40.29770234
384 7 50.84745763 45.4855807
384 8 50.94823168 45.5433293
384 9 27.81753131 31.83152078
555 2 38.51540616 38.36053404
555 3 30.40629096 33.46441661
555 5 14.97844828 22.7692019
555 8 24.54500738 29.69805503
555 9 25.93344156 30.61381055
555 10 27.49754661 31.62658394
;
run;
Proc sort data=ChlorophyllContents;
by variety;
run;
Proc means data=ChlorophyllContents mean n stderr std var clm alpha=0.01;
var spad;
by variety;
run;
proc glm data=ChlorophyllContents order=data;
class variety;
model spadarcsin = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;

dm'log;clear;output;clear';
Title 'Effect of yellow aphid feeding on leaf discoloration tolerance test 2007';
options nodate nonumber ps=55 ls=78;
data leafcolor;
input variety$ rep percentcolor rank percentcolor2 rank2;
cards;
128 2 40 2 20 1
128 4 5 1 2 1
128 5 30 2 20 1
128 6 80 4 40 2
128 9 5 1 0 0
128 12 5 1 5 1
128 13 100 5 100 5
384 1 50 3 50 3
384 4 90 5 90 5
384 5 50 3 50 3
384 6 100 5 100 5
384 7 95 5 95 5
384 8 90 5 90 5

```

```

384  9   25  2   25  2
555  2   40  2   40  2
555  3   25  2   25  2
555  4   60  3   60  3
555  5   25  2   25  2
555  7   30  2   30  2
555  8   60  3   60  3
555  9    5  1    5  1
;
Proc sort data=leafcolor;
by variety;
run;
Proc means data=leafcolor mean n stderr std var clm alpha=0.01;
var percentcolor;
by variety;
run;
Proc means data=leafcolor mean n stderr std var clm alpha=0.01;
var rank;
by variety;
run;
Proc means data=leafcolor mean n stderr std var clm alpha=0.01;
var percentcolor2;
by variety;
run;
Proc means data=leafcolor mean n stderr std var clm alpha=0.01;
var rank2;
by variety;
run;
proc glm data=leafcolor order=data;
class variety;
model percentcolor = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=leafcolor order=data;
class variety;
model rank = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=leafcolor order=data;
class variety;
model percentcolor2 = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=leafcolor order=data;
class variety;
model rank2 = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;

```

```

dm'log;clear;output;clear';
Title 'Effect of yellow aphid feeding on chlorophyll contents of 128 readings taken at removal and one wk after removal tolerance test 2007';
options nodate nonumber ps=55 ls=78;
data ChlorophyllContents;
input variety$ rep spad spadarsin;
cards;
1280 2      49.78723      44.87809
1280 3      2.864259      9.743705
1280 4      18.32461      25.3453
1280 5      59.13313      50.26244
1280 7      66.07516      54.37693
1280 9      25.83942      30.55231
1281 2      22.9443      28.62025
1281 3      59.63971      50.55795
1281 4      9.555126      18.00582
1281 5      1.394102      6.780851
1281 6      64.58333      53.47888
1281 9      1.232114      6.373002
;
Proc sort data=ChlorophyllContents;
by variety;
run;
Proc means data=ChlorophyllContents mean n stderr std var clm alpha=0.01;
var spad;
by variety;
run;
proc glm data=ChlorophyllContents order=data;
class variety;
model spadarsin = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;

```

```

dm'log;clear;output;clear';
Title 'Effect of yellow aphid feeding on 128 leaf discoloration tolerance test 2007';
options nodate nonumber ps=55 ls=78;
data leafcolor;
input variety$ rep percentcolor rank;
cards;
1280 2      40      2
1280 4      5      1
1280 5      30      2
1280 6      80      4
1280 9      5      1
1280 12     5      1
1280 13     100     5
1281 2      20      1

```

```

1281  4    2    1
1281  5   20    1
1281  6   40    2
1281  9    0    0
1281 12    5    1
1281 13  100    5
;
Proc sort data=leafcolor;
by variety;
run;
Proc means data=leafcolor mean n stderr std var clm alpha=0.01;
var percentcolor;
by variety;
run;
Proc means data=leafcolor mean n stderr std var clm alpha=0.01;
var rank;
by variety;
run;
proc glm data=leafcolor order=data;
class variety;
model percentcolor = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;
proc glm data=leafcolor order=data;
class variety;
model rank = variety / ss3;
means variety /alpha=0.05 tukey lsd;
run;

```

APPENDIX B: SAS CODES FOR CHAPTER 4

```

dm'log;clear;output;clear';
Title 'Effect of sugarcane cultivars on total and proportional time in pathway phase, xylem
phase, and SE1, SE2, SE. 1 represents values with 0 and 2 values without 0 readings ';
options nodate nonumber ps=55 ls=78;
data EPG;
input variety$ read$ aphid$ pwtime xyltime1 xyltime2 SE1time1 SE1time2 SE2time1 SE2time2
SEtime1 SEtime2 Proptimeinpw Proptimeinxyl ProptimeinSE;
cards;
128 1 3 4743 989 989 0 . 0 . 0 . 0.827
0.173 0
128 2 2 10415 971 971 20.142 20.142 4045 4045 4066 4066 0.674
0.062 0.263
128 2 3 10762 3446 3446 11.888 11.888 874 874 886 886 0.713
0.228 0.059
128 3 1 9260 2585 2585 7.875 7.875 2546 2546 2554 2554 0.643
0.18 0.177
128 3 2 13537 0 . 15.108 15.108 1344 1344 1358 1358 0.909
0 0.091
128 3 3 14977 0 . 7.604 7.604 332 332 340 340 0.978
0 0.022
128 3 4 11066 0 . 6.325 6.325 3305 3305 3311 3311 0.77
0 0.23
128 4 2 2928 0 . 10.045 10.045 13061 13061 13072 13072 0.183
0 0.817
128 4 3 8186 0 . 13.24 13.24 2762 2762 2776 2776 0.747
0 0.253
128 5 1 3322 0 . 8.75 8.75 7821 7821 7828 7828 0.298
0 0.702
128 5 2 9502 0 . 8.75 8.75 1710 1710 1719 1719 0.847
0 0.153
128 5 4 3857 2241 2241 9.167 9.167 2291 2291 2300 2300 0.459
0.267 0.274
128 6 1 6981 1447 1447 18.842 18.842 5072 5072 5089 5089 0.516
0.107 0.3716
128 6 2 8515 0 . 5.835 5.835 4964 4964 4969 4969 0.631
0 0.369
128 6 3 8263 0 . 10.688 10.688 3393 3393 3405 3405 0.706
0 0.29
128 6 4 4587 8757 8757 0 . 0 . 0 . 0.344
0.656 0
128 7 1 5420 0 . 17.162 17.162 1877 1877 1895 1895 0.741
0 0.259
128 7 4 1509 472 472 4.375 4.375 5205 5205 5209 5209 0.21
0.066 0.724
128 8 2 3378 0 . 7.125 7.125 12189 12189 12196 12196 0.217
0 0.783

```

128	9	1	300	0	.	8	8	10449	10449	10457	10457	0.028
	0	0.972										
128	9	2	6726	3514	3514	0	.	0	.	0	.	0.657
	0.343	0										
128	9	3	2767	3648	3648	8.938	8.938	9738	9738	9747	9747	0.247
	0.326	0.425										
128	9	4	10986	1477	1477	0	.	0	.	0	.	0.881
	0.119	0										
128	10	2	5523	1592	1592	0	.	0	.	0	.	0.776
	0.224	0										
128	10	3	7730	739	739	0	.	0	.	0	.	0.913
	0.087	0										
128	10	4	6117	407	407	14.875	14.875	4046	4046	4061	4061	0.429
	0.286	0.285										
128	10	1	2441	882	882	0	.	0	.	0	.	0.735
	0.265	0										
128	11	1	7002	0	.	7	7	8848	8848	8885	8885	0.442
	0	0.558										
128	11	2	10876	2024	2024	4	4	1961	1961	1965	1965	0.732
	0.136	0.132										
128	11	4	13002	267	267	6	6	984	984	990	990	0.912
	0.019	0.069										
555	1	1	8574	0	.	5.625	5.625	4560	4560	4566	4566	0.653
	0	0.347										
555	1	4	11033	4458	4458	0	.	0	.	0	.	0.712
	0.288	0										
555	2	1	2894	0	.	7	7	319	319	326	326	0.899
	0	0.101										
555	2	2	9446	2253	2253	0	.	0	.	0	.	0.807
	0.193	0										
555	2	3	6452	0	.	7.563	7.563	8581	8581	8588	8588	0.429
	0	0.571										
555	3	1	14478	0	.	6.437	6.437	749	749	755	755	0.95
	0	0.05										
555	3	3	10831	0	.	33.689	33.689	941	941	974.68	974.68	0.74
	0	0.26										
555	4	1	1885	12989	12989	0	.	0	.	0	.	0.127
	0.873	0										
555	4	2	1456	0	.	0	.	0	.	0	.	1
	0	0										
555	4	3	8306	1359	1359	23.75	23.75	6045	6045	6069	6069	0.528
	0.086	0.386										
555	5	2	5804	6487	6487	7	7	1125	1125	1132	1132	0.432
	0.483	0.084										
555	5	4	12994	2083	2083	5	5	441	441	446	446	0.837
	0.134	0.029										
555	6	1	11744	0	.	8	8	121	121	129	129	0.989
	0	0.011										

555	6	2	11820	0	.	21.751	21.751	3821	3821	3843	3843	0.755
	0	0.245										
555	7	1	9703	0	.	6.625	6.625	3800	3800	3807	3807	0.718
	0	0.282										
555	8	2	725	14516	14516	0	.	0	.	0	.	0.048
	0.952	0										
555	8	3	634	7906	7906	0	.	0	.	0	.	0.074
	0.926	0										
555	8	4	12664	2161	2161	7	7	189	189	196	196	0.843
	0.144	0.013										
555	9	3	4166	3282	3282	8	8	129	129	137	137	0.549
	0.433	0.018										
555	9	4	4663	0	.	9.2	9.2	430	430	439	439	0.914
	0	0.086										
555	11	2	10323	3532	3532	14.313	14.313	1298	1298	1312	1312	0.679
	0.233	0.087										
555	11	3	11915	0	.	16.148	16.148	3095	3095	3113	3113	0.793
	0	0.207										
555	11	4	10279	1606	1606	29.188	29.188	4030	4030	4059	4059	0.645
	0.101	0.257										
555	12	2	13572	0	.	25.626	25.626	647	647	671	671	0.953
	0	0.047										
555	12	4	11896	2641	2641	0	.	0	.	0	.	0.818
	0.182	0										
555	13	2	12029	0	.	16.603	16.603	1517	1517	1532	1532	0.887
	0	0.113										
555	13	3	12379	0	.	8.5	8.5	144	144	152	152	0.988
	0	0.012										
555	14	4	6516	0	.	8.125	8.125	9451	9451	9459	9459	0.407
	0	0.592										
555	6.928571429	2.535714286	8542.178571	2331.178571	5021	9.826535714						
	13.10204762	1836.892857	2449.190476	1846.631429	2462.175238	0.684785714						
	0.179571429	0.135642857										
384	1	2	4995	3613	3613	0	.	0	.	0	.	0.58
	0.42	0										
384	1	4	13119	0	.	8.625	8.625	296	296	305	305	0.977
	0	0.023										
384	2	3	11858	0	.	12.437	12.437	487	487	500	500	0.96
	0	0.04										
384	3	1	8256	0	.	6.812	6.812	144	144	151	151	0.982
	0	0.018										
384	3	2	8572	0	.	8.437	8.437	3607	3607	3616	3616	0.703
	0	0.297										
384	3	4	4226	0	.	5.25	5.25	6440	6440	6445	6445	0.394
	0	0.604										
384	4	1	3167	993	993	5.687	5.687	6230	6230	6236	6236	0.305
	0.096	0.6										

384	4	3	2465	0	.	7	7	1194	1194	1201	1201	0.672
	0	0.328										
384	4	4	1315	1553	1553	0	.	0	.	0	.	0.459
	0.541	0										
384	5	1	3555	0	.	9.937	9.937	10443	10443	10453	10453	0.254
	0	0.746										
384	5	2	12288	0	.	7.125	7.125	1848	1848	1855	1855	0.869
	0	0.131										
384	5	4	5963	0	.	18	18	5163	5163	5181	5181	0.535
	0	0.465										
384	6	2	2608	0	.	30	30	828	828	858	858	0.752
	0	0.248										
384	6	4	12579	0	.	40.617	40.617	2954	2954	2994	2994	0.807
	0	0.192										
384	7	1	7705	0	.	10.187	10.187	2404	2404	2414	2414	0.761
	0	0.238										
384	7	2	6195	0	.	19.437	19.437	6022	6022	6041	6041	0.506
	0	0.494										
384	7	4	10938	0	.	21.875	21.875	2039	2039	2060	2060	0.835
	0	0.157										
384	8	1	9697	0	.	4.625	4.625	3230	3230	3234	3234	0.749
	0	0.25										
384	8	2	9587	0	.	11.438	11.438	1341	1341	1353	1353	0.879
	0	0.124										
384	8	4	11256	0	.	12.75	12.75	803	803	816	816	0.932
	0	0.068										
384	9	1	3508	0	.	36.044	36.044	10855	10855	10891	10891	0.244
	0	0.756										
384	9	3	8807	990	990	0	.	0	.	0	.	0.899
	0.101	0										
384	10	3	7026	0	.	0	.	0	.	0	.	1
	0	0										
384	10	1	6197	0	.	5.021	5.021	5099	5099	5104	5104	0.548
	0	0.452										
384	11	1	6983	0	.	8.438	8.438	420	420	429	429	0.942
	0	0.059										
384	11	4	10428	0	.	6.312	6.312	710	710	716	716	0.936
	0	0.064										
384	12	1	11644	0	.	12.562	12.562	1836	1836	1848	1848	0.863
	0	0.137										
384	13	2	11933	0	.	10.831	10.831	1580	1580	1591	1591	0.882
	0	0.118										
384	13	1	2888	1487	1487	0	.	0	.	0	.	0.66
	0.34	0										
384	13	3	8684	5156	5156	0	.	0	.	0	.	0.627
	0.373	0										
384	14	2	9630	0	.	28.212	28.212	3578	3578	3608	3608	0.727
	0	0.273										

384	14	3	5553	7732	7732	4.687	4.687	350	350	355	355	0.407
			0.567	0.026								
384	7.375	2.375	7613.28125	672.625		3074.857143	11.0108125	13.55176923				
			2496.90625	3073.115385	2507.96875	3086.730769	0.7076875	0.0761875				
			0.215875									

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var pwtime xyltime1 xyltime2 SE1time1 SE1time2 SE2time1 SE2time2 SEtime1 SEtime2
Proptimeinpw Proptimeinxyl ProptimeinSE;
by variety;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var pwtime;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var xyltime1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var xyltime2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE1time1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE1time2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE2time1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE2time2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SEtime1;
run;
proc npar1way data = EPG wilcoxon;

```

```

class variety;
var Setime2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var Proptimeinpw;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var Proptimeinxyl;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var ProptimeinSE;
run;
dm'log;clear;output;clear';

```

Title 'Effect of sugarcane cultivars on total and proportional time.1 represents values with 0 and 2 values without 0 readings;'

```
options nodate nonumber ps=55 ls=78;
```

```
data EPG;
```

```
input variety$ read$ aphid$ pwtime xyltime1 xyltime2 SE1time1 SE1time2 SE2time1 SE2time2
SEtime1 SEtime2 Proptimeinpw Proptimeinxyl ProptimeinSE;
```

```
cards;
```

555	1	1	8574	0	.	5.625	5.625	4560	4560	4566	4566	0.653
		0										0.347
555	1	4	11033	4458	4458	0	.	0	.	0	.	0.712
		0.288										0
555	2	1	2894	0	.	7	7	319	319	326	326	0.899
		0										0.101
555	2	2	9446	2253	2253	0	.	0	.	0	.	0.807
		0.193										0
555	2	3	6452	0	.	7.563	7.563	8581	8581	8588	8588	0.429
		0										0.571
555	3	1	14478	0	.	6.437	6.437	749	749	755	755	0.95
		0										0.05
555	3	3	10831	0	.	33.689	33.689	941	941	974.68	974.68	0.74
		0										0.26
555	4	1	1885	12989	12989	0	.	0	.	0	.	0.127
		0.873										0
555	4	2	1456	0	.	0	.	0	.	0	.	1
		0										0
555	4	3	8306	1359	1359	23.75	23.75	6045	6045	6069	6069	0.528
		0.086										0.386
555	5	2	5804	6487	6487	7	7	1125	1125	1132	1132	0.432
		0.483										0.084
555	5	4	12994	2083	2083	5	5	441	441	446	446	0.837
		0.134										0.029

555	6	1	11744	0	.	8	8	121	121	129	129	0.989
	0	0.011										
555	6	2	11820	0	.	21.751	21.751	3821	3821	3843	3843	0.755
	0	0.245										
555	7	1	9703	0	.	6.625	6.625	3800	3800	3807	3807	0.718
	0	0.282										
555	8	2	725	14516	14516	0	.	0	.	0	.	0.048
	0.952	0										
555	8	3	634	7906	7906	0	.	0	.	0	.	0.074
	0.926	0										
555	8	4	12664	2161	2161	7	7	189	189	196	196	0.843
	0.144	0.013										
555	9	3	4166	3282	3282	8	8	129	129	137	137	0.549
	0.433	0.018										
555	9	4	4663	0	.	9.2	9.2	430	430	439	439	0.914
	0	0.086										
555	11	2	10323	3532	3532	14.313	14.313	1298	1298	1312	1312	0.679
	0.233	0.087										
555	11	3	11915	0	.	16.148	16.148	3095	3095	3113	3113	0.793
	0	0.207										
555	11	4	10279	1606	1606	29.188	29.188	4030	4030	4059	4059	0.645
	0.101	0.257										
555	12	2	13572	0	.	25.626	25.626	647	647	671	671	0.953
	0	0.047										
555	12	4	11896	2641	2641	0	.	0	.	0	.	0.818
	0.182	0										
555	13	2	12029	0	.	16.603	16.603	1517	1517	1532	1532	0.887
	0	0.113										
555	13	3	12379	0	.	8.5	8.5	144	144	152	152	0.988
	0	0.012										
555	14	4	6516	0	.	8.125	8.125	9451	9451	9459	9459	0.407
	0	0.592										
384	1	2	4995	3613	3613	0	.	0	.	0	.	0.58
	0.42	0										
384	1	4	13119	0	.	8.625	8.625	296	296	305	305	0.977
	0	0.023										
384	2	3	11858	0	.	12.437	12.437	487	487	500	500	0.96
	0	0.04										
384	3	1	8256	0	.	6.812	6.812	144	144	151	151	0.982
	0	0.018										
384	3	2	8572	0	.	8.437	8.437	3607	3607	3616	3616	0.703
	0	0.297										
384	3	4	4226	0	.	5.25	5.25	6440	6440	6445	6445	0.394
	0	0.604										
384	4	1	3167	993	993	5.687	5.687	6230	6230	6236	6236	0.305
	0.096	0.6										
384	4	3	2465	0	.	7	7	1194	1194	1201	1201	0.672
	0	0.328										

384	4	4	1315	1553	1553	0	.	0	.	0	.	0.459	
	0.541	0											
384	5	1	3555	0			9.937	9.937	10443	10443	10453	10453	0.254
	0	0.746											
384	5	2	12288	0			7.125	7.125	1848	1848	1855	1855	0.869
	0	0.131											
384	5	4	5963	0			18	18	5163	5163	5181	5181	0.535
	0	0.465											
384	6	2	2608	0			30	30	828	828	858	858	0.752
	0	0.248											
384	6	4	12579	0			40.617	40.617	2954	2954	2994	2994	0.807
	0	0.192											
384	7	1	7705	0			10.187	10.187	2404	2404	2414	2414	0.761
	0	0.238											
384	7	2	6195	0			19.437	19.437	6022	6022	6041	6041	0.506
	0	0.494											
384	7	4	10938	0			21.875	21.875	2039	2039	2060	2060	0.835
	0	0.157											
384	8	1	9697	0			4.625	4.625	3230	3230	3234	3234	0.749
	0	0.25											
384	8	2	9587	0			11.438	11.438	1341	1341	1353	1353	0.879
	0	0.124											
384	8	4	11256	0			12.75	12.75	803	803	816	816	0.932
	0	0.068											
384	9	1	3508	0			36.044	36.044	10855	10855	10891	10891	0.244
	0	0.756											
384	9	3	8807	990	990	0	.	0	.	0	.	0	0.899
	0.101	0											
384	10	3	7026	0			0	.	0	.	0	.	1
	0	0											
384	10	1	6197	0			5.021	5.021	5099	5099	5104	5104	0.548
	0	0.452											
384	11	1	6983	0			8.438	8.438	420	420	429	429	0.942
	0	0.059											
384	11	4	10428	0			6.312	6.312	710	710	716	716	0.936
	0	0.064											
384	12	1	11644	0			12.562	12.562	1836	1836	1848	1848	0.863
	0	0.137											
384	13	2	11933	0			10.831	10.831	1580	1580	1591	1591	0.882
	0	0.118											
384	13	1	2888	1487	1487	0	.	0	.	0	.	0	0.66
	0.34	0											
384	13	3	8684	5156	5156	0	.	0	.	0	.	0	0.627
	0.373	0											
384	14	2	9630	0			28.212	28.212	3578	3578	3608	3608	0.727
	0	0.273											
384	14	3	5553	7732	7732	4.687	4.687	350	350	355	355	355	0.407
	0.567	0.026											

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var pwtime xyltime1 xyltime2 SE1time1 SE1time2 SE2time1 SE2time2 SEtime1 SEtime2
Proptimeinpw Proptimeinxyl ProptimeinSE;
by variety;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var pwtime;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var xyltime1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var xyltime2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE1time1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE1time2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE2time1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE2time2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SEtime1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SEtime2;
run;
proc npar1way data = EPG wilcoxon;
class variety;

```

```

var Proptimeinpw;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var Proptimeinxyl;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var ProptimeinSE;
run;

```

```
dm'log;clear;output;clear';
```

Title 'Effect of sugarcane cultivars on total and proportional time.1 represents values with 0 and 2 values without 0 readings;'

```
options nodate nonumber ps=55 ls=78;
```

```
data EPG;
```

```
input variety$ read$ aphid$ pwtime xyltime1 xyltime2 SE1time1 SE1time2 SE2time1 SE2time2
SEtime1 SEtime2 Proptimeinpw Proptimeinxyl ProptimeinSE;
```

```
cards;
```

128	1	3	4743	989	989	0	.	0	.	0	.	0.827
			0.173	0								
128	2	2	10415	971	971	20.142	20.142	4045	4045	4066	4066	0.674
			0.062	0.263								
128	2	3	10762	3446	3446	11.888	11.888	874	874	886	886	0.713
			0.228	0.059								
128	3	1	9260	2585	2585	7.875	7.875	2546	2546	2554	2554	0.643
			0.18	0.177								
128	3	2	13537	0	.	15.108	15.108	1344	1344	1358	1358	0.909
			0	0.091								
128	3	3	14977	0	.	7.604	7.604	332	332	340	340	0.978
			0	0.022								
128	3	4	11066	0	.	6.325	6.325	3305	3305	3311	3311	0.77
			0	0.23								
128	4	2	2928	0	.	10.045	10.045	13061	13061	13072	13072	0.183
			0	0.817								
128	4	3	8186	0	.	13.24	13.24	2762	2762	2776	2776	0.747
			0	0.253								
128	5	1	3322	0	.	8.75	8.75	7821	7821	7828	7828	0.298
			0	0.702								
128	5	2	9502	0	.	8.75	8.75	1710	1710	1719	1719	0.847
			0	0.153								
128	5	4	3857	2241	2241	9.167	9.167	2291	2291	2300	2300	0.459
			0.267	0.274								
128	6	1	6981	1447	1447	18.842	18.842	5072	5072	5089	5089	0.516
			0.107	0.3716								
128	6	2	8515	0	.	5.835	5.835	4964	4964	4969	4969	0.631
			0	0.369								

128	6	3	8263	0	.	10.688	10.688	3393	3393	3405	3405	0.706
	0	0.29										
128	6	4	4587	8757	8757	0	.	0	.	0	.	0.344
	0.656	0										
128	7	1	5420	0	.	17.162	17.162	1877	1877	1895	1895	0.741
	0	0.259										
128	7	4	1509	472	472	4.375	4.375	5205	5205	5209	5209	0.21
	0.066	0.724										
128	8	2	3378	0	.	7.125	7.125	12189	12189	12196	12196	0.217
	0	0.783										
128	9	1	300	0	.	8	8	10449	10449	10457	10457	0.028
	0	0.972										
128	9	2	6726	3514	3514	0	.	0	.	0	.	0.657
	0.343	0										
128	9	3	2767	3648	3648	8.938	8.938	9738	9738	9747	9747	0.247
	0.326	0.425										
128	9	4	10986	1477	1477	0	.	0	.	0	.	0.881
	0.119	0										
128	10	2	5523	1592	1592	0	.	0	.	0	.	0.776
	0.224	0										
128	10	3	7730	739	739	0	.	0	.	0	.	0.913
	0.087	0										
128	10	4	6117	407	407	14.875	14.875	4046	4046	4061	4061	0.429
	0.286	0.285										
128	10	1	2441	882	882	0	.	0	.	0	.	0.735
	0.265	0										
128	11	1	7002	0	.	7	7	8848	8848	8885	8885	0.442
	0	0.558										
128	11	2	10876	2024	2024	4	4	1961	1961	1965	1965	0.732
	0.136	0.132										
128	11	4	13002	267	267	6	6	984	984	990	990	0.912
	0.019	0.069										
555	1	1	8574	0	.	5.625	5.625	4560	4560	4566	4566	0.653
	0	0.347										
555	1	4	11033	4458	4458	0	.	0	.	0	.	0.712
	0.288	0										
555	2	1	2894	0	.	7	7	319	319	326	326	0.899
	0	0.101										
555	2	2	9446	2253	2253	0	.	0	.	0	.	0.807
	0.193	0										
555	2	3	6452	0	.	7.563	7.563	8581	8581	8588	8588	0.429
	0	0.571										
555	3	1	14478	0	.	6.437	6.437	749	749	755	755	0.95
	0	0.05										
555	3	3	10831	0	.	33.689	33.689	941	941	974.68	974.68	0.74
	0	0.26										
555	4	1	1885	12989	12989	0	.	0	.	0	.	0.127
	0.873	0										

```

555 4 2 1456 0 . 0 . 0 . 0 . 1
0 0
555 4 3 8306 1359 1359 23.75 23.75 6045 6045 6069 6069 0.528
0.086 0.386
555 5 2 5804 6487 6487 7 7 1125 1125 1132 1132 0.432
0.483 0.084
555 5 4 12994 2083 2083 5 5 441 441 446 446 0.837
0.134 0.029
555 6 1 11744 0 . 8 8 121 121 129 129 0.989
0 0.011
555 6 2 11820 0 . 21.751 21.751 3821 3821 3843 3843 0.755
0 0.245
555 7 1 9703 0 . 6.625 6.625 3800 3800 3807 3807 0.718
0 0.282
555 8 2 725 14516 14516 0 . 0 . 0 . 0.048
0.952 0
555 8 3 634 7906 7906 0 . 0 . 0 . 0.074
0.926 0
555 8 4 12664 2161 2161 7 7 189 189 196 196 0.843
0.144 0.013
555 9 3 4166 3282 3282 8 8 129 129 137 137 0.549
0.433 0.018
555 9 4 4663 0 . 9.2 9.2 430 430 439 439 0.914
0 0.086
555 11 2 10323 3532 3532 14.313 14.313 1298 1298 1312 1312 0.679
0.233 0.087
555 11 3 11915 0 . 16.148 16.148 3095 3095 3113 3113 0.793
0 0.207
555 11 4 10279 1606 1606 29.188 29.188 4030 4030 4059 4059 0.645
0.101 0.257
555 12 2 13572 0 . 25.626 25.626 647 647 671 671 0.953
0 0.047
555 12 4 11896 2641 2641 0 . 0 . 0 . 0.818
0.182 0
555 13 2 12029 0 . 16.603 16.603 1517 1517 1532 1532 0.887
0 0.113
555 13 3 12379 0 . 8.5 8.5 144 144 152 152 0.988
0 0.012
555 14 4 6516 0 . 8.125 8.125 9451 9451 9459 9459 0.407
0 0.592

```

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var pwtime xyltime1 xyltime2 SE1time1 SE1time2 SE2time1 SE2time2 SEtime1 SEtime2
Proptimeinpw Proptimeinxyl ProptimeinSE;

```



```

by variety;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var pwtime;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var xyltime1;
run;
roc npar1way data = EPG wilcoxon;
class variety;
var xyltime2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE1time1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE1time2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE2time1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE2time2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SEtime1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SEtime2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var Proptimeinpw;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var Proptimeinxyl;
run;
proc npar1way data = EPG wilcoxon;
class variety;

```

```
var ProptimeinSE;
run;
```

```
dm'log;clear;output;clear';
```

Title 'Effect of sugarcane cultivars on total and proportional time.1 represents values with 0 and 2 values without 0 readings;'

```
options nodate nonumber ps=55 ls=78;
```

```
data EPG;
```

```
input variety$ read$ aphid$ pwtime xyltime1 xyltime2 SE1time1 SE1time2 SE2time1 SE2time2
SEtime1 SEtime2 Proptimeinpw Proptimeinxyl ProptimeinSE;
```

```
cards;
```

128	1	3	4743	989	989	0	.	0	.	0	.	0.827
			0.173	0								
128	2	2	10415	971	971	20.142	20.142	4045	4045	4066	4066	0.674
			0.062	0.263								
128	2	3	10762	3446	3446	11.888	11.888	874	874	886	886	0.713
			0.228	0.059								
128	3	1	9260	2585	2585	7.875	7.875	2546	2546	2554	2554	0.643
			0.18	0.177								
128	3	2	13537	0	.	15.108	15.108	1344	1344	1358	1358	0.909
			0	0.091								
128	3	3	14977	0	.	7.604	7.604	332	332	340	340	0.978
			0	0.022								
128	3	4	11066	0	.	6.325	6.325	3305	3305	3311	3311	0.77
			0	0.23								
128	4	2	2928	0	.	10.045	10.045	13061	13061	13072	13072	0.183
			0	0.817								
128	4	3	8186	0	.	13.24	13.24	2762	2762	2776	2776	0.747
			0	0.253								
128	5	1	3322	0	.	8.75	8.75	7821	7821	7828	7828	0.298
			0	0.702								
128	5	2	9502	0	.	8.75	8.75	1710	1710	1719	1719	0.847
			0	0.153								
128	5	4	3857	2241	2241	9.167	9.167	2291	2291	2300	2300	0.459
			0.267	0.274								
128	6	1	6981	1447	1447	18.842	18.842	5072	5072	5089	5089	0.516
			0.107	0.3716								
128	6	2	8515	0	.	5.835	5.835	4964	4964	4969	4969	0.631
			0	0.369								
128	6	3	8263	0	.	10.688	10.688	3393	3393	3405	3405	0.706
			0	0.29								
128	6	4	4587	8757	8757	0	.	0	.	0	.	0.344
			0.656	0								
128	7	1	5420	0	.	17.162	17.162	1877	1877	1895	1895	0.741
			0	0.259								
128	7	4	1509	472	472	4.375	4.375	5205	5205	5209	5209	0.21
			0.066	0.724								

128	8	2	3378	0	.	7.125	7.125	12189	12189	12196	12196	0.217
	0	0.783										
128	9	1	300	0	.	8	8	10449	10449	10457	10457	0.028
	0	0.972										
128	9	2	6726	3514	3514	0	.	0	.	0	.	0.657
	0.343	0										
128	9	3	2767	3648	3648	8.938	8.938	9738	9738	9747	9747	0.247
	0.326	0.425										
128	9	4	10986	1477	1477	0	.	0	.	0	.	0.881
	0.119	0										
128	10	2	5523	1592	1592	0	.	0	.	0	.	0.776
	0.224	0										
128	10	3	7730	739	739	0	.	0	.	0	.	0.913
	0.087	0										
128	10	4	6117	407	407	14.875	14.875	4046	4046	4061	4061	0.429
	0.286	0.285										
128	10	1	2441	882	882	0	.	0	.	0	.	0.735
	0.265	0										
128	11	1	7002	0	.	7	7	8848	8848	8885	8885	0.442
	0	0.558										
128	11	2	10876	2024	2024	4	4	1961	1961	1965	1965	0.732
	0.136	0.132										
128	11	4	13002	267	267	6	6	984	984	990	990	0.912
	0.019	0.069										
384	1	2	4995	3613	3613	0	.	0	.	0	.	0.58
	0.42	0										
384	1	4	13119	0	.	8.625	8.625	296	296	305	305	0.977
	0	0.023										
384	2	3	11858	0	.	12.437	12.437	487	487	500	500	0.96
	0	0.04										
384	3	1	8256	0	.	6.812	6.812	144	144	151	151	0.982
	0	0.018										
384	3	2	8572	0	.	8.437	8.437	3607	3607	3616	3616	0.703
	0	0.297										
384	3	4	4226	0	.	5.25	5.25	6440	6440	6445	6445	0.394
	0	0.604										
384	4	1	3167	993	993	5.687	5.687	6230	6230	6236	6236	0.305
	0.096	0.6										
384	4	3	2465	0	.	7	7	1194	1194	1201	1201	0.672
	0	0.328										
384	4	4	1315	1553	1553	0	.	0	.	0	.	0.459
	0.541	0										
384	5	1	3555	0	.	9.937	9.937	10443	10443	10453	10453	0.254
	0	0.746										
384	5	2	12288	0	.	7.125	7.125	1848	1848	1855	1855	0.869
	0	0.131										
384	5	4	5963	0	.	18	18	5163	5163	5181	5181	0.535
	0	0.465										

```

384 6 2 2608 0 . 30 30 828 828 858 858 0.752
0 0.248
384 6 4 12579 0 . 40.617 40.617 2954 2954 2994 2994 0.807
0 0.192
384 7 1 7705 0 . 10.187 10.187 2404 2404 2414 2414 0.761
0 0.238
384 7 2 6195 0 . 19.437 19.437 6022 6022 6041 6041 0.506
0 0.494
384 7 4 10938 0 . 21.875 21.875 2039 2039 2060 2060 0.835
0 0.157
384 8 1 9697 0 . 4.625 4.625 3230 3230 3234 3234 0.749
0 0.25
384 8 2 9587 0 . 11.438 11.438 1341 1341 1353 1353 0.879
0 0.124
384 8 4 11256 0 . 12.75 12.75 803 803 816 816 0.932
0 0.068
384 9 1 3508 0 . 36.044 36.044 10855 10855 10891 10891 0.244
0 0.756
384 9 3 8807 990 990 0 . 0 . 0 . 0.899
0.101 0
384 10 3 7026 0 . 0 . 0 . 0 . 1
0 0
384 10 1 6197 0 . 5.021 5.021 5099 5099 5104 5104 0.548
0 0.452
384 11 1 6983 0 . 8.438 8.438 420 420 429 429 0.942
0 0.059
384 11 4 10428 0 . 6.312 6.312 710 710 716 716 0.936
0 0.064
384 12 1 11644 0 . 12.562 12.562 1836 1836 1848 1848 0.863
0 0.137
384 13 2 11933 0 . 10.831 10.831 1580 1580 1591 1591 0.882
0 0.118
384 13 1 2888 1487 1487 0 . 0 . 0 . 0.66
0.34 0
384 13 3 8684 5156 5156 0 . 0 . 0 . 0.627
0.373 0
384 14 2 9630 0 . 28.212 28.212 3578 3578 3608 3608 0.727
0 0.273
384 14 3 5553 7732 7732 4.687 4.687 350 350 355 355 0.407
0.567 0.026

```

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var pwtime xyltime1 xyltime2 SE1time1 SE1time2 SE2time1 SE2time2 SEtime1 SEtime2
Proptimeinpw Proptimeinxyl ProptimeinSE;

```

```

by variety;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var pwtime;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var xyltime1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var xyltime2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE1time1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE1time2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE2time1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SE2time2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SEtime1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var SEtime2;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var Proptimeinpw;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var Proptimeinxyl;
run;
proc npar1way data = EPG wilcoxon;
class variety;

```

```
var ProptimeinSE;
run;
```

```
dm'log;clear;output;clear';
```

```
Title 'Effect of sugarcane cultivars on total probe time, mean probe duration, total
nonprobe time, time to reach SPP,G,and SEP';
```

```
options nodate nonumber ps=55 ls=78;
```

```
data EPG;
```

```
input variety$ read$ aphid$ Tprobetime MeProbeDuration Tnonprobetime TimetoreachSPP
TimetoreachG TimetoreachSEP;
```

```
cards;
```

128	1	3	5732	1433	10268	3266	3922	.
128	2	2	15452	7726	548	343	5347	7423
128	2	3	15094	5031	906	762	1334	11706
128	3	1	14399	2400	1601	642	972	7305
128	3	2	14895	4965	1105	295	.	12220
128	3	3	15317	15317	683	683	.	11623
128	3	4	14377	3594	1623	582	.	9137
128	4	2	16000	16000	0	0	.	2928
128	4	3	10962	1827	5043	0	.	8942
128	5	1	11158	11158	80	80	.	2705
128	5	2	11221	3610.5	17	1	.	2198
128	5	4	8398	4199	2846	2383	6620	2570
128	6	1	13517	2252.8	883	4	6832	1973
128	6	2	13484	6742	916	239	.	4205
128	6	3	11107	2926.8	2693	593	.	6583
128	6	4	13344	13344	1056	1056	3952	.
128	7	1	7315	7315	849	849	.	1864
128	7	4	7190	7190	926	926	911	1981
128	8	2	15574	7787	426	181	.	3623
128	9	1	10757	10757	5243	5243	.	300
128	9	2	10240	2048	5760	1312	7960	.
128	9	3	11162	5581	4838	1829	3468	9424
128	9	4	12463	1557.9	3537	13	10234	.
128	10	2	7115	2371.7	8885	1798	1942	.
128	10	3	8469	2823	7531	6115	6812	.
128	10	4	14249	4749.7	1751	1444	8592	1136
128	10	1	3323	3323	12677	593	1156	.
128	11	1	15857	5285.7	143	11	.	7134
128	11	2	14865	7432.5	1135	813	423	12064
128	11	4	14259	2851.8	1741	731	14569	10606
555	1	1	13140	2628	2860	175	.	11259
555	1	4	15491	5163.7	509	206	750	.
555	2	1	3220	536.7	12780	7708	.	2812
555	2	2	11699	1169.9	4301	1250	692	.
555	2	3	15040	5013	960	613	.	6799
555	3	1	15233	15233	767	767	.	1652
555	3	3	14634	3658.5	1366	435	.	1881

555	4	1	14874	14874	1126	1126	762	.	.
555	4	2	1456	1456	14544	14544	.	.	.
555	4	3	15134	2622.3	266	0.25	1442.75	977.75	.
555	5	2	13423	2237.2	2577	58	506	10503	.
555	5	4	15523	2217.6	477	18	7275	11939	.
555	6	1	11873	3957.7	4127	1582	.	12247	.
555	6	2	15663	3915.8	337	0.18	.	2915	.
555	7	1	13510	2702	2490	1446	.	7012	.
555	8	2	15241	15241	759	759	688	.	.
555	8	3	8540	4270	7460	7251	1653	.	.
555	8	4	15021	3755.3	979	754	1752	8380	.
555	9	3	7585	2528.3	8415	202	2718	8034	.
555	9	4	5102	1700.7	10298	179	.	4567	.
555	11	2	15167	5055.7	833	343	930	6904	.
555	11	3	15028	5009	972	774	.	1192	.
555	11	4	15944	15944	56	56	6062	1728	.
555	12	2	14243	2034.7	1757	836	.	6729	.
555	12	4	14537	7268.5	1463	1144	2181	.	.
555	13	2	13561	1937	2439	261	.	9441	.
555	13	3	12531	6265	3469	3338	.	12245	.
555	14	4	15975	15975	25	25	.	3962	.

;

run;

Proc sort;

by variety;

run;

Proc means mean n stderr clm;

var Tprobetime MeProbeDuration Tnonprobetime TimetoreachSPP TimetoreachG

TimetoreachSEP;

by variety;

run;

proc npar1way data = EPG wilcoxon;

class variety;

var Tprobetime;

run;

proc npar1way data = EPG wilcoxon;

class variety;

var MeProbeDuration;

run;

proc npar1way data = EPG wilcoxon;

class variety;

var Tnonprobetime;

run;

proc npar1way data = EPG wilcoxon;

class variety;

var TimetoreachSPP;

run;

proc npar1way data = EPG wilcoxon;

```

class variety;
var TimetoreachG;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var TimetoreachSEP;
run;

dm'log;clear;output;clear';
Title 'Effect of sugarcane cultivars on total probe time, mean probe duration, total
nonprobe time, time to reach SPP,G,and SEP';
options nodate nonumber ps=55 ls=78;
data EPG;
input variety$ read$ aphid$ Tprobetime MeProbeDuration Tnonprobetime TimetoreachSPP
TimetoreachG TimetoreachSEP;
cards;
128 1 3 5732 1433 10268 3266 3922 .
128 2 2 15452 7726 548 343 5347 7423
128 2 3 15094 5031 906 762 1334 11706
128 3 1 14399 2400 1601 642 972 7305
128 3 2 14895 4965 1105 295 . 12220
128 3 3 15317 15317 683 683 . 11623
128 3 4 14377 3594 1623 582 . 9137
128 4 2 16000 16000 0 0 . 2928
128 4 3 10962 1827 5043 0 . 8942
128 5 1 11158 11158 80 80 . 2705
128 5 2 11221 3610.5 17 1 . 2198
128 5 4 8398 4199 2846 2383 6620 2570
128 6 1 13517 2252.8 883 4 6832 1973
128 6 2 13484 6742 916 239 . 4205
128 6 3 11107 2926.8 2693 593 . 6583
128 6 4 13344 13344 1056 1056 3952 .
128 7 1 7315 7315 849 849 . 1864
128 7 4 7190 7190 926 926 911 1981
128 8 2 15574 7787 426 181 . 3623
128 9 1 10757 10757 5243 5243 . 300
128 9 2 10240 2048 5760 1312 7960 .
128 9 3 11162 5581 4838 1829 3468 9424
128 9 4 12463 1557.9 3537 13 10234 .
128 10 2 7115 2371.7 8885 1798 1942 .
128 10 3 8469 2823 7531 6115 6812 .
128 10 4 14249 4749.7 1751 1444 8592 1136
128 10 1 3323 3323 12677 593 1156 .
128 11 1 15857 5285.7 143 11 . 7134
128 11 2 14865 7432.5 1135 813 423 12064
128 11 4 14259 2851.8 1741 731 14569 10606
384 1 2 8608 1721 6058 5451 4905 .
384 1 4 13424 3356 1242 334 . 1985

```


384	2	3	12358	4119.3	2308	236	.	3117
384	3	1	8407	4203.5	7807	254	.	4459
384	3	2	12188	12188	2212	2212	.	8572
384	3	4	10671	3557	3729	612	.	7343
384	4	1	10396	5198	4004	1238	604	3607
384	4	3	3666	1222	10734	589	.	2115
384	4	4	2868	2868	11532	587	1315	.
384	5	1	14008	14008	392	392	.	3555
384	5	2	14143	7071.5	257	0	.	12545
384	5	4	11144	5572	3256	1323	.	6532
384	6	2	3466	3466	12534	12534	.	503
384	6	4	15573	3893.3	427	0	.	2936
384	7	1	10119	919	4281	497	.	4527
384	7	2	12236	4078	2164	1726	.	2865
384	7	4	13098	1190	1302	0	.	1733
384	8	1	12931	1847	1433	0	.	9553
384	8	2	10940	1823	3460	806	.	2752
384	8	4	12072	1509	2328	1087	.	4074
384	9	1	14399	14399	1	1	.	1329
384	9	3	9797	1959.4	4603	2657	9570	.
384	10	3	7026	1405.2	7374	3738	.	.
384	10	1	11301	2260.2	3099	0	.	9296
384	11	1	7412	1853	6988	0	.	5498
384	11	4	11144	2228.8	2356	163	.	7922
384	12	1	13492	1349.2	908	0	.	9134
384	13	2	13524	1690.5	876	0	.	5700
384	13	1	4375	546.9	10025	4465	4789	.
384	13	3	13840	1977.1	560	200	5875	.
384	14	2	13238	2647.6	1162	246	.	1604
384	14	3	13640	3410	760	331	6250	2340

;

run;

Proc sort;

by variety;

run;

Proc means mean n stderr clm;

var Tprobetime MeProbeDuration Tnonprobetime TimetoreachSPP TimetoreachG

TimetoreachSEP;

by variety;

run;

proc npar1 way data = EPG wilcoxon;

class variety;

var Tprobetime;

run;

proc npar1 way data = EPG wilcoxon;

class variety;

var MeProbeDuration;

run;

```

proc npar1way data = EPG wilcoxon;
class variety;
var Tnonprobetime;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var TimetoreachSPP;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var TimetoreachG;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var TimetoreachSEP;
run;

```

```
dm'log;clear;output;clear';
```

Title 'Effect of sugarcane cultivars on total probe time, mean probe duration, total nonprobe time, time to reach SPP,G,and SEP';

```
options nodate nonumber ps=55 ls=78;
```

```
data EPG;
```

```
input variety$ read$ aphid$ Tprobetime MeProbeDuration Tnonprobetime TimetoreachSPP
TimetoreachG TimetoreachSEP;
```

```
cards;
```

555	1	1	13140	2628	2860	175	.	11259	
555	1	4	15491	5163.7	509	206	750	.	
555	2	1	3220	536.7	12780	7708	.	2812	
555	2	2	11699	1169.9	4301	1250	692	.	
555	2	3	15040	5013	960	613	.	6799	
555	3	1	15233	15233	767	767	.	1652	
555	3	3	14634	3658.5	1366	435	.	1881	
555	4	1	14874	14874	1126	1126	762	.	
555	4	2	1456	1456	14544	14544	.	.	
555	4	3	15134	2622.3	266	0.25	1442.75	977.75	
555	5	2	13423	2237.2	2577	58	506	10503	
555	5	4	15523	2217.6	477	18	7275	11939	
555	6	1	11873	3957.7	4127	1582	.	12247	
555	6	2	15663	3915.8	337	0.18	.	2915	
555	7	1	13510	2702	2490	1446	.	7012	
555	8	2	15241	15241	759	759	688	.	
555	8	3	8540	4270	7460	7251	1653	.	
555	8	4	15021	3755.3	979	754	1752	8380	
555	9	3	7585	2528.3	8415	202	2718	8034	
555	9	4	5102	1700.7	10298	179	.	4567	
555	11	2	15167	5055.7	833	343	930	6904	
555	11	3	15028	5009	972	774	.	1192	
555	11	4	15944	15944	56	56	6062	1728	

555	12	2	14243	2034.7	1757	836	.	6729
555	12	4	14537	7268.5	1463	1144	2181	.
555	13	2	13561	1937	2439	261	.	9441
555	13	3	12531	6265	3469	3338	.	12245
555	14	4	15975	15975	25	25	.	3962
384	1	2	8608	1721	6058	5451	4905	.
384	1	4	13424	3356	1242	334	.	1985
384	2	3	12358	4119.3	2308	236	.	3117
384	3	1	8407	4203.5	7807	254	.	4459
384	3	2	12188	12188	2212	2212	.	8572
384	3	4	10671	3557	3729	612	.	7343
384	4	1	10396	5198	4004	1238	604	3607
384	4	3	3666	1222	10734	589	.	2115
384	4	4	2868	2868	11532	587	1315	.
384	5	1	14008	14008	392	392	.	3555
384	5	2	14143	7071.5	257	0	.	12545
384	5	4	11144	5572	3256	1323	.	6532
384	6	2	3466	3466	12534	12534	.	503
384	6	4	15573	3893.3	427	0	.	2936
384	7	1	10119	919	4281	497	.	4527
384	7	2	12236	4078	2164	1726	.	2865
384	7	4	13098	1190	1302	0	.	1733
384	8	1	12931	1847	1433	0	.	9553
384	8	2	10940	1823	3460	806	.	2752
384	8	4	12072	1509	2328	1087	.	4074
384	9	1	14399	14399	1	1	.	1329
384	9	3	9797	1959.4	4603	2657	9570	.
384	10	3	7026	1405.2	7374	3738	.	.
384	10	1	11301	2260.2	3099	0	.	9296
384	11	1	7412	1853	6988	0	.	5498
384	11	4	11144	2228.8	2356	163	.	7922
384	12	1	13492	1349.2	908	0	.	9134
384	13	2	13524	1690.5	876	0	.	5700
384	13	1	4375	546.9	10025	4465	4789	.
384	13	3	13840	1977.1	560	200	5875	.
384	14	2	13238	2647.6	1162	246	.	1604
384	14	3	13640	3410	760	331	6250	2340

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var Tprobetime MeProbeDuration Tnonprobetime TimetoreachSPP TimetoreachG
TimetoreachSEP;
by variety;
run;
proc npar1way data = EPG wilcoxon;

```

```

class variety;
var Tprobetime;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var MeProbeDuration;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var Tnonprobetime;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var TimetoreachSPP;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var TimetoreachG;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var TimetoreachSEP;
run;

dm'log;clear;output;clear';
Title 'Effect of sugarcane cultivars on mean duration of SPP, G, SEP, SE1, SE2 phases';
options nodate nonumber ps=55 ls=78;
data EPG;
input variety$ read$ aphid$ meandurationofSPP meandurationofG meandurationofSEP
meandurationofSE1 meandurationofSE2;
cards;
128 1 3 592 198 . . .
128 2 2 1735 486 1355 6.714 1348
128 2 3 1537 1149 443 5.944 437
128 3 1 1158 2585 2554 7.875 2546
128 3 2 2707 . 679 7.554 672
128 3 3 7489 . 340 7.604 332
128 3 4 2213.2 . 3311 6.325 3305
128 4 2 2928 . 13072 10.045 13061
128 4 3 1169.4 . 1388 6.625 1381
128 5 1 1661 . 7836 8.75 7821
128 5 2 3167.3 . 1719 8.75 1710
128 5 4 1285.7 2241 2300 9.167 2291
128 6 1 1332.3 1447 1696.3 6.281 1690.7
128 6 2 2838.3 . 4969 5.835 4964
128 6 3 1652.6 . 1702 5.344 1696.5
128 6 4 1146.8 2189.3 . . .
128 7 1 1806.7 . 631.7 5.721 625.7

```

128	7	4	754.5	472	5209	4.375	5205
128	8	2	1689	.	12196	7.125	12189
128	9	1	300	.	10457	8	10449
128	9	2	747.3	878.5	.	.	.
128	9	3	691.8	1824	9747	8.938	9738
128	9	4	1220.7	1477	.	.	.
128	10	2	920.5	530.7	.	.	.
128	10	3	1546	369.5	.	.	.
128	10	4	873.9	2035.5	2030.5	7.4	2023
128	10	1	1220.5	882	.	.	.
128	11	1	2334	.	8855	7	8848
128	11	2	2719	2024	1965	4	1961
128	11	4	1857.4	267	990	6	984
555	1	1	1714.8	.	4566	5.625	4560
555	1	4	1576.1	891.6	.	.	.
555	2	1	413.4	.	326	7	319
555	2	2	787.2	1126.5	.	.	.
555	2	3	2150.7	.	8588	7.563	8581
555	3	1	7239	.	755	6.437	749
555	3	3	1353.8	.	950.75	8.422	941
555	4	1	942.5	6494.5	.	.	.
555	4	2	1456
555	4	3	755	1359	1517.3	5.93	1511.3
555	5	2	527.6	1621.7	1132	7	1125
555	5	4	1181.3	694.3	446	5	441
555	6	1	2936	.	129	8	121
555	6	2	1688.6	.	1281	7.25	1273.7
555	7	1	1617.2	.	3807	6.625	3800
555	8	2	362.5	14516	.	.	.
555	8	3	317	7906	.	.	.
555	8	4	2110.7	2162	196	7	189
555	9	3	833.2	3282	137	8	129
555	9	4	1165.8	.	439	9.2	430
555	11	2	1290.4	883	656	7.156	649
555	11	3	1985.8	.	1037.7	5.382	1031.7
555	11	4	1713.2	803	1023.8	7.3	1007.5
555	12	2	1357.2	.	167.8	6.4	161.8
555	12	4	1487	440.2	.	.	.
555	13	2	1503	.	766	8.032	758
555	13	3	4126	.	152	8.5	144
555	14	4	3258	.	9459	8.125	9451

;

run;

Proc sort;

by variety;

run;

Proc means mean n stderr clm;

```

var meandurationofSPP meandurationofG meandurationofSEP meandurationofSE1
meandurationofSE2;
by variety;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var meandurationofSPP;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var meandurationofG;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var meandurationofSEP;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var meandurationofSE1;
run;
proc npar1way data = EPG wilcoxon;
class variety;
var meandurationofSE2;
run;

dm'log;clear;output;clear';
Title 'Effect of sugarcane cultivars on mean duration of SPP, G, SEP, SE1, SE2 phases';
options nodate nonumber ps=55 ls=78;
data EPG;
input variety$ read$ aphid$ meandurationofSPP meandurationofG meandurationofSEP
meandurationofSE1 meandurationofSE2;
cards;
128 1 3 592 198 . . .
128 2 2 1735 486 1355 6.714 1348
128 2 3 1537 1149 443 5.944 437
128 3 1 1158 2585 2554 7.875 2546
128 3 2 2707 . 679 7.554 672
128 3 3 7489 . 340 7.604 332
128 3 4 2213.2 . 3311 6.325 3305
128 4 2 2928 . 13072 10.045 13061
128 4 3 1169.4 . 1388 6.625 1381
128 5 1 1661 . 7836 8.75 7821
128 5 2 3167.3 . 1719 8.75 1710
128 5 4 1285.7 2241 2300 9.167 2291
128 6 1 1332.3 1447 1696.3 6.281 1690.7
128 6 2 2838.3 . 4969 5.835 4964
128 6 3 1652.6 . 1702 5.344 1696.5
128 6 4 1146.8 2189.3 . . .

```

128	7	1	1806.7	.	631.7	5.721	625.7
128	7	4	754.5	472	5209	4.375	5205
128	8	2	1689	.	12196	7.125	12189
128	9	1	300	.	10457	8	10449
128	9	2	747.3	878.5	.	.	.
128	9	3	691.8	1824	9747	8.938	9738
128	9	4	1220.7	1477	.	.	.
128	10	2	920.5	530.7	.	.	.
128	10	3	1546	369.5	.	.	.
128	10	4	873.9	2035.5	2030.5	7.4	2023
128	10	1	1220.5	882	.	.	.
128	11	1	2334	.	8855	7	8848
128	11	2	2719	2024	1965	4	1961
128	11	4	1857.4	267	990	6	984
384	1	2	832.5	3613	.	.	.
384	1	4	2623.8	.	305	8.625	296
384	2	3	2371.6	.	250	6.219	243.5
384	3	1	2752	.	151	6.812	144
384	3	2	8572	.	3616	8.437	3607
384	3	4	1408.7	.	6445	5.25	6440
384	4	1	791.8	993	6236	5.687	6230
384	4	3	616.3	.	1201	7	1194
384	4	4	1315	1553	.	.	.
384	5	1	3555	.	10453	9.937	10443
384	5	2	6144	.	1855	7.125	1848
384	5	4	1987.7	.	2590.5	9	2581.5
384	6	2	521.6	.	214.5	7.5	207
384	6	4	1572	.	598	8.123	592
384	7	1	642	.	1207	5.093	1202
384	7	2	1032.5	.	2013.7	6.479	2007.2
384	7	4	729	.	515	5.468	509
384	8	1	1212	.	3234	4.625	3230
384	8	2	1198.4	.	676.5	5.719	670.5
384	8	4	1125.6	.	408	6.375	401.5
384	9	1	501.1	.	1815.2	6.007	1809
384	9	3	2935.7	495	.	.	.
384	10	3	1405.2
384	10	1	1239.4	.	5104	5.021	5099
384	11	1	1745.8	.	429	8.438	420
384	11	4	2085.6	.	716	6.312	710
384	12	1	1164.4	.	924	6.281	918
384	13	2	1325.9	.	795.5	5.419	790
384	13	1	288.8	743.5	.	.	.
384	13	3	868.4	1289	.	.	.
384	14	2	802.5	.	721.6	5.642	715.6
384	14	3	925.5	3866	355	4.687	350

;
run;

```

Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var meandurationofSPP meandurationofG meandurationofSEP meandurationofSE1
meandurationofSE2;
by variety;
run;
proc npar1 way data = EPG wilcoxon;
class variety;
var meandurationofSPP;
run;
proc npar1 way data = EPG wilcoxon;
class variety;
var meandurationofG;
run;
proc npar1 way data = EPG wilcoxon;
class variety;
var meandurationofSEP;
run;
proc npar1 way data = EPG wilcoxon;
class variety;
var meandurationofSE1;
run;
proc npar1 way data = EPG wilcoxon;
class variety;
var meandurationofSE2;
run;

```


APPENDIX C: SAS CODES FOR CHAPTER 5

```
dm'output;clear;log;clear';
Title"amount of toal phenolics in 128 and 555";
data totalphenolics;
input variety$ rep$ phenolics;
cards;
128 1 13.94009217
128 2 14.22018349
128 3 14.7995283
128 4 13.96396396
128 5 12.14953271
128 6 15.55164319
128 7 15.53217822
128 8 12.32227488
128 9 13.1097561
128 10 17.39386792
128 11 18.36492891
128 12 18.50728155
128 13 21.96261682
128 14 18.60189573
128 15 18.53448276
555 1 11.41552511
555 2 12.55841121
555 3 11.77130045
555 4 18.77990431
555 5 19.77272727
555 6 22.12389381
555 7 12.73923445
555 8 10.4679803
555 9 10.10869565
555 10 16.45348837
555 11 13.49118943
555 12 18.03797468
555 13 13.71527778
555 14 13.73922414
555 15 15.5625
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var phenolics;
by variety;
run;
;
Proc ttest data=totalphenolics;
class variety;
```

```

var phenolics;
run;
;

title 'Effect of variety on TAC';
Data TAC;
input variety$ rep$ mggdw;
datalines;
128 1 165.1361473
128 2 159.3
128 3 185.2271045
128 4 184.935044
128 5 176.6819219
128 6 279.9224104
128 7 370.9401869
128 8 92.47245614
128 9 248.3826254
128 10 360.8250755
128 11 209.2810149
128 12 141.4795588
555 1 242.9758235
555 2 281.1730408
555 3 243.5211854
555 4 216.5079701
555 5 214.0271552
555 6 202.3560231
555 7 284.4254369
555 8 223.2533133
555 9 315.3412308
555 10 320.4472892
555 11 257.229764
555 12 198.4303395
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var mggdw;
by variety;
run;
;
Proc ttest data=TAC;
class variety;
var mggdw;
run;
;

dm'log;clear;output;clear';

```

Title 'Water potential readings of L 97-128 and HoCP 91-555';

options nodate nonumber ps=55 ls=78;

data wp;

input variety\$ rep potential;

cards;

128	1	8.5
128	2	7
128	3	4.5
128	4	7.5
128	5	5.5
128	6	3.5
128	7	4.5
128	8	11.5
128	9	4.5
128	10	7.5
128	11	2.5
128	12	3.5
128	13	3
128	14	3.5
128	15	4
555	1	4
555	2	6
555	3	4.5
555	4	3.5
555	5	3.5
555	6	3.5
555	7	2.5
555	8	1.5
555	9	1.5
555	10	2.5
555	11	2.5
555	12	5.5
555	13	8.5
555	14	3.5
555	15	5.5

Proc sort;

by variety;

run;

Proc means mean n stderr clm;

var potential;

by variety;

run;

Proc ttest cochran;

class variety;

var potential;

run;

dm'log;clear;output;clear';

Title Concentration of total FAAs, total essential FAAs, and total nonessential FAAs in the whole leaf tissue samples of L 97-128 and HoCP 91-555;

options nodate nonumber ps=55 ls=78;

data wholeleaf;

input variety\$ rep\$ totalFAA totalessential totalnonessential;
cards;

128	1	26405.06438	6695.308355	19709.75603
128	2	20989.30216	4309.439257	16679.8629
128	3	31916.95675	7832.48692	24084.46983
128	4	23566.62796	4577.478142	18989.14982
128	5	23450.18432	5164.178521	18286.0058
128	6	16213.12585	2785.88911	13427.23675
128	7	25459.65833	6761.530183	18698.12815
128	8	20471.12687	3552.267254	16918.85961
128	9	27237.24715	7391.51822	19845.72893
128	10	18376.42257	3677.878838	14698.54374
128	11	18014.82285	2913.028109	15101.79474
128	12	23855.98514	5829.212509	18026.77263
555	1	5011.699456	605.5432854	4406.15617
555	2	15531.32336	1505.904927	14025.41844
555	3	15951.59317	2740.617547	13210.97562
555	4	6673.582515	404.9778301	6268.604685
555	5	4309.813668	198.816614	4110.997054
555	6	9443.938475	1168.587339	8275.351137
555	7	6208.20021	359.0655883	5849.134622
555	8	6592.19178	421.0775039	6171.114276
555	9	13262.42626	1243.904095	12018.52216
555	10	22689.0037	1790.356368	20898.64733
555	11	11496.65212	528.7024123	10967.94971
555	12	6125.30407	319.6904477	5805.613622

;

run;

Proc sort;

by variety;

run;

Proc means mean n stderr clm;

var totalFAA totalessential totalnonessential;

by variety;

run;

;

Proc ttest data= wholeleaf;

class variety;

var totalFAA;

run;

Proc ttest data= wholeleaf;

class variety;

var totalessential;

run;

```
Proc ttest data= wholeleaf;
class variety;
var totalnonessential;
run;
quit;
```

```
dm'log;clear;output;clear';
```

Title Analysis of relative amounts of FAAs in wholeleaf tissue samples of L 97-128 and HoCP 91-555 to get SE;

```
options nodate nonumber ps=55 ls=78;
```

```
data wholeleaf;
```

```
input variety$ rep$ alanine aspartic cystine glutamic glycine proline serine tyrosine arginine
histidine isoleucine leucine lysine methionine phenyl threonine valine;
```

```
cards;
```

```
128 1 15.91522371 17.37123687 0 23.33627285 2.218265816 2.780802842
12.32499302 0.697052528 8.567744824 4.395031339 0.497281426 0.602045369
2.763109936 0 0.544730044 4.677551812 3.308657607
128 2 29.03002732 16.62085914 0 16.93211065 2.027345152 4.874597331
9.522247466 0.461214395 6.407091927 4.646371565 0.595403312 0.763367828
1.870999743 0 0.636083901 4.868693789 0.743586488
128 3 27.32965043 17.70070281 0 12.75418327 2.297245478 2.880495213
11.85588269 0.641634154 9.609803558 5.188998885 0.524815542 0.643003405
2.848054732 0 0.470922725 4.401725675 0.852881431
128 4 34.28906861 17.36865226 0 12.23353835 2.369611103 3.718729921
10.16242273 0.43441695 7.446326694 3.796356898 0.404651934 0.673940641
2.059171083 0 0.342277129 4.066281498 0.634554207
128 5 29.11174145 14.6496124 0 17.07553406 2.38806271 4.232365303
9.897076219 0.623697704 7.460713155 4.069647112 0.515837507 0.751299846
1.832884715 0 0.359431202 4.31118663 2.720909983
128 6 31.87478113 14.98581306 0 15.13631436 2.111671526 5.982581411
12.29576435 0.43015007 4.628141413 3.843081862 0.367882702 0.514008595
1.186916233 0 0.461722316 3.834240345 2.346930632
128 7 24.50964303 19.06516247 0 12.44663661 2.073369863 4.092503984
10.41079999 0.844064245 9.337992717 4.450807639 0.872747342 1.002051505
2.280832544 0 0.493418138 4.59157216 3.528397764
128 8 33.58853717 15.65076033 0 15.11129772 2.377974095 4.802417733
11.11643985 0 4.822334954 3.247437719 0.417061461 0.599382386
1.198844747 0 0.395750017 4.417120051 2.254641766
128 9 16.77490036 17.95492196 0 19.20969007 1.786312879 3.405515732
12.82702303 0.904098245 13.35465854 4.242846605 0.594443663 0.704257751
3.697904249 0.224654798 0.843433554 0 3.475338563
128 10 30.17659415 14.85922338 0 16.14946774 1.945860857 5.487461977
10.77859311 0.588681111 7.813154811 2.817613451 0.509849846 0.619146631
1.849403111 0 0.549652309 3.475753317 2.379544197
128 11 30.47794358 12.87286294 0 19.14719442 1.872801702 6.573062915
12.39261468 0.493346283 4.608501975 2.577942321 0.465796689 0.613819836
0.846321326 0 0.567197836 4.03271957 2.457873941
```

```

128 12 22.96524625 13.46853528 0 20.11842731 2.234366993 5.168244425
11.12877575 0.481393346 8.129937863 4.478098033 0.452224583 0.656396859
1.7677794 0 1.244327843 4.333392679 3.372853384
555 1 0 9.492240388 0 21.35259885 2.42358537 45.91027034
8.738711297 0 2.973647476 2.282723536 0 0 1.766645988 0
0 3.132863352 1.926713402
555 2 39.85081078 9.564057472 0 16.39524389 0 14.42485894
10.06910737 0 1.659416616 1.707714679 0 0.432066466 0.842802473
0 0.470720826 3.099820492 1.483379988
555 3 24.11622831 13.20576834 0 28.52440795 0 5.926468784
10.51598291 0.530304657 2.577342164 3.463540554 0.817635243 0.825121316
1.479698231 0 0.393323453 5.195768027 2.428410056
555 4 29.60612999 7.101853668 0 12.83218465 1.956990956 32.34042229
10.09404618 0 1.984253244 0 0 0 0.935350648 0 0
1.775810185 1.372958183
555 5 43.29251774 7.078032806 0 14.56372683 1.689914041 21.19123894
7.571455722 0 0 0 0 0 1.159859386 0 0
1.976469336 1.476785193
555 6 31.64882028 11.18832616 0 15.2779178 1.86252091 20.12082371
7.527650526 0 4.036875295 2.29060816 0 0 1.498377916 0
0 2.717043146 1.8310361
555 7 49.45419863 9.317632447 0 13.57825469 1.588165035 12.6062433
7.67177509 0 1.487723235 0 0 0 0.859738537 0 0
2.052160901 1.384108136
555 8 39.24763615 6.409136712 0 14.98042867 1.49966356 25.56733605
5.908279845 0 2.091423852 0 0 0 0.930679694 0 0
1.866680342 1.498735135
555 9 43.8879448 13.85619849 0 14.05855704 1.941423962 6.191987064
10.68472927 0 2.544783608 1.392662946 0 0 0.782633115 0
0 2.776481219 1.882598486
555 10 44.02291451 11.13783365 0 17.67592136 1.486955834 6.50511068
11.28040903 0 0 1.11568654 0.303595411 0.356034207 0.712877961
0 0.500087646 2.833884143 2.068689026
555 11 53.57783925 4.371576867 0 13.1433193 1.870069224 14.49882754
7.939616738 0 1.064230179 0 0 0 0 0 0
2.036291601 1.49822929

555 12 47.17951906 6.021750726 0 14.0251184 2.095556148 20.2741299
5.184748991 0 1.649096573 0 0 0 0 0 0
1.903660094 1.666420113

```

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;

```

```
var alanine aspartic cystine glutamic glycine proline serine tyrosine arginine histidine isoleucine
leucine lysine methionine phenyl threonine valine;
by variety;
run;
```

```
dm'log;clear;output;clear';
```

Title Analysis of relative amounts of FAAs in whole leaf tissue of L 97-128 and HoCP 91-555 data after arcsin transformation;

```
options nodate nonumber ps=55 ls=78;
```

```
data wholeleaf;
```

```
input variety$ rep$ alanine aspartic glutamic glycine proline serine tyrosine arginine histidine
isoleucine leucine lysine methionine phenyl threonine valine;
```

```
cards;
```

```
128 1 23.51186004 24.63197589 28.88651466 8.565412479 9.599340294
20.55275635 4.789180702 17.02009605 12.10144956 4.043750522 4.450143164
9.568465549 0 4.232611189 12.49042667 10.48028343
128 2 32.60166085 24.05961075 24.29824613 8.185872051 12.75513828
17.97375929 3.894112226 14.66235345 12.44805697 4.425477804 5.012376163
7.861821134 0 4.574475698 12.74728209 4.946842421
128 3 31.518758 24.88018077 20.92404339 8.717728171 9.771552298
20.14051884 4.594432718 18.05903955 13.16721482 4.15438349 4.599342921
9.715835675 0 3.934947225 12.11080192 5.298906271
128 4 35.84317524 24.63002146 20.47292713 8.85505886 11.11857504
18.58949603 3.779122387 15.83569094 11.23551841 3.647176452 4.708932663
8.250318384 0 3.353973546 11.63347656 4.568960195
128 5 32.65321321 22.50401181 24.40761941 8.889746385 11.872068
18.33643783 4.529624368 15.85138322 11.63835731 4.118633757 4.972497753
7.780829534 0 3.437090981 11.98373288 9.494435063
128 6 34.37296085 22.77511358 22.8956596 8.355573571 14.15779205
20.52727092 3.760490333 12.42322203 11.30535529 3.477314394 4.111313346
6.254542521 0 3.896259176 11.29217183 8.81224037
128 7 29.67450788 25.88948668 20.65854366 8.27891302 11.67145292
18.82372849 5.271366814 17.79313935 12.17916603 5.360442559 5.745074219
8.686288258 0 4.027986288 12.37326299 10.82677323
128 8 35.41933312 23.30404975 22.87565647 8.870796873 12.65877232
19.47607764 0 12.68543179 10.38178719 3.702755231 4.440270466
6.286019115 0 3.606782661 12.13228302 8.635888613
128 9 24.17793451 25.07046024 25.99473825 7.680737569 10.6343376
20.98652245 5.456159204 21.43467971 11.88697362 4.421902848 4.813927336
11.08700252 2.716709655 5.26939147 0 10.74408436
128 10 33.32121587 22.67333334 23.69475817 8.018574022 13.54761287
19.16609055 4.400375186 16.23157147 9.663271874 4.09461914 4.513033565
7.81603037 0 4.251726467 10.74473307 8.873748579
128 11 33.50902476 21.02576453 25.94926329 7.865630033 14.85536333
20.61161855 4.027692499 12.39641508 9.239385768 3.913438952 4.493537731
5.278430073 0 4.319180193 11.58469945 9.019817647
```

```

128 12 28.63451592 21.530412 26.64977465 8.596676622 13.14038338
19.4873176 3.978521733 16.5666469 12.21702216 3.855916238 4.647101338
7.64054968 0 6.404641929 12.01501542 10.58262686
555 1 0 17.94445156 27.52197136 8.956160006 42.65413676 17.19430598
0 9.929871579 8.68991618 0 0 7.6380853 0 0
10.19500654 7.978766425
555 2 39.14425145 18.01452607 23.885513 0 22.32133955 18.50084053
0 7.401314372 7.508863107 0 3.76886993 5.267414172 0
3.934102287 10.14052819 6.995655825
555 3 29.41177352 21.3089903 32.28170783 0 14.08986201 18.92217552
4.176090951 9.238300801 10.72561553 5.187953087 5.211714221 6.986925458
0 3.595693479 13.17595502 8.965143587
555 4 32.96421702 15.45568796 20.99094413 8.041625322 34.65867522
18.52456955 0 8.097817738 0 0 0 5.54995193 0 0
7.657988861 6.728993498
555 5 41.14527456 15.42909923 22.43434504 7.469401266 27.40901237
15.97171521 0 0 0 0 0 6.182561294 0 0
8.081812514 6.980010332
555 6 34.23391434 19.54150097 23.00862993 7.843874763 26.65148712
15.92421412 0 11.59074967 8.705027261 0 0 7.031109873 0
0 9.487623735 7.776880451
555 7 44.68727264 17.77308312 21.62232666 7.23980307 20.79667541
16.08002581 0 7.005941041 0 0 0 5.32022623 0 0
8.236165136 6.756388556
555 8 38.79084952 14.66474542 22.77079167 7.034140896 30.37394805
14.06777785 0 8.31513354 0 0 0 5.536033514 0 0
7.852683648 7.031952179
555 9 41.48926962 21.85377791 22.02106333 8.009366861 14.40870466
19.07921257 0 9.179255744 6.777333752 0 0 5.075395191 0
0 9.591807736 7.886306654
555 10 41.56717295 19.49556731 24.8615751 7.00412484 14.7766181
19.62503734 0 0 6.063237976 3.158567981 3.420790984 4.843369236
0 4.055163196 9.691401033 8.269496998
555 11 47.05170435 12.06862862 21.25609375 7.859853541 22.38158838
16.36606667 0 5.921254894 0 0 0 0 0 0
8.204038022 7.030759392
555 12 43.38312519 14.20503383 21.99349021 8.323402348 26.76088179
13.16172468 0 7.378135332 0 0 0 0 0 0
7.930580369 7.417004037

```

```

;
run;
Proc sort;
by variety;
run;
Proc ttest data=wholeleaf;
class variety;
var aspartic;
run;

```



```
Proc ttest data=wholeleaf;
class variety;
var glutamic;
run;
Proc ttest data=wholeleaf;
class variety;
var serine;
run;
Proc ttest data=wholeleaf;
class variety;
var histidine;
run;
Proc ttest data=wholeleaf;
class variety;
var glycine;
run;
Proc ttest data=wholeleaf;
class variety;
var threonine;
run;
Proc ttest data=wholeleaf;
class variety;
var arginine;
run;
Proc ttest data=wholeleaf;
class variety;
var alanine;
run;
Proc ttest data=wholeleaf;
class variety;
var valine;
run;
Proc ttest data=wholeleaf;
class variety;
var tyrosine;
run;
Proc ttest data=wholeleaf;
class variety;
var proline;
run;
Proc ttest data=wholeleaf;
class variety;
var isoleucine;
run;
Proc ttest data=wholeleaf;
class variety;
var leucine;
run;
```

```

Proc ttest data=wholeleaf;
class variety;
var lysine;
run;
Proc ttest data=wholeleaf;
class variety;
var phenyl;
run;
Proc ttest data=wholeleaf;
class variety;
var methionine;
run;

```

```
dm'log;clear;output;clear';
```

Title Analysis of relative amounts of FAAs in whole leaf tissue and Sap of L 97-128 after arcsin transformation;

```
options nodate nonumber ps=55 ls=78;
```

```
data leafvssap;
```

```
input variety$ rep$ alanine aspartic glutamic glycine proline serine tyrosine arginine histidine
isoleucine leucine lysine methionine phenyl threonine valine;
```

```
cards;
```

128-sap	1	21.8641153	0	19.37924022	0	0	16.24947296	0	
		22.69768654	46.37649805	0	0	0	0	0	0
128-sap	2	26.55842512	20.82106944	25.96159049	0	0	16.48207973		
		0	13.51651993	36.08986081	0	0	0	0	0
128-sap	3	21.32744228	27.15470249	24.18636454	19.39983329	0			
		18.46688708	0	15.6772843	20.71066975	0	0	0	0
		16.72531464	0						
128-sap	4	34.11482043	27.08304906	32.77072732	25.48820486	0	0		
		0	0	0	0	0	0	0	0
128-sap	5	31.83399464	30.44077271	29.38280728	28.27287459	0	0		
		0	0	0	0	0	0	0	0
128-sap	6	32.16920613	0	18.2485968	23.21372554	0	35.60258932		
		0	0	0	0	0	20.63513571	0	
128-sap	7	28.82851445	14.32400105	16.09055977	21.027599	0			
		29.91728499	0	15.93715473	16.68881157	0	0	0	0
		17.86421106	0						
128-sap	8	32.69738534	18.80012587	23.17287251	0	0	29.53132791		
		0	17.19872217	20.18820098	0	0	0	0	0
128-sap	9	36.7624943	28.77310887	24.57281188	0	0	29.1441623		
		0	0	0	0	0	0	0	0
128-sap	10	33.34138287	27.52114242	34.43523385	0	0	23.93832764		
		0	0	0	0	0	0	0	0
128-sap	11	35.26171824	28.09299945	29.68339905	0	0	26.54570994		
		0	0	0	0	0	0	0	0
128-leaf	1	23.51186004	24.63197589	28.88651466	8.565412479	9.599340294			
		20.55275635	4.789180702	17.02009605	12.10144956	4.043750522	4.450143164		
		9.568465549	0	4.232611189	12.49042667	10.48028343			

128-leaf	2	32.60166085	24.05961075	24.29824613	8.185872051	12.75513828
		17.97375929	3.894112226	14.66235345	12.44805697	4.425477804
		7.861821134	0	4.574475698	12.74728209	4.946842421
128-leaf	3	31.518758	24.88018077	20.92404339	8.717728171	9.771552298
		20.14051884	4.594432718	18.05903955	13.16721482	4.15438349
		9.715835675	0	3.934947225	12.11080192	5.298906271
128-leaf	4	35.84317524	24.63002146	20.47292713	8.85505886	11.11857504
		18.58949603	3.779122387	15.83569094	11.23551841	3.647176452
		8.250318384	0	3.353973546	11.63347656	4.568960195
128-leaf	5	32.65321321	22.50401181	24.40761941	8.889746385	11.872068
		18.33643783	4.529624368	15.85138322	11.63835731	4.118633757
		7.780829534	0	3.437090981	11.98373288	9.494435063
128-leaf	6	34.37296085	22.77511358	22.8956596	8.355573571	14.15779205
		20.52727092	3.760490333	12.42322203	11.30535529	3.477314394
		6.254542521	0	3.896259176	11.29217183	8.81224037
128-leaf	7	29.67450788	25.88948668	20.65854366	8.27891302	11.67145292
		18.82372849	5.271366814	17.79313935	12.17916603	5.360442559
		8.686288258	0	4.027986288	12.37326299	10.82677323
128-leaf	8	35.41933312	23.30404975	22.87565647	8.870796873	12.65877232
		19.47607764	0	12.68543179	10.38178719	3.702755231
		6.286019115	0	3.606782661	12.13228302	8.635888613
128-leaf	9	24.17793451	25.07046024	25.99473825	7.680737569	10.6343376
		20.98652245	5.456159204	21.43467971	11.88697362	4.421902848
		11.08700252	2.716709655	5.26939147	0	10.74408436
128-leaf	10	33.32121587	22.67333334	23.69475817	8.018574022	13.54761287
		19.16609055	4.400375186	16.23157147	9.663271874	4.09461914
		7.81603037	0	4.251726467	10.74473307	8.873748579
128-leaf	11	33.50902476	21.02576453	25.94926329	7.865630033	14.85536333
		20.61161855	4.027692499	12.39641508	9.239385768	3.913438952
		5.278430073	0	4.319180193	11.58469945	9.019817647
128-leaf	12	28.63451592	21.530412	26.64977465	8.596676622	13.14038338
		19.4873176	3.978521733	16.5666469	12.21702216	3.855916238
		7.64054968	0	6.404641929	12.01501542	10.58262686

```

;
run;
Proc sort;
by variety;
run;
Proc ttest data=leafvssap;
class variety;
var aspartic;
run;
Proc ttest data=leafvssap;
class variety;
var glutamic;
run;
Proc ttest data=leafvssap;
class variety;

```

```

var serine;
run;
Proc ttest data=leafvssap;
class variety;
var histidine;
run;
Proc ttest data=leafvssap;
class variety;
var glycine;
run;
Proc ttest data=leafvssap;
class variety;
var threonine;
run;
Proc ttest data=leafvssap;
class variety;
var arginine;
run;
Proc ttest data=leafvssap;
class variety;
var alanine;
run;
Proc ttest data=leafvssap;
class variety;
var valine;
run;
Proc ttest data=leafvssap;
class variety;
var tyrosine;
run;
Proc ttest data=leafvssap;
class variety;
var proline;
run;
Proc ttest data=leafvssap;
class variety;
var isoleucine;
run;
Proc ttest data=leafvssap;
class variety;
var leucine;
run;
Proc ttest data=leafvssap;
class variety;
var lysine;
run;
Proc ttest data=leafvssap;
class variety;

```

```

var phenyl;
run;
Proc ttest data=leafvssap;
class variety;
var methionine;
run;

dm'log;clear;output;clear';
Title Analysis of relative amounts of FAAs in whole leaf tissue and Sap of HoCP 91-555
after arcsin transformation;
options nodate nonumber ps=55 ls=78;
data leafvssap;
input variety$ rep$ alanine aspartic glutamic glycine proline serine tyrosine arginine histidine
isoleucine leucine lysine methionine phenyl threonine valine;
cards;

```

```

555-sap      1      35.53144377  25.06557182  25.82959131  16.51828764  0
              21.94151484  0      0      0      0      0      0      0      0      0      15.61901821
              0
555-sap      2      26.97256605  31.02667985  28.55303687  0      0      23.37382361
              0      0      0      0      0      0      0      0      13.89339672  16.96067378
555-sap      3      33.43626179  26.33765485  26.64828908  12.67299482  0
              20.04723359  0      0      0      0      0      0      0      0      0      14.31547192
              15.5220564
555-sap      4      35.06034294  29.40397114  29.49693792  0      0      25.58830818
              0      0      0      0      0      0      0      0      0      0
555-sap      5      36.42789427  25.6723764   31.49781043  0      0      25.60268537
              0      0      0      0      0      0      0      0      0      0
555-sap      6      36.98673889  26.97195933  28.01048238  13.03680601  0
              18.90478241  0      0      0      0      0      0      0      0      0      13.67799782
              0
555-sap      7      36.50967051  27.67118373  30.81634535  0      0      24.19113996
              0      0      0      0      0      0      0      0      0      0
555-sap      8      36.38171761  27.46105414  31.49367083  0      0      23.78047523
              0      0      0      0      0      0      0      0      0      0
555-sap      9      34.14000755  25.70144429  32.81535481  26.79808364  0      0
              0      0      0      0      0      0      0      0      0      0
555-sap     10      43.25670995  20.58940716  21.67605812  16.30327556  0
              25.95176441  0      0      0      0      0      0      0      0      0      0

```

555-sap	11	34.14952836	27.16580321	28.01408675	18.4995816	0					
	23.196211	0	0	0	0	0	0	0	0	0	0
555-sap	12	40.26026437	22.41916572	23.65750639	19.97991689	0					
	23.50993602	0	0	0	0	0	0	0	0	0	0
555-sap	13	39.86641446	22.92830176	25.92723204	18.54392838	0					
	22.38530093	0	0	0	0	0	0	0	0	0	0
555-sap	14	38.70014175	0	28.66689529	24.58310981	0					26.98350942
	0	0	0	0	0	0	0	0	0	0	
555-leaf	1	0	17.94445156	27.52197136	8.956160006	42.65413676					
	17.19430598	0	9.929871579	8.68991618	0	0	7.6380853	0			
	0	10.19500654	7.978766425								
555-leaf	2	39.14425145	18.01452607	23.885513	0	22.32133955					
	18.50084053	0	7.401314372	7.508863107	0	3.76886993	5.267414172				
	0	3.934102287	10.14052819	6.995655825							
555-leaf	3	29.41177352	21.3089903	32.28170783	0	14.08986201					
	18.92217552	4.176090951	9.238300801	10.72561553	5.187953087	5.211714221					
	6.986925458	0	3.595693479	13.17595502	8.965143587						
555-leaf	4	32.96421702	15.45568796	20.99094413	8.041625322	34.65867522					
	18.52456955	0	8.097817738	0	0	0	5.54995193	0	0		
	7.657988861	6.728993498									
555-leaf	5	41.14527456	15.42909923	22.43434504	7.469401266	27.40901237					
	15.97171521	0	0	0	0	6.182561294	0	0			
	8.081812514	6.980010332									
555-leaf	6	34.23391434	19.54150097	23.00862993	7.843874763	26.65148712					
	15.92421412	0	11.59074967	8.705027261	0	0	7.031109873	0			
	0	9.487623735	7.776880451								
555-leaf	7	44.68727264	17.77308312	21.62232666	7.23980307	20.79667541					
	16.08002581	0	7.005941041	0	0	0	5.32022623	0	0		
	8.236165136	6.756388556									
555-leaf	8	38.79084952	14.66474542	22.77079167	7.034140896	30.37394805					
	14.06777785	0	8.31513354	0	0	0	5.536033514	0	0		
	7.852683648	7.031952179									
555-leaf	9	41.48926962	21.85377791	22.02106333	8.009366861	14.40870466					
	19.07921257	0	9.179255744	6.777333752	0	0	5.075395191	0			
	0	9.591807736	7.886306654								
555-leaf	10	41.56717295	19.49556731	24.8615751	7.00412484	14.7766181					
	19.62503734	0	0	6.063237976	3.158567981	3.420790984	4.843369236				
	0	4.055163196	9.691401033	8.269496998							
555-leaf	11	47.05170435	12.06862862	21.25609375	7.859853541	22.38158838					
	16.36606667	0	5.921254894	0	0	0	0	0	0		
	8.204038022	7.030759392									

```
555-leaf      12      43.38312519  14.20503383  21.99349021  8.323402348  26.76088179
              13.16172468  0          7.378135332  0          0          0          0          0
              7.930580369  7.417004037
```

```
;  
run;  
Proc sort;  
by variety;  
run;  
Proc ttest data=leafvssap;  
class variety;  
var aspartic;  
  
run;  
Proc ttest data=leafvssap;  
class variety;  
var glutamic;  
run;  
Proc ttest data=leafvssap;  
class variety;  
var serine;  
run;  
Proc ttest data=leafvssap;  
class variety;  
var histidine;  
run;  
Proc ttest data=leafvssap;  
class variety;  
var glycine;  
run;  
Proc ttest data=leafvssap;  
class variety;  
var threonine;  
run;  
Proc ttest data=leafvssap;  
class variety;  
var arginine;  
run;  
Proc ttest data=leafvssap;  
class variety;  
var alanine;  
run;  
Proc ttest data=leafvssap;  
class variety;  
var valine;  
run;  
Proc ttest data=leafvssap;  
class variety;  
var tyrosine;
```

```

run;
Proc ttest data=leafvssap;
class variety;
var proline;
run;
Proc ttest data=leafvssap;
class variety;
var isoleucine;
run;
Proc ttest data=leafvssap;
class variety;
var leucine;
run;
Proc ttest data=leafvssap;
class variety;
var lysine;
run;
Proc ttest data=leafvssap;
class variety;
var phenyl;
run;
Proc ttest data=leafvssap;
class variety;
var methionine;
run;

```

```
dm'log;clear;output;clear';
```

Title Concentration of total FAAs, total essential FAAs, and total nonessential FAAs in the sap of L 97-128 and HoCP 91-555;

```
options nodate nonumber ps=55 ls=78;
```

```
data sap;
```

```
input variety$ rep$ totalFAA totalessential totalnonessential;
```

```
cards;
```

128	1	821.5663125	552.8401092	268.7262033
128	2	829.6458006	333.1953575	496.4504431
128	3	1004.067941	282.0479692	722.0199718
128	4	424.1205962	0	424.1205962
128	5	314.3887905	0	314.3887905
128	6	791.6177077	98.31635373	693.301354
128	7	1097.899738	276.6348924	821.2648459
128	8	631.7631085	130.47681	501.2862985
128	9	586.3651958	0	586.3651958
128	10	558.3634179	0	558.3634179
128	11	510.9095227	0	510.9095227
555	1	855.8564878	62.04096243	793.8155254
555	2	1431.196865	204.3084527	1226.888412
555	3	1449.259917	192.3929994	1256.866918


```

555 4 401.0672549 0 401.0672549
555 5 476.5404517 0 476.5404517
555 6 1616.836358 90.40656389 1526.429794
555 7 437.0765779 0 437.0765779
555 8 520.8581264 0 520.8581264
555 9 344.4582353 0 344.4582353
555 10 1093.255698 0 1093.255698
555 11 716.7388319 0 716.7388319
555 12 549.0355405 0 549.0355405
555 13 649.7162294 0 649.7162294
555 14 393.7858056 0 393.7858056

```

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var totalFAA totalessential totalnonessential;
by variety;
run;
;
Proc ttest data= sap;
class variety;
var totalFAA;
run;
Proc ttest data=sap;
class variety;
var totalessential;
run;
Proc ttest data=sap;
class variety;
var totalnonessential;
run;
quit;

```

```
dm'log;clear;output;clear';
```

Title Analysis of relative amounts of FAAs in phloem sap samples of L 97-128 and HoCP 91-555-after transformation;

```
options nodate nonumber ps=55 ls=78;
```

```
data sap;
```

```
input variety$ rep$ alanine aspartic glutamic glycine serine arginine histidine threonine valine;
cards;
```

128	1	21.8641153	0	19.37924022	0	16.24947296	22.69768654
		46.37649805	0	0			
128	2	26.55842512	20.82106944	25.96159049	0	16.48207973	
		13.51651993	36.08986081	0	0		
128	3	21.32744228	27.15470249	24.18636454	19.39983329	18.46688708	
		15.6772843	20.71066975	16.72531464	0		

128	4	34.11482043	27.08304906	32.77072732	25.48820486	0	0
0	0	0					
128	5	31.83399464	30.44077271	29.38280728	28.27287459	0	0
0	0	0					
128	6	32.16920613	0	18.2485968	23.21372554	35.60258932	0
0	20.63513571	0					
128	7	28.82851445	14.32400105	16.09055977	21.027599	29.91728499	
15.93715473		16.68881157	17.86421106	0			
128	8	32.69738534	18.80012587	23.17287251	0	29.53132791	
17.19872217		20.18820098	0	0			
128	9	36.7624943	28.77310887	24.57281188	0	29.1441623	0
0	0	0					
128	10	33.34138287	27.52114242	34.43523385	0	23.93832764	0
0	0	0					
128	11	35.26171824	28.09299945	29.68339905	0	26.54570994	0
0	0	0					
555	1	35.53144377	25.06557182	25.82959131	16.51828764	21.94151484	
0	0	15.61901821	0				
555	2	26.97256605	31.02667985	28.55303687	0	23.37382361	0
0	13.89339672	16.96067378					
555	3	33.43626179	26.33765485	26.64828908	12.67299482	20.04723359	
0	0	14.31547192	15.5220564				
555	4	35.06034294	29.40397114	29.49693792	0	25.58830818	0
0	0	0					
555	5	36.42789427	25.6723764	31.49781043	0	25.60268537	0
0	0	0					
555	6	36.98673889	26.97195933	28.01048238	13.03680601	18.90478241	
0	0	13.67799782	0				
555	7	36.50967051	27.67118373	30.81634535	0	24.19113996	0
0	0	0					
555	8	36.38171761	27.46105414	31.49367083	0	23.78047523	0
0	0	0					
555	9	34.14000755	25.70144429	32.81535481	26.79808364	0	0
0	0	0					
555	10	43.25670995	20.58940716	21.67605812	16.30327556	25.95176441	
0	0	0	0				
555	11	34.14952836	27.16580321	28.01408675	18.4995816	23.196211	
0	0	0	0				
555	12	40.26026437	22.41916572	23.65750639	19.97991689	23.50993602	
0	0	0	0				
555	13	39.86641446	22.92830176	25.92723204	18.54392838	22.38530093	
0	0	0	0				
555	14	38.70014175	0	28.66689529	24.58310981	26.98350942	0
0	0	0					

```

;
run;
Proc sort;
by variety;

```

```

run;
Proc ttest data=sap;
class variety;
var aspartic;
run;
Proc ttest data=sap;
class variety;
var glutamic;
run;
Proc ttest data=sap;
class variety;
var serine;
run;
Proc ttest data=sap;
class variety;
var histidine;
run;
Proc ttest data=sap;
class variety;
var glycine;
run;
Proc ttest data=sap;
class variety;
var threonine;
run;
Proc ttest data=sap;
class variety;
var arginine;
run;
Proc ttest data=sap;
class variety;
var alanine;
run;
Proc ttest data=sap;
class variety;
var valine;
run;

```

```
dm'log;clear;output;clear';
```

Title Concentration of total FAAs, total essential AAs, and total nonessential AAs per mg in the honeydew of aphids feeding on L 97-128 and HoCP 91-555;

```
options nodate nonumber ps=55 ls=78;
```

```
data hd;
```

```
input variety$ rep$ totalFAA totalessential totalnonessential;
```

```
cards;
```

128	1	619.0670361	118.1363806	500.9306555
128	2	229.7342485	26.35635242	203.377896
128	3	404.0508652	0	404.0508652

```

128 4 303.5556883 164.9031835 138.6525048
128 5 447.942551 146.4821269 301.4604241
555 1 68.39213476 13.11335828 55.27877649
555 2 98.11026517 42.88010203 55.23016314
555 3 63.78528704 14.88573921 48.89954783
555 4 172.9164677 84.51892538 88.39754235
555 5 85.301965 30.0788349 55.2231301

```

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var totalFAA totalessential totalnonessential;
by variety;
run;
;
Proc ttest data= hd;
class variety;
var totalFAA;
run;
Proc ttest data=hd;
class variety;
var totalessential;
run;
Proc ttest data=hd;
class variety;
var totalnonessential;
run;
quit;

```

```
dm'log;clear;output;clear';
```

Title Analysis of relative amounts of FAAs in honeydew of aphids feeding on L 97-128 and HoCP 91-555 data after arcsin transformation;

```
options nodate nonumber ps=55 ls=78;
```

```
data hd;
```

```
input variety$ rep$ alanine aspartic glutamic glycine serine tyrosine proline arginine histidine
isoleucine leucine lysine phenyl threonine valine;
```

```
cards;
```

```

128 1 8.799048913 30.70403969 32.4773006 14.0459895 20.05657218
6.944876786 12.32801594 15.35729499 7.677463411 6.597876982 11.62353087
5.482465993 0 11.5250181 0
128 2 16.45894052 22.49741228 31.7713548 0 21.71597675 0
29.63117356 19.79838347 0 0 0 0 0 0 0
128 3 0 29.02376043 26.44200823 0 22.90630771 0 40.09601453
0 0 0 0 0 0 0 0

```

```

128 4 9.468660751 24.38738884 21.69686925 12.31174006 13.64611766
8.41302855 0 25.62959527 23.39576375 7.868668597 9.043348369
9.249074758 8.881760204 9.611128865 16.16081168
128 5 12.18225562 26.20071983 25.39986065 11.2387036 25.57600456
5.727750496 7.086800468 19.29239364 13.74337505 6.449775192 5.86241329
8.964165094 6.963347262 14.69806258 10.78393208
555 1 0 26.94149424 37.43749926 17.04390334 22.58789421 0 0
20.85703358 0 0 0 0 0 14.76780142 0
555 2 12.18851958 21.09101371 28.59525571 13.33979811 19.05267264 0
0 18.74928952 26.23722108 0 0 10.72138089 10.80605648
12.47557151 8.505755734
555 3 0 27.35063315 31.1996977 18.57336841 25.52991104 0 0
12.53378252 12.46281561 0 0 11.8769412 13.02428996 12.46087082
0
555 4 10.68006076 27.15512462 28.12329951 0 12.43636194 0 0
10.15984884 21.96967855 0 0 15.81424651 14.2289194 20.90699024
13.64865569
555 5 18.61546649 23.51130721 38.43036229 0 0 0 0 0
26.89228419 0 0 18.4541169 12.63228024 0 0

```

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var alanine aspartic glutamic glycine serine tyrosine proline arginine histidine isoleucine leucine
lysine phenyl threonine valine;
by variety;
run;
Proc ttest data=hd;
class variety;
var aspartic;
run;
Proc ttest data=hd;
class variety;
var glutamic;
run;
Proc ttest data=hd;
class variety;
var serine;
run;
Proc ttest data=hd;
class variety;
var histidine;
run;

Proc ttest data=hd;
class variety;

```

```

var glycine;
run;
Proc ttest data=hd;
class variety;
var threonine;
run;
Proc ttest data=hd;
class variety;
var arginine;
run;
Proc ttest data=hd;
class variety;
var alanine;
run;
Proc ttest data=hd;
class variety;
var valine;
run;
Proc ttest data=hd;
class variety;
var tyrosine;
run;
Proc ttest data=hd;
class variety;
var proline;
run;
Proc ttest data=hd;
class variety;
var isoleucine;
run;
Proc ttest data=hd;
class variety;
var leucine;
run;
Proc ttest data=hd;
class variety;
var lysine;
run;
Proc ttest data=hd;
class variety;
var phenyl;
run;

```

```
dm'log;clear;output;clear';
```

Title Analysis of relative amounts of FAAs in the sap and honeydew of aphids feeding on L 97-128 after arcsin transformation;

```
options nodate nonumber ps=55 ls=78;
```

```
data saphd;
```

```

input variety$ rep$ alanine aspartic glutamic glycine serine tyrosine proline arginine histidine
isoleucine leucine lysine phenyl threonine valine;
cards;
128-HD      1      8.799048913 30.70403969 32.4773006 14.0459895 20.05657218
             6.944876786 12.32801594 15.35729499 7.677463411 6.597876982 11.62353087
             5.482465993 0      11.5250181 0
128-HD      2      16.45894052 22.49741228 31.7713548 0      21.71597675 0
             29.63117356 19.79838347 0      0      0      0      0      0      0
128-HD      3      0      29.02376043 26.44200823 0      22.90630771 0
             40.09601453 0      0      0      0      0      0      0
128-HD      4      9.468660751 24.38738884 21.69686925 12.31174006 13.64611766
             8.41302855 0      25.62959527 23.39576375 7.868668597 9.043348369
             9.249074758 8.881760204 9.611128865 16.16081168
128-HD      5      12.18225562 26.20071983 25.39986065 11.2387036 25.57600456
             5.727750496 7.086800468 19.29239364 13.74337505 6.449775192 5.86241329
             8.964165094 6.963347262 14.69806258 10.78393208
128-SAP     1      21.8641153 0      19.37924022 0      16.24947296 0      0
             22.69768654 46.37649805 0      0      0      0      0      0
128-SAP     2      26.55842512 20.82106944 25.96159049 0      16.48207973 0
             0      13.51651993 36.08986081 0      0      0      0      0      0
128-SAP     3      21.32744228 27.15470249 24.18636454 19.39983329 18.46688708
             0      0      15.6772843 20.71066975 0      0      0      0      16.72531464
             0
128-SAP     4      34.11482043 27.08304906 32.77072732 25.48820486 0      0
             0      0      0      0      0      0      0
128-SAP     5      31.83399464 30.44077271 29.38280728 28.27287459 0      0
             0      0      0      0      0      0      0
128-SAP     6      32.16920613 0      18.2485968 23.21372554 35.60258932 0
             0      0      0      0      0      20.63513571 0
128-SAP     7      28.82851445 14.32400105 16.09055977 21.027599 29.91728499
             0      0      15.93715473 16.68881157 0      0      0      0      17.86421106
             0
128-SAP     8      32.69738534 18.80012587 23.17287251 0      29.53132791 0
             0      17.19872217 20.18820098 0      0      0      0      0      0
128-SAP     9      36.7624943 28.77310887 24.57281188 0      29.1441623 0
             0      0      0      0      0      0      0
128-SAP     10     33.34138287 27.52114242 34.43523385 0      23.93832764 0
             0      0      0      0      0      0      0
128-SAP     11     35.26171824 28.09299945 29.68339905 0      26.54570994 0
             0      0      0      0      0      0      0
;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;

```

```

var alanine aspartic glutamic glycine serine tyrosine proline arginine histidine isoleucine leucine
lysine phenyl threonine valine;
by variety;
run;
Proc ttest data=saphd;
class variety;
var aspartic;
run;
Proc ttest data=saphd;
class variety;
var glutamic;
run;
Proc ttest data=saphd;
class variety;
var serine;
run;
Proc ttest data=saphd;
class variety;
var histidine;
run;
Proc ttest data=saphd;
class variety;
var glycine;
run;
Proc ttest data=saphd;
class variety;
var threonine;
run;
Proc ttest data=saphd;
class variety;
var arginine;
run;
Proc ttest data=saphd;
class variety;
var alanine;
run;
Proc ttest data=saphd;
class variety;
var valine;
run;
run;
Proc ttest data=saphd;
class variety;
var tyrosine;
run;
Proc ttest data=saphd;
class variety;
var proline;

```



```
run;
Proc ttest data=saphd;
class variety;
var isoleucine;
run;
Proc ttest data=saphd;
class variety;
var leucine;
run;
Proc ttest data=saphd;
class variety;
var lysine;
run;
Proc ttest data=saphd;
class variety;
var phenyl;
run;
```

```
dm'log;clear;output;clear';
```

Title Analysis of relative amounts of FAAs in the sap and honeydew of aphids feeding on HoCP 91-555 after arcsin transformation;

```
options nodate nonumber ps=55 ls=78;
```

```
data saphd;
```

```
input variety$ rep$ alanine aspartic glutamic glycine serine arginine histidine lysine phenyl
threonine valine;
```

```
cards;
```

```
555-HD      1      0      26.94149424  37.43749926  17.04390334  22.58789421
            20.85703358  0      0      0      14.76780142  0
555-HD      2      12.18851958  21.09101371  28.59525571  13.33979811  19.05267264
            18.74928952  26.23722108  10.72138089  10.80605648  12.47557151  8.505755734
555-HD      3      0      27.35063315  31.1996977   18.57336841  25.52991104
            12.53378252  12.46281561  11.8769412   13.02428996  12.46087082  0
555-HD      4      10.68006076  27.15512462  28.12329951  0      12.43636194
            10.15984884  21.96967855  15.81424651  14.2289194  20.90699024  13.64865569
555-HD      5      18.61546649  23.51130721  38.43036229  0      0      0
            26.89228419  18.4541169   12.63228024  0      0
555-SAP     1      35.53144377  25.06557182  25.82959131  16.51828764  21.94151484
            0      0      0      0      15.61901821  0
555-SAP     2      26.97256605  31.02667985  28.55303687  0      23.37382361  0
            0      0      0      13.89339672  16.96067378
555-SAP     3      33.43626179  26.33765485  26.64828908  12.67299482  20.04723359
            0      0      0      0      14.31547192  15.5220564
555-SAP     4      35.06034294  29.40397114  29.49693792  0      25.58830818  0
            0      0      0      0      0
555-SAP     5      36.42789427  25.6723764   31.49781043  0      25.60268537  0
            0      0      0      0      0
555-SAP     6      36.98673889  26.97195933  28.01048238  13.03680601  18.90478241
            0      0      0      0      13.67799782  0
```

555-SAP	7	36.50967051	27.67118373	30.81634535	0	24.19113996	0
0	0	0	0	0			
555-SAP	8	36.38171761	27.46105414	31.49367083	0	23.78047523	0
0	0	0	0	0			
555-SAP	9	34.14000755	25.70144429	32.81535481	26.79808364	0	0
0	0	0	0	0			
555-SAP	10	43.25670995	20.58940716	21.67605812	16.30327556	25.95176441	
0	0	0	0	0			
555-SAP	11	34.14952836	27.16580321	28.01408675	18.4995816	23.196211	
0	0	0	0	0			
555-SAP	12	40.26026437	22.41916572	23.65750639	19.97991689	23.50993602	
0	0	0	0	0			
555-SAP	13	39.86641446	22.92830176	25.92723204	18.54392838	22.38530093	
0	0	0	0	0			
555-SAP	14	38.70014175	0	28.66689529	24.58310981	26.98350942	0
0	0	0	0	0			

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var alanine aspartic glutamic glycine serine arginine histidine lysine phenyl threonine valine;
by variety;
run;
Proc ttest data=saphd;
class variety;
var aspartic;
run;
Proc ttest data=saphd;
class variety;
var glutamic;
run;
Proc ttest data=saphd;
class variety;
var serine;
run;
Proc ttest data=saphd;
class variety;
var histidine;
run;
Proc ttest data=saphd;
class variety;
var glycine;
run;
Proc ttest data=saphd;
class variety;
var threonine;

```

```

run;
Proc ttest data=saphd;
class variety;
var arginine;
run;
Proc ttest data=saphd;
class variety;
var alanine;
run;
Proc ttest data=saphd;
class variety;
var valine;
run;
run;
Proc ttest data=saphd;
class variety;
var lysine;
run;
Proc ttest data=saphd;
class variety;
var phenyl;
run;

```

```
dm'log;clear;output;clear';
```

Title Analysis of relative amounts of FAAs in the sap and honeydew of aphids feeding on L 97-128 after arcsin transformation;

```
options nodate nonumber ps=55 ls=78;
```

```
data saphd;
```

```
input variety$ rep$ alanine aspartic glutamic glycine serine tyrosine proline arginine histidine
isoleucine leucine lysine phenyl threonine valine;
```

```
cards;
```

128-HD	1	8.799048913	30.70403969	32.4773006	14.0459895	20.05657218				
		6.944876786	12.32801594	15.35729499	7.677463411	6.597876982	11.62353087			
		5.482465993	0	11.5250181	0					
128-HD	2	16.45894052	22.49741228	31.7713548	0	21.71597675	0			
		29.63117356	19.79838347	0	0	0	0	0	0	0
128-HD	3	0	29.02376043	26.44200823	0	22.90630771	0			
		40.09601453	0	0	0	0	0	0	0	
128-HD	4	9.468660751	24.38738884	21.69686925	12.31174006	13.64611766				
		8.41302855	0	25.62959527	23.39576375	7.868668597	9.043348369			
		9.249074758	8.881760204	9.611128865	16.16081168					
128-HD	5	12.18225562	26.20071983	25.39986065	11.2387036	25.57600456				
		5.727750496	7.086800468	19.29239364	13.74337505	6.449775192	5.86241329			
		8.964165094	6.963347262	14.69806258	10.78393208					
128-SAP	1	21.8641153	0	19.37924022	0	16.24947296	0	0		
		22.69768654	46.37649805	0	0	0	0	0	0	
128-SAP	2	26.55842512	20.82106944	25.96159049	0	16.48207973	0			
		0	13.51651993	36.08986081	0	0	0	0	0	0

128-SAP	3	21.32744228	27.15470249	24.18636454	19.39983329	18.46688708		
0	0	15.6772843	20.71066975	0	0	0	0	16.72531464
0								
128-SAP	4	34.11482043	27.08304906	32.77072732	25.48820486	0	0	
0	0	0	0	0	0	0	0	
0								
128-SAP	5	31.83399464	30.44077271	29.38280728	28.27287459	0	0	
0	0	0	0	0	0	0	0	
0								
128-SAP	6	32.16920613	0	18.2485968	23.21372554	35.60258932	0	
0	0	0	0	0	20.63513571	0		
0								
128-SAP	7	28.82851445	14.32400105	16.09055977	21.027599	29.91728499		
0	0	15.93715473	16.68881157	0	0	0	0	17.86421106
0								
128-SAP	8	32.69738534	18.80012587	23.17287251	0	29.53132791	0	
0	17.19872217	20.18820098	0	0	0	0	0	0
0								
128-SAP	9	36.7624943	28.77310887	24.57281188	0	29.1441623	0	
0	0	0	0	0	0	0	0	
0								
128-SAP	10	33.34138287	27.52114242	34.43523385	0	23.93832764	0	
0	0	0	0	0	0	0	0	
0								
128-SAP	11	35.26171824	28.09299945	29.68339905	0	26.54570994	0	
0	0	0	0	0	0	0	0	
0								

```

;
run;
Proc sort;
by variety;
run;
Proc means mean n stderr clm;
var alanine aspartic glutamic glycine serine tyrosine proline arginine histidine isoleucine leucine
lysine phenyl threonine valine;
by variety;
run;
Proc ttest data=saphd;
class variety;
var aspartic;
run;
Proc ttest data=saphd;
class variety;
var glutamic;
run;
Proc ttest data=saphd;
class variety;
var serine;
run;
Proc ttest data=saphd;
class variety;
var histidine;
run;
Proc ttest data=saphd;

```

```

class variety;
var glycine;
run;
Proc ttest data=saphd;
class variety;
var threonine;
run;
Proc ttest data=saphd;
class variety;
var arginine;
run;
Proc ttest data=saphd;
class variety;
var alanine;
run;
Proc ttest data=saphd;
class variety;
var valine;
run;
run;
Proc ttest data=saphd;
class variety;
var tyrosine;
run;
Proc ttest data=saphd;
class variety;
var proline;
run;
Proc ttest data=saphd;
class variety;
var isoleucine;
run;
Proc ttest data=saphd;
class variety;
var leucine;
run;
Proc ttest data=saphd;
class variety;
var lysine;
run;
Proc ttest data=saphd;
class variety;
var phenyl;
run;

```

```
dm'log;clear;output;clear';
```

Title Analysis of relative amounts of FAAs in the sap and honeydew of aphids feeding on HoCP 91-555 after arcsin transformation;

```

options nodate nonumber ps=55 ls=78;
data saphd;
input variety$ rep$ alanine aspartic glutamic glycine serine arginine histidine lysine phenyl
threonine valine;
cards;
555-HD      1      0      26.94149424  37.43749926  17.04390334  22.58789421
            20.85703358  0      0      0      14.76780142  0
555-HD      2      12.18851958  21.09101371  28.59525571  13.33979811  19.05267264
            18.74928952  26.23722108  10.72138089  10.80605648  12.47557151  8.505755734
555-HD      3      0      27.35063315  31.1996977   18.57336841  25.52991104
            12.53378252  12.46281561  11.8769412   13.02428996  12.46087082  0
555-HD      4      10.68006076  27.15512462  28.12329951  0      12.43636194
            10.15984884  21.96967855  15.81424651  14.2289194  20.90699024  13.64865569
555-HD      5      18.61546649  23.51130721  38.43036229  0      0      0
            26.89228419  18.4541169  12.63228024  0      0
555-SAP     1      35.53144377  25.06557182  25.82959131  16.51828764  21.94151484
            0      0      0      0      15.61901821  0
555-SAP     2      26.97256605  31.02667985  28.55303687  0      23.37382361  0
            0      0      0      13.89339672  16.96067378
555-SAP     3      33.43626179  26.33765485  26.64828908  12.67299482  20.04723359
            0      0      0      0      14.31547192  15.5220564
555-SAP     4      35.06034294  29.40397114  29.49693792  0      25.58830818  0
            0      0      0      0      0
555-SAP     5      36.42789427  25.6723764   31.49781043  0      25.60268537  0
            0      0      0      0      0
555-SAP     6      36.98673889  26.97195933  28.01048238  13.03680601  18.90478241
            0      0      0      0      13.67799782  0
555-SAP     7      36.50967051  27.67118373  30.81634535  0      24.19113996  0
            0      0      0      0      0
555-SAP     8      36.38171761  27.46105414  31.49367083  0      23.78047523  0
            0      0      0      0      0
555-SAP     9      34.14000755  25.70144429  32.81535481  26.79808364  0      0
            0      0      0      0      0
555-SAP    10      43.25670995  20.58940716  21.67605812  16.30327556  25.95176441
            0      0      0      0      0
555-SAP    11      34.14952836  27.16580321  28.01408675  18.4995816   23.196211
            0      0      0      0      0
555-SAP    12      40.26026437  22.41916572  23.65750639  19.97991689  23.50993602
            0      0      0      0      0
555-SAP    13      39.86641446  22.92830176  25.92723204  18.54392838  22.38530093
            0      0      0      0      0
555-SAP    14      38.70014175  0      28.66689529  24.58310981  26.98350942  0
            0      0      0      0      0
;
run;
Proc sort;
by variety;
run;

```

```

Proc means mean n stderr clm;
var alanine aspartic glutamic glycine serine arginine histidine lysine phenyl threonine valine;
by variety;
run;
Proc ttest data=saphd;
class variety;
var aspartic;
run;
Proc ttest data=saphd;
class variety;
var glutamic;
run;
Proc ttest data=saphd;
class variety;
var serine;
run;
Proc ttest data=saphd;
class variety;
var histidine;
run;
Proc ttest data=saphd;
class variety;
var glycine;
run;
Proc ttest data=saphd;
class variety;
var threonine;
run;
Proc ttest data=saphd;
class variety;
var arginine;
run;
Proc ttest data=saphd;
class variety;
var alanine;
run;
Proc ttest data=saphd;
class variety;
var valine;
run;
Proc ttest data=saphd;
class variety;
var lysine;
run;
Proc ttest data=saphd;
class variety;
var phenyl;
run;

```

APPENDIX D: SAS CODES FOR CHAPTER 6

```
dm'log;clear;output;clear';
options nodate nonumber ps=55 ls=78;
title Effect of variety and week on total aphid numbers per plant include. 2007 data;
```

```
Data totalaphids2007;
input year$ week variety$ rep aphidsperplant logaphidsperplant;
cards;
2007 1 128 1 2 0.477121255
2007 1 128 2 5.9 0.838849091
2007 1 128 3 1.9 0.462397998
2007 1 128 4 2 0.477121255
2007 1 128 5 1.3 0.361727836
2007 1 384 1 3.4 0.643452676
2007 1 384 2 2 0.477121255
2007 1 384 3 2.5 0.544068044
2007 1 384 4 2.9 0.591064607
2007 1 384 5 10 1.041392685
2007 1 540 1 2.2 0.505149978
2007 1 540 2 1.4 0.380211242
2007 1 540 3 1.5 0.397940009
2007 1 540 4 0.3 0.113943352
2007 1 540 5 1.2 0.342422681
2007 1 555 1 3.3 0.633468456
2007 1 555 2 0.8 0.255272505
2007 1 555 3 4.5 0.740362689
2007 1 555 4 2.3 0.51851394
2007 1 555 5 0 0
2007 1 988 1 1.2 0.342422681
2007 1 988 2 5.7 0.826074803
2007 1 988 3 4 0.698970004
2007 1 988 4 8.1 0.959041392
2007 1 988 5 22.3 1.367355921
2007 2 128 1 2 0.477121255
2007 2 128 2 1.9 0.462397998
2007 2 128 3 1.4 0.380211242
2007 2 128 4 2 0.477121255
2007 2 128 5 1 0.301029996
2007 2 384 1 1 0.301029996
2007 2 384 2 3.2 0.62324929
2007 2 384 3 2.4 0.531478917
2007 2 384 4 5.8 0.832508913
2007 2 384 5 2 0.477121255
2007 2 540 1 0.1 0.041392685
2007 2 540 2 0 0
2007 2 540 3 0.1 0.041392685
2007 2 540 4 0.1 0.041392685
```


2007	2	540	5	0.1	0.041392685
2007	2	555	1	0	0
2007	2	555	2	0.3	0.113943352
2007	2	555	3	0.9	0.278753601
2007	2	555	4	0	0
2007	2	555	5	0.2	0.079181246
2007	2	988	1	2.3	0.51851394
2007	2	988	2	1.3	0.361727836
2007	2	988	3	2.2	0.505149978
2007	2	988	4	1.1	0.322219295
2007	2	988	5	1.3	0.361727836
2007	3	128	1	5.6	0.819543936
2007	3	128	2	10.8	1.071882007
2007	3	128	3	5.5	0.812913357
2007	3	128	4	9.1	1.004321374
2007	3	128	5	11.4	1.093421685
2007	3	384	1	16	1.230448921
2007	3	384	2	9.6	1.025305865
2007	3	384	3	6.8	0.892094603
2007	3	384	4	9.3	1.012837225
2007	3	384	5	9	1
2007	3	540	1	8	0.954242509
2007	3	540	2	7.8	0.944482672
2007	3	540	3	6.1	0.851258349
2007	3	540	4	5.6	0.819543936
2007	3	540	5	8.1	0.959041392
2007	3	555	1	9.5	1.021189299
2007	3	555	2	7	0.903089987
2007	3	555	3	8.2	0.963787827
2007	3	555	4	5.1	0.785329835
2007	3	555	5	5.8	0.832508913
2007	3	988	1	7.2	0.913813852
2007	3	988	2	17	1.255272505
2007	3	988	3	6.7	0.886490725
2007	3	988	4	11.2	1.086359831
2007	3	988	5	12	1.113943352
2007	4	128	1	10.6	1.064457989
2007	4	128	2	50.7	1.713490543
2007	4	128	3	12.5	1.130333768
2007	4	128	4	11.9	1.11058971
2007	4	128	5	67.5	1.835690571
2007	4	384	1	33.1	1.532754379
2007	4	384	2	17.1	1.257678575
2007	4	384	3	34.3	1.547774705
2007	4	384	4	14.9	1.201397124
2007	4	384	5	11.6	1.100370545
2007	4	540	1	14.7	1.195899652
2007	4	540	2	10	1.041392685

2007	4	540	3	12.2	1.120573931
2007	4	540	4	13.2	1.152288344
2007	4	540	5	19.1	1.303196057
2007	4	555	1	10.6	1.064457989
2007	4	555	2	10.2	1.049218023
2007	4	555	3	12.4	1.127104798
2007	4	555	4	11.8	1.10720997
2007	4	555	5	12.2	1.120573931
2007	4	988	1	10	1.041392685
2007	4	988	2	17.6	1.269512944
2007	4	988	3	26	1.431363764
2007	4	988	4	37.4	1.584331224
2007	4	988	5	17.7	1.271841607
2007	5	128	1	20.5	1.33243846
2007	5	128	2	63.9	1.812244697
2007	5	128	3	20.8	1.338456494
2007	5	128	4	85.7	1.938019097
2007	5	128	5	16.3	1.238046103
2007	5	384	1	20.8	1.338456494
2007	5	384	2	22.2	1.365487985
2007	5	384	3	53.7	1.737987326
2007	5	384	4	22.5	1.371067862
2007	5	384	5	24.6	1.408239965
2007	5	540	1	10.2	1.049218023
2007	5	540	2	19.7	1.315970345
2007	5	540	3	17.7	1.271841607
2007	5	540	4	11.1	1.08278537
2007	5	540	5	15.8	1.225309282
2007	5	555	1	10.2	1.049218023
2007	5	555	2	10	1.041392685
2007	5	555	3	11.3	1.089905111
2007	5	555	4	10.1	1.045322979
2007	5	555	5	10.6	1.064457989
2007	5	988	1	23.5	1.389166084
2007	5	988	2	36.4	1.572871602
2007	5	988	3	39.6	1.608526034
2007	5	988	4	65.9	1.825426118
2007	5	988	5	22.9	1.378397901
2007	6	128	1	42.7	1.640481437
2007	6	128	2	66.8	1.831229694
2007	6	128	3	32	1.51851394
2007	6	128	4	61.5	1.795880017
2007	6	128	5	71.6	1.860936621
2007	6	384	1	24	1.397940009
2007	6	384	2	57	1.763427994
2007	6	384	3	36	1.568201724
2007	6	384	4	58.9	1.777426822
2007	6	384	5	23.3	1.385606274

2007	6	540	1	16.8	1.250420002
2007	6	540	2	11.3	1.089905111
2007	6	540	3	11.6	1.100370545
2007	6	540	4	16.5	1.243038049
2007	6	540	5	26.6	1.440909082
2007	6	555	1	10.2	1.049218023
2007	6	555	2	24.3	1.403120521
2007	6	555	3	11.2	1.086359831
2007	6	555	4	10.8	1.071882007
2007	6	555	5	11.2	1.086359831
2007	6	988	1	46.2	1.673941999
2007	6	988	2	124.9	2.10002573
2007	6	988	3	11.6	1.100370545
2007	6	988	4	26.7	1.442479769
2007	6	988	5	32.2	1.521138084
2007	7	128	1	45.4	1.666517981
2007	7	128	2	76.5	1.889301703
2007	7	128	3	22.5	1.371067862
2007	7	128	4	83	1.924279286
2007	7	128	5	46.4	1.675778342
2007	7	384	1	42.5	1.638489257
2007	7	384	2	74.5	1.877946952
2007	7	384	3	47.6	1.686636269
2007	7	384	4	10.9	1.075546961
2007	7	384	5	80.6	1.911690159
2007	7	540	1	11.4	1.093421685
2007	7	540	2	14.2	1.181843588
2007	7	540	3	13.5	1.161368002
2007	7	540	4	13	1.146128036
2007	7	540	5	31.7	1.514547753
2007	7	555	1	11.1	1.08278537
2007	7	555	2	11.9	1.11058971
2007	7	555	3	12.8	1.139879086
2007	7	555	4	11.4	1.093421685
2007	7	555	5	12.2	1.120573931
2007	7	988	1	49.9	1.706717782
2007	7	988	2	89.9	1.958563883
2007	7	988	3	45.9	1.671172843
2007	7	988	4	57.4	1.766412847
2007	7	988	5	42.3	1.636487896
2007	8	128	1	43.3	1.646403726
2007	8	128	2	141.9	2.155032229
2007	8	128	3	22.1	1.36361198
2007	8	128	4	63.2	1.807535028
2007	8	128	5	53.2	1.733999287
2007	8	384	1	6.6	0.880813592
2007	8	384	2	12.4	1.127104798
2007	8	384	3	27.4	1.45331834

2007	8	384	4	10.5	1.06069784
2007	8	384	5	58.7	1.775974331
2007	8	540	1	11	1.079181246
2007	8	540	2	14.2	1.181843588
2007	8	540	3	12.5	1.130333768
2007	8	540	4	10.4	1.056904851
2007	8	540	5	11.1	1.08278537
2007	8	555	1	7.1	0.908485019
2007	8	555	2	5.3	0.799340549
2007	8	555	3	25.1	1.416640507
2007	8	555	4	2	0.477121255
2007	8	555	5	2.5	0.544068044
2007	8	988	1	43.8	1.651278014
2007	8	988	2	12	1.113943352
2007	8	988	3	25	1.414973348
2007	8	988	4	22	1.361727836
2007	8	988	5	5.7	0.826074803
2007	9	128	1	10	1.041392685
2007	9	128	2	22	1.361727836
2007	9	128	3	87.5	1.946943271
2007	9	128	4	4.2	0.716003344
2007	9	128	5	54.4	1.743509765
2007	9	384	1	0.1	0.041392685
2007	9	384	2	1	0.301029996
2007	9	384	3	0.1	0.041392685
2007	9	384	4	3.9	0.69019608
2007	9	384	5	1.3	0.361727836
2007	9	540	1	14	1.176091259
2007	9	540	2	2.7	0.568201724
2007	9	540	3	1.4	0.380211242
2007	9	540	4	0.7	0.230448921
2007	9	540	5	0.8	0.255272505
2007	9	555	1	0.2	0.079181246
2007	9	555	2	2	0.477121255
2007	9	555	3	5.5	0.812913357
2007	9	555	4	2.2	0.505149978
2007	9	555	5	0	0
2007	9	988	1	37.6	1.586587305
2007	9	988	2	10.8	1.071882007
2007	9	988	3	53	1.73239376
2007	9	988	4	18	1.278753601
2007	9	988	5	6.8	0.892094603
2007	10	128	1	21	1.342422681
2007	10	128	2	5	0.77815125
2007	10	128	3	42	1.633468456
2007	10	128	4	7.6	0.934498451
2007	10	128	5	7.5	0.929418926
2007	10	384	1	2.2	0.505149978

```

2007 10 384 2 0.5 0.176091259
2007 10 384 3 0 0
2007 10 384 4 0.6 0.204119983
2007 10 384 5 0 0
2007 10 540 1 0 0
2007 10 540 2 0 0
2007 10 540 3 0 0
2007 10 540 4 0 0
2007 10 540 5 0 0
2007 10 555 1 0.2 0.079181246
2007 10 555 2 2 0.477121255
2007 10 555 3 0.1 0.041392685
2007 10 555 4 2.2 0.505149978
2007 10 555 5 0 0
2007 10 988 1 17.3 1.26245109
2007 10 988 2 21 1.342422681
2007 10 988 3 12 1.113943352
2007 10 988 4 4.2 0.716003344
2007 10 988 5 0 0
;
proc sort;
by variety week;
run;
proc means n mean var stderr;
var aphidsperplant;
by variety week;
run;
proc mixed data=totalaphids2007;
class rep variety week;
model logaphidsperplant = variety week variety*week;
random rep rep*variety;
repeated / subject= rep*variety type=ar(1) rcorr=1;
lsmeans variety*week / slice=week;
%include 'C:\Documents and Settings\wakbar\Desktop\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;

dm'log;clear;output;clear';
options nodate nonumber ps=55 ls=78;
title Effect of variety and week on M. sacchari numbers per plant 2007 data;
Data SAphids2007;
input year$ week variety$ rep aphidsperplant logaphidsperplant;
cards;
2007 1 128 1 1 0.301029996
2007 1 128 2 1.1 0.322219295
2007 1 128 3 1.7 0.431363764
2007 1 128 4 1.4 0.380211242
2007 1 128 5 1.2 0.342422681

```

2007	1	384	1	1.1	0.322219295
2007	1	384	2	1	0.301029996
2007	1	384	3	0.5	0.176091259
2007	1	384	4	1	0.301029996
2007	1	384	5	0.9	0.278753601
2007	1	540	1	0	0
2007	1	540	2	0	0
2007	1	540	3	0	0
2007	1	540	4	0	0
2007	1	540	5	0	0
2007	1	555	1	0	0
2007	1	555	2	0	0
2007	1	555	3	4.5	0.740362689
2007	1	555	4	2	0.477121255
2007	1	555	5	0	0
2007	1	988	1	0.1	0.041392685
2007	1	988	2	5.3	0.799340549
2007	1	988	3	2.8	0.579783597
2007	1	988	4	2	0.477121255
2007	1	988	5	21.7	1.356025857
2007	2	128	1	1	0.301029996
2007	2	128	2	1.2	0.342422681
2007	2	128	3	0.6	0.204119983
2007	2	128	4	1	0.301029996
2007	2	128	5	0	0
2007	2	384	1	1	0.301029996
2007	2	384	2	1.2	0.342422681
2007	2	384	3	1.3	0.361727836
2007	2	384	4	5.8	0.832508913
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run;
proc means n mean var stderr;
var aphidsperplant;
by variety week;
run;
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class rep variety week;
model logaphidsperplant = variety week variety*week;
random rep rep*variety;
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2007	9	555	1	0	0
2007	9	555	2	0	0
2007	9	555	3	0.1	0.041392685
2007	9	555	4	0	0
2007	9	555	5	0	0
2007	9	988	1	0	0
2007	9	988	2	0	0
2007	9	988	3	0	0
2007	9	988	4	0	0
2007	9	988	5	0	0
2007	10	128	1	0	0
2007	10	128	2	0	0
2007	10	128	3	0	0
2007	10	128	4	0.1	0.041392685
2007	10	128	5	0	0
2007	10	384	1	0.1	0.041392685
2007	10	384	2	0	0
2007	10	384	3	0	0
2007	10	384	4	0	0
2007	10	384	5	0	0
2007	10	540	1	0	0
2007	10	540	2	0.5	0.176091259
2007	10	540	3	0	0
2007	10	540	4	0	0
2007	10	540	5	0	0
2007	10	555	1	0	0
2007	10	555	2	0	0
2007	10	555	3	0.1	0.041392685
2007	10	555	4	0	0
2007	10	555	5	0	0
2007	10	988	1	0	0
2007	10	988	2	0	0
2007	10	988	3	0	0
2007	10	988	4	0	0
2007	10	988	5	0	0

```

;
proc sort;
by variety week;
run;
proc means n mean var stderr;
var aphidsperplant;
by variety week;
run;
proc mixed data=yaphids2007;

```



```

class rep variety week;
model logaphidsperplant = variety week variety*week;
random rep rep*variety;
repeated / subject= rep*variety type=ar(1) rcorr=1;
lsmeans variety*week / slice=week;
%include 'C:\Documents and Settings\wakbar\Desktop\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;

```

```

dm'log;clear;output;clear';
options nodate nonumber ps=55 ls=78;
title Effect of variety and week on M. sacchari and S. flava numbers per plant during June and July aphids 2007 data;

```

```

Data totalaphids2007;
input species$ week variety$ rep aphidsperplant logaphidsperplant;
cards;

```

SA	5	128	1	10.3	1.053078443
SA	5	128	2	60	1.785329835
SA	5	128	3	20	1.322219295
SA	5	128	4	85.7	1.938019097
SA	5	128	5	11	1.079181246
SA	5	384	1	18.6	1.292256071
SA	5	384	2	8.7	0.986771734
SA	5	384	3	50	1.707570176
SA	5	384	4	18.5	1.290034611
SA	5	384	5	14.6	1.193124598
SA	5	540	1	10	1.041392685
SA	5	540	2	17.5	1.267171728
SA	5	540	3	15.5	1.217483944
SA	5	540	4	10.5	1.06069784
SA	5	540	5	5.4	0.806179974
SA	5	555	1	1	0.301029996
SA	5	555	2	5	0.77815125
SA	5	555	3	5	0.77815125
SA	5	555	4	1	0.301029996
SA	5	555	5	10	1.041392685
SA	5	988	1	19.2	1.305351369
SA	5	988	2	35	1.556302501
SA	5	988	3	38	1.591064607
SA	5	988	4	63.9	1.812244697
SA	5	988	5	20	1.322219295
YSA	5	128	1	10.2	1.049218023
YSA	5	128	2	3.9	0.69019608
YSA	5	128	3	0.8	0.255272505
YSA	5	128	4	0	0
YSA	5	128	5	5.3	0.799340549
YSA	5	384	1	2.2	0.505149978
YSA	5	384	2	13.5	1.161368002

YSA	5	384	3	3.7	0.672097858
YSA	5	384	4	4	0.698970004
YSA	5	384	5	10	1.041392685
YSA	5	540	1	0.2	0.079181246
YSA	5	540	2	2.2	0.505149978
YSA	5	540	3	2.2	0.505149978
YSA	5	540	4	0.6	0.204119983
YSA	5	540	5	10.4	1.056904851
YSA	5	555	1	9.2	1.008600172
YSA	5	555	2	5	0.77815125
YSA	5	555	3	6.3	0.86332286
YSA	5	555	4	9.1	1.004321374
YSA	5	555	5	0.6	0.204119983
YSA	5	988	1	4.3	0.72427587
YSA	5	988	2	1.4	0.380211242
YSA	5	988	3	1.6	0.414973348
YSA	5	988	4	2	0.477121255
YSA	5	988	5	2.9	0.591064607

```

;
proc sort;
by species variety;
run;
proc means n mean var stderr;
var aphidsperplant;
by species variety;
run;
proc mixed data=totalaphids2007;
class species variety rep;
model logaphidsperplant= species variety species*variety/ htype=3;
random rep;
lsmeans species*variety/ diff cl adjust=tukey;
contrast 'SA vs. YSA 128' species 1 -1 species*variety 1 0 0 0 0 -1 0 0 0 0;
contrast 'SA vs. YSA 384' species 1 -1 species*variety 0 1 0 0 0 0 -1 0 0 0;
contrast 'SA vs. YSA 540' species 1 -1 species*variety 0 0 1 0 0 0 0 -1 0 0;
contrast 'SA vs. YSA 555' species 1 -1 species*variety 0 0 0 1 0 0 0 0 -1 0;
contrast 'SA vs. YSA 988' species 1 -1 species*variety 0 0 0 0 1 0 0 0 0 -1;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'C:\Documents and Settings\wakbar\Desktop\Pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;

dm'log;clear;output;clear';
options nodate nonumber ps=55 ls=78;
title Effect of variety and week on total aphid numbers per plant include. 2008 data;
Data totalaphids2008;

```

input year\$ week variety\$ rep aphidsperplant logaphidsperplant whiteperplant logwhiteperplant
 yelperplant logyelperplant;

cards;

2008	1	128	1	9	1	0	0	9	1		
2008	1	128	2	9.4	1.017033339	3	0.602059991	6.4	0.86923172		
2008	1	128	3	10	1.041392685	2.8	0.579783597	7.2	0.913813852		
2008	1	128	4	7.2	0.913813852	0	0	7.2	0.913813852		
2008	1	128	5	5	0.77815125	3.6	0.662757832	1.4	0.380211242		
2008	1	384	1	3.9	0.69019608	0.3	0.113943352	3.6	0.662757832		
2008	1	384	2	8.1	0.959041392	0	0	8.1	0.959041392		
2008	1	384	3	8.4	0.973127854	0	0	8.4	0.973127854		
2008	1	384	4	13	1.146128036	11.5	1.096910013	1.5	0.397940009		
2008	1	384	5	1.5	0.397940009	0	0	1.5	0.397940009		
2008	1	540	1	12.3	1.123851641	8.2	0.963787827	4.1	0.707570176		
2008	1	540	2	4.3	0.72427587	0	0	4.3	0.72427587		
2008	1	540	3	12	1.113943352	3.2	0.62324929	8.8	0.991226076		
2008	1	540	4	4.6	0.748188027	0	0	4.6	0.748188027		
2008	1	540	5	10.2	1.049218023	0	0	10.2	1.049218023		
2008	1	555	1	13.6	1.164352856	10	1.041392685	3.6	0.662757832		
2008	1	555	2	5.4	0.806179974	0	0	5.4	0.806179974		
2008	1	555	3	9.6	1.025305865	0	0	9.6	1.025305865		
2008	1	555	4	3.7	0.672097858	0	0	3.7	0.672097858		
2008	1	555	5	4.5	0.740362689	0	0	4.5	0.740362689		
2008	1	988	1	3.6	0.662757832	0	0	3.6	0.662757832		
2008	1	988	2	20.2	1.326335861	8.5	0.977723605	11.7	1.103803721		
2008	1	988	3	7.7	0.939519253	1.2	0.342422681	6.5	0.875061263		
2008	1	988	4	14.5	1.190331698	11	1.079181246	3.5	0.653212514		
2008	1	988	5	5.6	0.819543936	1.8	0.447158031	3.8	0.681241237		
2008	2	128	1	13.1	1.149219113	13	1.146128036	0.1	0.041392685		
2008	2	128	2	1.3	0.361727836	0	0	1.3	0.361727836		
2008	2	128	3	7.6	0.934498451	0	0	7.6	0.934498451		
2008	2	128	4	1.4	0.380211242	1	0.301029996	0.4	0.146128036		
2008	2	128	5	13.9	1.173186268	12.6	1.133538908	1.3	0.361727836		
2008	2	384	1	4	0.698970004	4	0.698970004	0	0		
2008	2	384	2	1.1	0.322219295	0.7	0.230448921	0.4	0.146128036		
2008	2	384	3	0	0	0	0	0			
2008	2	384	4	1.4	0.380211242	0	0	1.4	0.380211242		
2008	2	384	5	0.6	0.204119983	0	0	0.6	0.204119983		
2008	2	540	1	0	0	0	0	0			
2008	2	540	2	1.7	0.431363764	0	0	1.7	0.431363764		
2008	2	540	3	2.9	0.591064607	2.7	0.568201724	0.2	0.079181246		
2008	2	540	4	0	0	0	0	0			
2008	2	540	5	1.8	0.447158031	0	0	1.8	0.447158031		
2008	2	555	1	1.3	0.361727836	0	0	1.3	0.361727836		
2008	2	555	2	0.7	0.230448921	0.1	0.041392685	0.6	0.204119983		
2008	2	555	3	2.7	0.568201724	0	0	2.7	0.568201724		
2008	2	555	4	0	0	0	0	0			
2008	2	555	5	2.1	0.491361694	0	0	2.1	0.491361694		

2008	2	988	1	80.4	1.910624405	77.4	1.894316063	3	0.602059991
2008	2	988	2	49.6	1.704150517	49.5	1.703291378	0.1	0.041392685
2008	2	988	3	15.1	1.206825876	14.5	1.190331698	0.6	0.204119983
2008	2	988	4	25.3	1.419955748	25.2	1.418301291	0.1	0.041392685
2008	2	988	5	29	1.477121255	27.9	1.460897843	1.1	0.322219295
2008	3	128	1	16.5	1.243038049	10	1.041392685	6.5	0.875061263
2008	3	128	2	6.5	0.875061263	6.1	0.851258349	0.4	0.146128036
2008	3	128	3	7.5	0.929418926	6.9	0.897627091	0.6	0.204119983
2008	3	128	4	19.4	1.309630167	19.2	1.305351369	0.2	0.079181246
2008	3	128	5	46.3	1.674861141	46.2	1.673941999	0.1	0.041392685
2008	3	384	1	7.8	0.944482672	7.4	0.924279286	0.4	0.146128036
2008	3	384	2	8.8	0.991226076	8.8	0.991226076	0	0
2008	3	384	3	1.4	0.380211242	1.2	0.342422681	0.2	0.079181246
2008	3	384	4	4.1	0.707570176	4.1	0.707570176	0	0
2008	3	384	5	0	0	0	0	0	0
2008	3	540	1	12.1	1.117271296	12	1.113943352	0.1	0.041392685
2008	3	540	2	0.7	0.230448921	0	0	0.7	0.230448921
2008	3	540	3	14.6	1.193124598	12.9	1.1430148	1.7	0.431363764
2008	3	540	4	0.1	0.041392685	0	0	0.1	0.041392685
2008	3	540	5	0.9	0.278753601	0	0	0.9	0.278753601
2008	3	555	1	0.5	0.176091259	0.2	0.079181246	0.3	0.113943352
2008	3	555	2	1.5	0.397940009	0.3	0.113943352	1.2	0.342422681
2008	3	555	3	2.4	0.531478917	0.5	0.176091259	1.9	0.462397998
2008	3	555	4	2.2	0.505149978	0	0	2.2	0.505149978
2008	3	555	5	13.5	1.161368002	13.3	1.155336037	0.2	0.079181246
2008	3	988	1	48.2	1.691965103	47	1.681241237	1.2	0.342422681
2008	3	988	2	33.3	1.53529412	33	1.531478917	0.3	0.113943352
2008	3	988	3	21.3	1.348304863	20.9	1.340444115	0.4	0.146128036
2008	3	988	4	85.4	1.936513742	84.8	1.933487288	0.6	0.204119983
2008	3	988	5	65.9	1.825426118	64.8	1.818225894	1.1	0.322219295
2008	4	128	1	26	1.431363764	24	1.397940009	2	0.477121255
2008	4	128	2	42	1.633468456	42	1.633468456	0	0
2008	4	128	3	38.7	1.598790507	34.3	1.547774705	4.4	0.73239376
2008	4	128	4	17.4	1.264817823	17.4	1.264817823	0	0
2008	4	128	5	39.6	1.608526034	39.6	1.608526034	0	0
2008	4	384	1	9.8	1.033423755	9.7	1.029383778	0.1	0.041392685
2008	4	384	2	12.9	1.1430148	11.7	1.103803721	1.2	0.342422681
2008	4	384	3	15.3	1.212187604	15	1.204119983	0.3	0.113943352
2008	4	384	4	7.8	0.944482672	5	0.77815125	2.8	0.579783597
2008	4	384	5	3.2	0.62324929	3.2	0.62324929	0	0
2008	4	540	1	0	0	0	0	0	0
2008	4	540	2	8.9	0.995635195	6.6	0.880813592	2.3	0.51851394
2008	4	540	3	2.8	0.579783597	0.4	0.146128036	2.4	0.531478917
2008	4	540	4	6.2	0.857332496	6.2	0.857332496	0	0
2008	4	540	5	12.2	1.120573931	12.2	1.120573931	0	0
2008	4	555	1	1.4	0.380211242	1.4	0.380211242	0	0
2008	4	555	2	5.8	0.832508913	0.9	0.278753601	4.9	0.770852012
2008	4	555	3	10	1.041392685	0.1	0.041392685	9.9	1.037426498

2008	4	555	4	1	0.301029996	0	0	1	0.301029996
2008	4	555	5	16.7	1.247973266	16.6	1.245512668	0.1	0.041392685
2008	4	988	1	316.3	2.501470072	316.2	2.501333179	0.1	0.041392685
2008	4	988	2	41.9	1.632457292	41.4	1.627365857	0.5	0.176091259
2008	4	988	3	156.1	2.196176185	156	2.195899652	0.1	0.041392685
2008	4	988	4	13.2	1.152288344	13.2	1.152288344	0	0
2008	4	988	5	107	2.033423755	107	2.033423755	0	0
2008	5	128	1	44.3	1.656098202	40.4	1.617000341	3.9	0.69019608
2008	5	128	2	35.8	1.565847819	35.8	1.565847819	0	0
2008	5	128	3	56.7	1.761175813	47.5	1.685741739	9.2	1.008600172
2008	5	128	4	10.7	1.068185862	10.7	1.068185862	0	0
2008	5	128	5	52	1.72427587	52	1.72427587	0	0
2008	5	384	1	39.6	1.608526034	38.8	1.599883072	0.8	0.255272505
2008	5	384	2	8.3	0.968482949	8.2	0.963787827	0.1	0.041392685
2008	5	384	3	13.5	1.161368002	12.7	1.136720567	0.8	0.255272505
2008	5	384	4	17.3	1.26245109	11.4	1.093421685	5.9	0.838849091
2008	5	384	5	33	1.531478917	10	1.041392685	23	1.380211242
2008	5	540	1	5.6	0.819543936	5.6	0.819543936	0	0
2008	5	540	2	19.3	1.307496038	19.3	1.307496038	0	0
2008	5	540	3	11.5	1.096910013	11.3	1.089905111	0.2	0.079181246
2008	5	540	4	7.8	0.944482672	7.8	0.944482672	0	0
2008	5	540	5	0.4	0.146128036	0.4	0.146128036	0	0
2008	5	555	1	29.7	1.487138375	29.7	1.487138375	0	0
2008	5	555	2	7	0.903089987	5.8	0.832508913	1.2	0.342422681
2008	5	555	3	0.7	0.230448921	0	0	0.7	0.230448921
2008	5	555	4	0.2	0.079181246	0.1	0.041392685	0.1	0.041392685
2008	5	555	5	0.2	0.079181246	0.1	0.041392685	0.1	0.041392685
2008	5	988	1	224.8	2.353723938	224.8	2.353723938	0	0
2008	5	988	2	151.1	2.182129214	151.1	2.182129214	0	0
2008	5	988	3	15	1.204119983	15	1.204119983	0	0
2008	5	988	4	91.8	1.967547976	91.8	1.967547976	0	0
2008	5	988	5	93.7	1.976349979	93.7	1.976349979	0	0
2008	6	128	1	97	1.991226076	97	1.991226076	0	0
2008	6	128	2	55.1	1.748962861	49	1.698970004	6.1	0.851258349
2008	6	128	3	80.5	1.911157609	75.2	1.881954971	5.3	0.799340549
2008	6	128	4	72.9	1.868644438	72.9	1.868644438	0	0
2008	6	128	5	28.9	1.475671188	25.9	1.42975228	3	0.602059991
2008	6	384	1	59.6	1.782472624	59.6	1.782472624	0	0
2008	6	384	2	5.3	0.799340549	5.3	0.799340549	0	0
2008	6	384	3	102.4	2.014520539	102.4	2.014520539	0	0
2008	6	384	4	4.3	0.72427587	4.3	0.72427587	0	0
2008	6	384	5	2.4	0.531478917	0.2	0.079181246	2.2	0.505149978
2008	6	540	1	32.2	1.521138084	32.2	1.521138084	0	0
2008	6	540	2	88.3	1.950851459	86.8	1.943494516	1.5	0.397940009
2008	6	540	3	31.8	1.515873844	21.2	1.346352974	10.6	1.064457989
2008	6	540	4	5.8	0.832508913	2.2	0.505149978	3.6	0.662757832
2008	6	540	5	0.9	0.278753601	0.9	0.278753601	0	0
2008	6	555	1	46.9	1.680335513	46.9	1.680335513	0	0

2008	6	555	2	3.9	0.69019608	2	0.477121255	1.9	0.462397998
2008	6	555	3	26.4	1.437750563	23.3	1.385606274	3.1	0.612783857
2008	6	555	4	3.2	0.62324929	2.9	0.591064607	0.3	0.113943352
2008	6	555	5	1.7	0.431363764	1	0.301029996	0.7	0.230448921
2008	6	988	1	86.6	1.942504106	86.6	1.942504106	0	0
2008	6	988	2	246.9	2.394276527	246.9	2.394276527	0	0
2008	6	988	3	121.6	2.08849047	121.6	2.08849047	0	0
2008	6	988	4	90.8	1.962842681	90.8	1.962842681	0	0
2008	6	988	5	108.6	2.039810554	108.6	2.039810554	0	0
2008	7	128	1	56	1.755874856	56	1.755874856	0	0
2008	7	128	2	59.2	1.779596491	59.2	1.779596491	0	0
2008	7	128	3	81.9	1.918554531	76.3	1.888179494	5.6	0.819543936
2008	7	128	4	142.2	2.155943018	142.2	2.155943018	0	0
2008	7	128	5	159.1	2.204391332	152	2.184691431	7.1	0.908485019
2008	7	384	1	13.5	1.161368002	13.5	1.161368002	0	0
2008	7	384	2	32.7	1.527629901	32.6	1.526339277	0.1	0.041392685
2008	7	384	3	30	1.491361694	30	1.491361694	0	0
2008	7	384	4	7	0.903089987	7	0.903089987	0	0
2008	7	384	5	21	1.342422681	21	1.342422681	0	0
2008	7	540	1	46.2	1.673941999	46.2	1.673941999	0	0
2008	7	540	2	15.8	1.225309282	14.6	1.193124598	1.2	0.342422681
2008	7	540	3	26.5	1.439332694	24.8	1.411619706	1.7	0.431363764
2008	7	540	4	1.2	0.342422681	1.2	0.342422681	0	0
2008	7	540	5	2.4	0.531478917	2.4	0.531478917	0	0
2008	7	555	1	8.4	0.973127854	6.2	0.857332496	2.2	0.505149978
2008	7	555	2	15.1	1.206825876	14.9	1.201397124	0.2	0.079181246
2008	7	555	3	4.4	0.73239376	0.4	0.146128036	4	0.698970004
2008	7	555	4	0	0	0	0	0	
2008	7	555	5	0.6	0.204119983	0.6	0.204119983	0	0
2008	7	988	1	78.6	1.900913068	78.6	1.900913068	0	0
2008	7	988	2	57.7	1.768638101	57.7	1.768638101	0	0
2008	7	988	3	46	1.672097858	46	1.672097858	0	0
2008	7	988	4	85	1.934498451	85	1.934498451	0	0
2008	7	988	5	63.6	1.810232518	63.6	1.810232518	0	0
2008	8	128	1	72.4	1.86569606	72.4	1.86569606	0	0
2008	8	128	2	40.5	1.618048097	30.4	1.496929648	10.1	1.045322979
2008	8	128	3	44.3	1.656098202	33.2	1.534026106	11.1	1.08278537
2008	8	128	4	48.8	1.697229343	48.8	1.697229343	0	0
2008	8	128	5	140.7	2.15136985	59.2	1.779596491	81.5	1.916453949
2008	8	384	1	7	0.903089987	7	0.903089987	0	0
2008	8	384	2	14	1.176091259	14	1.176091259	0	0
2008	8	384	3	2.5	0.544068044	2.5	0.544068044	0	0
2008	8	384	4	0	0	0	0	0	
2008	8	384	5	9.5	1.021189299	9.5	1.021189299	0	0
2008	8	540	1	2.3	0.51851394	2.3	0.51851394	0	0
2008	8	540	2	15.8	1.225309282	11.4	1.093421685	4.4	0.73239376
2008	8	540	3	4.6	0.748188027	1.7	0.431363764	2.9	0.591064607
2008	8	540	4	6.7	0.886490725	6.5	0.875061263	0.2	0.079181246

2008	8	540	5	23.4	1.387389826	23.4	1.387389826	0	0
2008	8	555	1	15.4	1.214843848	14	1.176091259	1.4	0.380211242
2008	8	555	2	3.4	0.643452676	0.2	0.079181246	3.2	0.62324929
2008	8	555	3	6	0.84509804	2	0.477121255	4	0.698970004
2008	8	555	4	7.5	0.929418926	7.5	0.929418926	0	0
2008	8	555	5	0.4	0.146128036	0.4	0.146128036	0	0
2008	8	988	1	51	1.716003344	51	1.716003344	0	0
2008	8	988	2	125	2.100370545	125	2.100370545	0	0
2008	8	988	3	81.4	1.915927212	81.4	1.915927212	0	0
2008	8	988	4	67.6	1.836324116	67.6	1.836324116	0	0
2008	8	988	5	104.5	2.02325246	104.5	2.02325246	0	0
2008	9	128	1	67.8	1.837588438	67.8	1.837588438	0	0
2008	9	128	2	45.5	1.667452953	45.5	1.667452953	0	0
2008	9	128	3	21	1.342422681	18.5	1.290034611	2.5	0.544068044
2008	9	128	4	3.5	0.653212514	3.5	0.653212514	0	0
2008	9	128	5	29.8	1.488550717	18.8	1.29666519	11	1.079181246
2008	9	384	1	3.2	0.62324929	3.2	0.62324929	0	0
2008	9	384	2	10	1.041392685	10	1.041392685	0	0
2008	9	384	3	5.5	0.812913357	5.5	0.812913357	0	0
2008	9	384	4	15	1.204119983	15	1.204119983	0	0
2008	9	384	5	2.6	0.556302501	2.6	0.556302501	0	0
2008	9	540	1	0	0	0	0	0	
2008	9	540	2	10.2	1.049218023	7.6	0.934498451	2.6	0.556302501
2008	9	540	3	6	0.84509804	6	0.84509804	0	0
2008	9	540	4	23.4	1.387389826	23.4	1.387389826	0	0
2008	9	540	5	7	0.903089987	7	0.903089987	0	0
2008	9	555	1	0	0	0	0	0	
2008	9	555	2	2.8	0.579783597	2.8	0.579783597	0	0
2008	9	555	3	7.5	0.929418926	7.5	0.929418926	0	0
2008	9	555	4	4	0.698970004	4	0.698970004	0	0
2008	9	555	5	0	0	0	0	0	
2008	9	988	1	125	2.100370545	125	2.100370545	0	0
2008	9	988	2	43.2	1.645422269	43.2	1.645422269	0	0
2008	9	988	3	5.6	0.819543936	5.6	0.819543936	0	0
2008	9	988	4	111	2.049218023	111	2.049218023	0	0
2008	9	988	5	65	1.819543936	65	1.819543936	0	0
2008	10	128	1	15	1.204119983	15	1.204119983	0	0
2008	10	128	2	7.5	0.929418926	7.5	0.929418926	0	0
2008	10	128	3	32.2	1.521138084	32.2	1.521138084	0	0
2008	10	128	4	58.7	1.775974331	58.7	1.775974331	0	0
2008	10	128	5	21	1.342422681	10	1.041392685	11	1.079181246
2008	10	384	1	0	0	0	0	0	
2008	10	384	2	3.5	0.653212514	3.5	0.653212514	0	0
2008	10	384	3	1	0.301029996	1	0.301029996	0	0
2008	10	384	4	4	0.698970004	4	0.698970004	0	0
2008	10	384	5	3.5	0.653212514	3.5	0.653212514	0	0
2008	10	540	1	0	0	0	0	0	
2008	10	540	2	15	1.204119983	15	1.204119983	0	0

2008	10	540	3	1.8	0.447158031	1.8	0.447158031	0	0
2008	10	540	4	0	0	0	0	0	0
2008	10	540	5	4.5	0.740362689	4.5	0.740362689	0	0
2008	10	555	1	0	0	0	0	0	0
2008	10	555	2	2.8	0.579783597	2.8	0.579783597	0	0
2008	10	555	3	4.5	0.740362689	4.5	0.740362689	0	0
2008	10	555	4	1.2	0.342422681	1.2	0.342422681	0	0
2008	10	555	5	0	0	0	0	0	0
2008	10	988	1	38.8	1.599883072	38.8	1.599883072	0	0
2008	10	988	2	69	1.84509804	69	1.84509804	0	0
2008	10	988	3	31	1.505149978	31	1.505149978	0	0
2008	10	988	4	67.6	1.836324116	67.6	1.836324116	0	0
2008	10	988	5	87	1.944482672	87	1.944482672	0	0

```

;
proc sort;
by variety week;
run;
proc means n mean var stderr;
var aphidsperplant;
by variety week;
run;
proc means n mean var stderr;
var whiteperplant;
by variety week;
run;
proc means n mean var stderr;
var yelperplant;
by variety week;
run;
proc mixed data=totalaphids2008;
class rep variety week;
model logaphidsperplant = variety week variety*week;

random rep rep*variety;
repeated / subject= rep*variety type=ar(1) rcorr=1;

lsmeans variety*week / slice=week;
lsmeans variety / diff cl adjust=tukey;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'c:\Documents and Settings\wakbar\Desktop\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
proc mixed data=totalaphids2008;
class rep variety week;
model logwhiteperplant = variety week variety*week;
random rep rep*variety;

```



```

repeated / subject= rep*variety type=ar(1) rcorr=1;
lsmeans variety*week / slice=week;
lsmeans variety / diff cl adjust=tukey;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'c:\Documents and Settings\wakbar\Desktop\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;
proc mixed data=totalaphids2008;
class rep variety week;
model logyelperplant = variety week variety*week;
random rep rep*variety;
repeated / subject= rep*variety type=ar(1) rcorr=1;
lsmeans variety*week / slice=week;
lsmeans variety / diff cl adjust=tukey;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
run;
%include 'c:\Documents and Settings\wakbar\Desktop\pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
run;

dm'log;clear;output;clear';
options nodate nonumber ps=55 ls=78;
title Effect of variety and week on M. sacchari and S. flava numbers per plant during June and July 2008 data;
Data totalaphids2008;
input species$ week variety$ rep aphidsperplant logaphidsperplant;
cards;
SA 5 128 1 40.4 1.617000341
SA 5 128 2 35.8 1.565847819
SA 5 128 3 47.5 1.685741739
SA 5 128 4 10.7 1.068185862
SA 5 128 5 52 1.72427587
SA 5 384 1 38.8 1.599883072
SA 5 384 2 8.2 0.963787827
SA 5 384 3 12.7 1.136720567
SA 5 384 4 11.4 1.093421685
SA 5 384 5 10 1.041392685
SA 5 540 1 5.6 0.819543936
SA 5 540 2 19.3 1.307496038
SA 5 540 3 11.3 1.089905111
SA 5 540 4 7.8 0.944482672
SA 5 540 5 0.4 0.146128036
SA 5 555 1 29.7 1.487138375
SA 5 555 2 5.8 0.832508913
SA 5 555 3 0 0

```

SA	5	555	4	0.1	0.041392685
SA	5	555	5	0.1	0.041392685
SA	5	988	1	224.8	2.353723938
SA	5	988	2	151.1	2.182129214
SA	5	988	3	15	1.204119983
SA	5	988	4	91.8	1.967547976
SA	5	988	5	93.7	1.976349979
YSA	5	128	1	3.9	0.69019608
YSA	5	128	2	0	0
YSA	5	128	3	9.2	1.008600172
YSA	5	128	4	0	0
YSA	5	128	5	0	0
YSA	5	384	1	0.8	0.255272505
YSA	5	384	2	0.1	0.041392685
YSA	5	384	3	0.8	0.255272505
YSA	5	384	4	5.9	0.838849091
YSA	5	384	5	23	1.380211242
YSA	5	540	1	0	0
YSA	5	540	2	0	0
YSA	5	540	3	0.2	0.079181246
YSA	5	540	4	0	0
YSA	5	540	5	0	0
YSA	5	555	1	0	0
YSA	5	555	2	1.2	0.342422681
YSA	5	555	3	0.7	0.230448921
YSA	5	555	4	0.1	0.041392685
YSA	5	555	5	0.1	0.041392685
YSA	5	988	1	0	0
YSA	5	988	2	0	0
YSA	5	988	3	0	0
YSA	5	988	4	0	0
YSA	5	988	5	0	0

```

;
proc sort;
by species variety;
run;
proc means n mean var stderr;
var aphidsperplant;
by species variety;
run;
proc mixed data=totalaphids2008;
class species variety rep;
model logaphidsperplant= species variety species*variety/ htype=3;
random rep;
lsmeans species*variety/ diff cl adjust=tukey;
contrast 'SA vs. YSA 128' species 1 -1 species*variety 1 0 0 0 0 -1 0 0 0 0;
contrast 'SA vs. YSA 384' species 1 -1 species*variety 0 1 0 0 0 0 -1 0 0 0;
contrast 'SA vs. YSA 540' species 1 -1 species*variety 0 0 1 0 0 0 0 -1 0 0;

```

```
contrast 'SA vs. YSA 555' species 1 -1 species*variety 0 0 0 1 0 0 0 0 -1 0;  
contrast 'SA vs. YSA 988' species 1 -1 species*variety 0 0 0 0 1 0 0 0 0 -1;  
ods output diffs=ppp lsmeans=mmm;  
ods listing exclude diffs lsmeans;  
run;  
%include 'C:\Documents and Settings\wakbar\Desktop\Pdmix800.sas';  
%pdmix800(ppp,mmm,alpha=.05,sort=yes);  
run;
```

APPENDIX E: SAS CODES FOR CHAPTER 7

```
dm'log;clear;output;clear';
Title 'average number of days for egg hatch';
options nodate nonumber ps=55 ls=78;
data eggs;
input egg days;
cards;
1      5
2      5
3      4
4      4
5      4
6      4
7      4
8      4
9      4
10     4
11     4
12     4
13     4
14     4
15     4
16     4
17     5
18     5
19     5
20     5
21     5
22     5
23     5
24     5
25     5
26     5
27     5
28     5
run;
Proc means mean n stderr clm;
var days;
run;

dm'log;clear;output;clear';
Title 'average egg size';
options nodate nonumber ps=55 ls=78;
data eggs;
input egg size;
cards;
1      1
```

```
2      1
3      1
4      0.9
5      0.9
6      1
7      1
8      0.9
9      0.9
10     1
11     1
12     1
13     1
14     1
15     0.9
16     0.9
17     0.9
18     0.9
19     0.9
20     0.9
21     0.9
22     0.9
23     0.9
24     0.9
25     0.9
26     0.9
```

```
run;
```

```
Proc means mean n stderr clm;
```

```
var size;
```

```
run;
```

```
dm'log;clear;output;clear';
```

```
Title 'first instar larvae size';
```

```
options nodate nonumber ps=55 ls=78;
```

```
data eggs;
```

```
input larva size;
```

```
cards;
```

```
1      1
2      1
3      1
4      0.75
5      0.75
6      1
7      1
8      0.9
9      0.75
10     1
11     1
12     1
```

```
13 1
14 1
15 0.9
16 0.9
17 0.9
18 0.9
19 0.9
20 0.9
21 0.9
22 0.9
23 0.9
24 0.9
25 0.9
26 0.9
27 0.9
28 0.9
29 0.9
30 0.9
31 0.9
32 0.9
33 0.9
34 0.9
35 0.9
36 0.9
37 0.9
38 0.9
39 0.9
40 0.9
41 0.9
```

```
run;
```

```
Proc means mean n stderr clm;
```

```
var size;
```

```
run;
```

```
dm'log;clear;output;clear';
```

```
Title 'first instar larvaal days';
```

```
options nodate nonumber ps=55 ls=78;
```

```
data eggs;
```

```
input larva days;
```

```
cards;
```

```
1 1
2 1
3 2
16 1
17 1
18 1
19 1
20 2
```

```
22 2
24 2
25 2
26 2
27 2
28 2
29 2
30 2
36 2
37 2
38 2
39 2
40 1
```

```
run;
```

```
Proc means mean n stderr clm;
```

```
var days;
```

```
run;
```

```
dm'log;clear;output;clear';
```

```
Title 'last instar larvaal length';
```

```
options nodate nonumber ps=55 ls=78;
```

```
data larvae;
```

```
input larva length;
```

```
cards;
```

```
1 3
2 3
3 3
4 2
5 3
6 2
7 3
8 3
9 3
10 2
11 3
12 2
13 2
14 2.5
15 2
16 3
17 3
18 2
19 3
20 2
21 3
22 2.5
23 2
24 2
```

```

25      2.5
run;
Proc means mean n stderr clm;
var length;
run;

dm'log;clear;output;clear';
Title 'last instar larvaal days';
options nodate nonumber ps=55 ls=78;
data larvae;
input larva days;
cards;
6      2
7      3
8      3
11     2
19     1
20     1
21     1
22     1
23     1
24     1
27     2
29     3
31     1
36     1
37     2
38     2
40     2
run;
Proc means mean n stderr clm;
var days;
run;

dm'log;clear;output;clear';
Title 'total larvaal days';
options nodate nonumber ps=55 ls=78;
data larvae;
input larva days;
cards;
1      8
2      11
3      12
4      8
5      13
6      4
7      4
8      4

```



```
9      5
10     4
11     6
12     9
13     9
14     5
15     5
16     9
17     8
18     6
19     4
20     5
21     4
22     7
23     5
24     8
```

```
run;
```

```
Proc means mean n stderr clm;
```

```
var days;
```

```
run;
```

```
dm'log;clear;output;clear';
```

```
Title 'total pupal days';
```

```
options nodate nonumber ps=55 ls=78;
```

```
data Pupae;
```

```
input pupa days;
```

```
cards;
```

```
1      6
2      5
3      5
4      5
5      4
6      5
7      6
8      6
9      4
10     4
11     4
12     6
13     4
14     5
15     6
16     4
17     4
18     5
19     5
```

```
run;
```

```
Proc means mean n stderr clm;
```

```

var days;
run;

dm'log;clear;output;clear';
Title 'total pupal size';
options nodate nonumber ps=55 ls=78;
data Pupae;
input pupa size;
cards;
1      1.5
2      1.25
3      2
4      1.25
5      1
6      1.25
7      2
8      2
9      1.5
10     1.5
11     1
12     1.25
13     1.25
14     1.25
15     1.5
16     1.25
17     1.5
18     1
19     1.5
run;
Proc means mean n stderr clm;
var size;
run;

```

```

dm'log;clear;output;clear';
Title 'total lar to adult days';
options nodate nonumber ps=55 ls=78;
data Pupae;
input pupa days;
cards;
1      14
2      16
3      17
4      13
5      9
6      10
7      11
8      13

```

```
9      13
10     9
11     13
12     11
13     9
14     12
15     11
16     13
```

```
run;
```

```
Proc means mean n stderr clm;
```

```
var days;
```

```
run;
```

```
dm'log;clear;output;clear';
```

```
Title 'aphids eaten by larvae for complete development';
```

```
options nodate nonumber ps=55 ls=78;
```

```
data Pupae;
```

```
input larva aphids;
```

```
cards;
```

```
1      25
2      26
3      18
4      22
5      38
6      36
7      32
8      38
9      36
10     38
11     37
12     27
13     40
14     24
15     30
16     21
17     20
```

```
run;
```

```
Proc means mean n stderr clm;
```

```
var aphids;
```

```
run;
```

```
dm'log;clear;output;clear';
```

```
Title 'aphids eaten by first instar larvae';
```

```
options nodate nonumber ps=55 ls=78;
```

```
data Pupae;
```

```
input larva aphids;
```

```
cards;
```

```
19     10
```

```
24 3
25 5
26 8
27 8
28 7
29 9
30 10
31 11
34 4
37 9
38 10
39 4
40 10
```

```
run;
```

```
Proc means mean n stderr clm;
```

```
var aphids;
```

```
run;
```

```
dm'log;clear;output;clear';
```

```
Title 'aphids eaten per day by larvae';
```

```
options nodate nonumber ps=55 ls=78;
```

```
data Pupae;
```

```
input larva aphids;
```

```
cards;
```

```
19 6.25
21 6.5
22 3.6
23 5.5
24 6.333333333
27 4.222222222
29 4.222222222
31 5.4
36 8
37 2.666666667
38 3.75
40 3.333333333
42 8
43 6
44 3.333333333
45 3.333333333
48 2.666666667
50 2.666666667
52 4
53 3.333333333
```

```
run;
```

```
Proc means mean n stderr clm;
```

```
var aphids;
```

```
run;
```

```

dm'log;clear;output;clear';
Title 'adult voracity test';
options nodate nonumber ps=55 ls=78;
data Pupae;
input adult aphids;
cards;
1      18
2      11
3      18
4      18
5      18
6      23
7      20
8      21
9      19
10     20
11     23
12     20
run;
Proc means mean n stderr clm;
var aphids;
run;

```

```

dm'log;clear;output;clear';
Title 'adult size';
options nodate nonumber ps=55 ls=78;
data Pupae;
input adult size;
cards;
1      1.5
2      1.5
3      2
4      2
5      2
6      2
7      2
8      1.5
9      1.5
10     1.5
11     1.5
12     1.5
13     2
14     1.5
15     2
run;
Proc means mean n stderr clm;
var size;

```

```

run;

dm'log;clear;output;clear';
Title 'pupae width';
options nodate nonumber ps=55 ls=78;
data Pupae;
input pupae width;
cards;
1      1
2      0.75
3      1
4      0.75
5      0.5
6      0.5
7      1
8      1
9      0.5
10     0.5
11     0.5
12     1
13     1
14     0.5
15     1
16     0.75
17     0.75
18     0.5
19     1
run;
Proc means mean n stderr clm;
var width;
run;

```

```

dm'log;clear;output;clear';
Title 'average number of days for adult life';
options nodate nonumber ps=55 ls=78;
data adult;
input rep days;
cards;
1      15
2      25
3      32
4      24
5      29
6      30
7      24
8      20
9      27
10     35

```

```
run;  
Proc means mean n stderr clm;  
var days;  
run;
```

APPENDIX F: SUPPLEMENTARY DATA

Chapter 3- Antibiosis data for sugarcane aphid

Variety	Dm	d	Md	Dm	Dr	total nymph
128	9	10	8	9	23	20
128	5	8	9	5	25	24
128	6	12	8	6	16	15
128	11	9	6	11	19	14
128	7	12	14	7	23	25
384	9	12	7	9	7	7
384	7	16	10	7	18	11
384	9	15	19	9	12	19
384	8	14	16	8	19	20
384	7	15	20	7	16	22
540	11	14	8	11	9	8
540	9	12	9	9	13	12
540	10	11	12	10	18	15
540	13	14	9	13	17	13
540	9	14	9	9	16	11
555	11	8	2	11	8	2
555	7	14	3	7	13	3
555	11	10	3	11	12	5
555	7	9	2	7	10	5
555	15	19	2	15	10	2
988	7	14	4	7	15	4
988	11	14	6	11	16	7
988	9	16	20	9	19	25
988	11	14	16	11	16	17
988	11	10	5	11	12	6

Yellow sugarcane aphid antibiosis data

Var.	rep	Dm	Dr	TNym	d	Md
128	1	9	17	19	10	14
128	2	11	12	16	10	16
128	3	11	16	21	12	21
128	4	7	26	27	10	17
128	5	11	28	31	8	20
128	6	14	7	20	10	5
128	7	11		15	8	7
384	1	7	20	10	8	10
384	2	12	22	15	13	15
384	3	15	6	10	12	8
384	4	14	9	5	13	5
384	5	9	14	15	13	15
384	6	12	18	14	10	11
384	7	15	20	12	10	8
555	1	13	5	8	22	8

555	2	8	12	14	19	14
555	3	12	14	12	18	12
555	4	14	2	1	19	1
555	5	17	10	7	15	7
555	6	18	14	8	22	8

Tolerance test- Sugarcane aphid data

VarietyRep	C	T
128 1	39.8	28.9
128 2	62.2	50.3
128 3	32.9	19.5
128 4	42	33.8
128 5	9.3	5.5
384 1	47.6	43.6
384 2	41.2	40.6
384 3	41.6	32.6
384 4	43.8	15.8
384 5	24.6	16.3
540 1	35.9	30.5
540 2	48.3	44.9
540 3	46.2	44.1
540 4	46.5	40.5
540 5	35.3	18.9
555 1	34	31.1
555 2	42.6	35.5
555 3	40.3	25.8
555 4	33.6	29.1
555 5	38.8	14.3
988 1	44.2	40.6
988 2	44	32
988 3	41.2	32.9
988 4	28.1	24.8
988 5	36.5	24

Tolerance test- Yellow sugarcane aphid data

VarietyRep	C	T
128 1	28.2	14.16
128 2	48.18	46.8
128 3	45.84	37.44
128 4	19.38	7.92
128 5	6.5	7.72
128 6	19.16	6.5
128 7	41.1	30.48
384 1	35.26	28.86
384 2	37.32	23.1
384 3	39.26	18.38

384	4	26.8	14.94
384	5	27.82	22
384	6	31.98	11
384	7	13.02	4.62
555	1	38.96	26.18
555	2	39.02	26.26
555	3	43.92	31.78
555	4	37.92	34.66
555	5	38.2	34.8
555	6	39.08	30.32
555	7	38.74	24.86

Chapter 4- EPG Data

Var	Ap#	TProtime	MeProDur	T nonproti	TitoreachPW
128	1	5732	1433	10268	3266
128	2	15452	7726	548	343
128	3	15094	5031	906	762
128	4	14399	2400	1601	642
128	5	14895	4965	1105	295
128	6	15317	15317	683	683
128	7	14377	3594	1623	582
128	8	16000	16000	0	0
128	9	10962	1827	5043	0
128	10	11158	11158	80	80
128	11	11221	3610.5	17	1
128	12	8398	4199	2846	2383
128	13	13517	2252.8	883	4
128	14	13484	6742	916	239
128	15	11707	2926.8	2693	593
128	16	13344	13344	1056	1056
128	17	7315	7315	849	849
128	18	7190	7190	926	926
128	19	15574	7787	426	181
128	20	10757	10757	5243	5243
128	21	10240	2048	5760	1312
128	22	11162	5581	4838	1829
128	23	12463	1557.9	3537	13
128	24	7115	2371.7	8885	1798
128	25	8469	2823	7531	6115
128	26	14249	4749.7	1751	1444
128	27	3323	3323	12677	593
128	28	15857	5285.7	143	11
128	29	14865	7432.5	1135	813
128	30	14259	2851.8	1741	731
555	1	13140	2628	2860	175
555	2	15491	5163.7	509	206
555	3	3220	536.7	12780	7708
555	4	11699	1169.9	4301	1250

555	5	15040	5013	960	613
555	6	15233	15233	767	767
555	7	14634	3658.5	1366	435
555	8	14874	14874	1126	1126
555	9	1456	1456	14544	14544
555	10	15734	2622.3	266	0.25
555	11	13423	2237.2	2577	58
555	12	15523	2217.6	477	18
555	13	11873	3957.7	4127	1582
555	14	15663	3915.8	337	0.18
555	15	13510	2702	2490	1446
555	16	15241	15241	759	759
555	17	8540	4270	7460	7251
555	18	15021	3755.3	979	754
555	19	7585	2528.3	8415	202
555	20	5102	1700.7	10898	179
555	21	15167	5055.7	833	343
555	22	15028	5009	972	774
555	23	15944	15944	56	56
555	24	14243	2034.7	1757	836
555	25	14537	7268.5	1463	1144
555	26	13561	1937	2439	261
555	27	12531	6265	3469	3338
555	28	15975	15975	25	25
384	1	8608	1721	6058	5451
384	2	13424	3356	1242	334
384	3	12358	4119.3	2308	236
384	4	8407	4203.5	7807	254
384	5	12188	12188	2212	2212
384	6	10671	3557	3729	612
384	7	10396	5198	4004	1238
384	8	3666	1222	10734	589
384	9	2868	2868	11532	587
384	10	14008	14008	392	392
384	11	14143	7071.5	257	0
384	12	11144	5572	3256	1323
384	13	3466	3466	12534	12534
384	14	15573	3893.3	427	0
384	15	10119	919	4281	497
384	16	12236	4078	2164	1726
384	17	13098	1190	1302	0
384	18	12931	1847	1433	0
384	19	10940	1823	3460	806
384	20	12072	1509	2328	1087
384	21	14399	14399	1	1
384	22	9797	1959.4	4603	2657
384	23	7026	1405.2	7374	3738
384	24	11301	2260.2	3099	0

384	25	7412	1853	6988	0
384	26	11144	2228.8	2356	163
384	27	13492	1349.2	908	0
384	28	13524	1690.5	876	0
384	29	4375	546.9	10025	4465
384	30	13840	1977.1	560	200
384	31	13238	2647.6	1162	246
384	32	13640	3410	760	331
384	33	10797	3735.5156	3755.375	1302.4688

Var	Ap#	Ti1toreachXY	Ti2toreachXY	Ti1toreachSE	Ti2toreachSE
128	1	7188	3922	.	.
128	2	5690	5347	7766	7423
128	3	2096	1334	12468	11706
128	4	1614	972	7947	7305
128	5	.	.	12515	12220
128	6	.	.	12306	11623
128	7	.	.	9719	9137
128	8	.	.	2928	2928
128	9	.	.	8942	8942
128	10	.	.	2785	2705
128	11	.	.	2199	2198
128	12	9003	6620	4953	2570
128	13	6836	6832	1977	1973
128	14	.	.	4444	4205
128	15	.	.	7176	6583
128	16	5008	3952	.	.
128	17	.	.	2713	1864
128	18	1837	911	2907	1981
128	19	.	.	3804	3623
128	20	.	.	5543	300
128	21	9272	7960	.	.
128	22	5297	3468	11253	9424
128	23	10247	10234	.	.
128	24	9740	1942	.	.
128	25	12927	6812	.	.
128	26	10036	8592	4580	1136
128	27	1749	1156	.	.
128	28	.	.	7145	7134
128	29	1236	423	12877	12064
128	30	15300	14569	11337	10606
555	1	.	.	11434	11259
555	2	956	750	.	.
555	3	.	.	10520	2812
555	4	2942	692	.	.
555	5	.	.	7412	6799
555	6	.	.	2419	1652
555	7	.	.	2316	1881

555	8	1888	762	.	.
555	9
555	10	1443	1442.75	978	977.75
555	11	564	506	10561	10503
555	12	7293	7275	11957	11939
555	13	.	.	13829	12247
555	14	.	.	2915	2915
555	15	.	.	8458	7012
555	16	1447	688	.	.
555	17	8094	1653	.	.
555	18	2506	1752	9134	8380
555	19	2902	2718	8236	8034
555	20	.	.	4746	4567
555	21	1273	930	7307	6904
555	22	.	.	1466	1192
555	23	6118	6062	1784	1728
555	24	.	.	7565	6729
555	25	3325	2181	.	.
555	26	.	.	9702	9441
555	27	.	.	15583	12245
555	28	.	.	3987	3962
384	1	10356	4905	.	.
384	2	.	.	2319	1985
384	3	.	.	3443	3117
384	4	.	.	4713	4459
384	5	.	.	10784	8572
384	6	.	.	7955	7343
384	7	1848	604	4845	3607
384	8	.	.	2704	2115
384	9	1902	1315	.	.
384	10	.	.	3947	3555
384	11	.	.	12545	12545
384	12	.	.	7855	6532
384	13	.	.	13037	503
384	14	.	.	2936	2936
384	15	.	.	5024	4527
384	16	.	.	4591	2865
384	17	.	.	1733	1733
384	18	.	.	9553	9553
384	19	.	.	3558	2752
384	20	.	.	5161	4074
384	21	.	.	1330	1329
384	22	12227	9570	.	.
384	23
384	24	.	.	9296	9296
384	25	.	.	5498	5498
384	26	.	.	8085	7922
384	27	.	.	9134	9134

384	28	.	.	5700	5700
384	29	9254	4789	.	.
384	30	6075	5875	.	.
384	31	.	.	1850	1604
384	32	6581	6250	2671	2340
384	33	6891.8571	4758.2857	5779.5	4830.6154

Var	Ap#	MedurOfPW	MedurOfXY	MedurOfSE
128	1	592	198	.
128	2	1735	486	1355
128	3	1537	1149	443
128	4	1158	2585	2554
128	5	2707	.	679
128	6	7489	.	340
128	7	2213.2	.	3311
128	8	2928	.	13072
128	9	1169.4	.	1388
128	10	1661	.	7836
128	11	3167.3	.	1719
128	12	1285.7	2241	2300
128	13	1332.3	1447	1696.3
128	14	2838.3	.	4969
128	15	1652.6	.	1702
128	16	1146.8	2189.3	.
128	17	1806.7	.	631.7
128	18	754.5	472	5209
128	19	1689	.	12196
128	20	300	.	10457
128	21	747.3	878.5	.
128	22	691.8	1824	9747
128	23	1220.7	1477	.
128	24	920.5	530.7	.
128	25	1546	369.5	.
128	26	873.9	2035.5	2030.5
128	27	1220.5	882	.
128	28	2334	.	8855
128	29	2719	2024	1965
128	30	1857.4	267	990
555	1	1714.8	.	4566
555	2	1576.1	891.6	.
555	3	413.4	.	326
555	4	787.2	1126.5	.
555	5	2150.7	.	8588
555	6	7239	.	755
555	7	1353.8	.	950.75
555	8	942.5	6494.5	.
555	9	1456	.	.
555	10	755	1359	1517.3

555	11	527.6	1621.7	1132
555	12	1181.3	694.3	446
555	13	2936	.	129
555	14	1688.6	.	1281
555	15	1617.2	.	3807
555	16	362.5	14516	.
555	17	317	7906	.
555	18	2110.7	2162	196
555	19	833.2	3282	137
555	20	1165.8	.	439
555	21	1290.4	883	656
555	22	1985.8	.	1037.7
555	23	1713.2	803	1023.8
555	24	1357.2	.	167.8
555	25	1487	440.2	.
555	26	1503	.	766
555	27	4126	.	152
555	28	3258	.	9459
384	1	832.5	3613	.
384	2	2623.8	.	305
384	3	2371.6	.	250
384	4	2752	.	151
384	5	8572	.	3616
384	6	1408.7	.	6445
384	7	791.8	993	6236
384	8	616.3	.	1201
384	9	1315	1553	.
384	10	3555	.	10453
384	11	6144	.	1855
384	12	1987.7	.	2590.5
384	13	521.6	.	214.5
384	14	1572	.	598
384	15	642	.	1207
384	16	1032.5	.	2013.7
384	17	729	.	515
384	18	1212	.	3234
384	19	1198.4	.	676.5
384	20	1125.6	.	408
384	21	501.1	.	1815.2
384	22	2935.7	495	.
384	23	1405.2	.	.
384	24	1239.4	.	5104
384	25	1745.8	.	429
384	26	2085.6	.	716
384	27	1164.4	.	924
384	28	1325.9	.	795.5
384	29	288.8	743.5	.
384	30	868.4	1289	.

384	31	802.5	.	721.6
384	32	925.5	3866	355
384	33	1759.1188	1793.2143	2031.9038

Var	Ap#	TotttimeinSE1	TotttimeinSE1	MedurOfSE1	TotttimeinSE2
128	1	.	0	.	.
128	2	20.142	20.142	6.714	4045
128	3	11.888	11.888	5.944	874
128	4	7.875	7.875	7.875	2546
128	5	15.108	15.108	7.554	1344
128	6	7.604	7.604	7.604	332
128	7	6.325	6.325	6.325	3305
128	8	10.045	10.045	10.045	13061
128	9	13.24	13.24	6.625	2762
128	10	8.75	8.75	8.75	7821
128	11	8.75	8.75	8.75	1710
128	12	9.167	9.167	9.167	2291
128	13	18.842	18.842	6.281	5072
128	14	5.835	5.835	5.835	4964
128	15	10.688	10.688	5.344	3393
128	16	.	0	.	.
128	17	17.162	17.162	5.721	1877
128	18	4.375	4.375	4.375	5205
128	19	7.125	7.125	7.125	12189
128	20	8	8	8	10449
128	21	.	0	.	.
128	22	8.938	8.938	8.938	9738
128	23	.	0	.	.
128	24	.	0	.	.
128	25	.	0	.	.
128	26	14.875	14.875	7.4	4046
128	27	.	0	.	.
128	28	7	7	7	8848
128	29	4	4	4	1961
128	30	6	6	6	984
555	1	5.625	5.625	5.625	4560
555	2	.	0	.	.
555	3	7	7	7	319
555	4	.	0	.	.
555	5	7.563	7.563	7.563	8581
555	6	6.437	6.437	6.437	749
555	7	33.689	33.689	8.422	941
555	8	.	0	.	.
555	9	.	0	.	.
555	10	23.75	23.75	5.93	6045
555	11	7	7	7	1125
555	12	5	5	5	441
555	13	8	8	8	121

555	14	21.751	21.751	7.25	3821
555	15	6.625	6.625	6.625	3800
555	16	.	0	.	.
555	17	.	0	.	.
555	18	7	7	7	189
555	19	8	8	8	129
555	20	9.2	9.2	9.2	430
555	21	14.313	14.313	7.156	1298
555	22	16.148	16.148	5.382	3095
555	23	29.188	29.188	7.3	4030
555	24	25.626	25.626	6.4	647
555	25	.	0	.	.
555	26	16.603	16.603	8.032	1517
555	27	8.5	8.5	8.5	144
555	28	8.125	8.125	8.125	9451
384	1	.	0	.	.
384	2	8.625	8.625	8.625	296
384	3	12.437	12.437	6.219	487
384	4	6.812	6.812	6.812	144
384	5	8.437	8.437	8.437	3607
384	6	5.25	5.25	5.25	6440
384	7	5.687	5.687	5.687	6230
384	8	7	7	7	1194
384	9	.	0	.	.
384	10	9.937	9.937	9.937	10443
384	11	7.125	7.125	7.125	1848
384	12	18	18	9	5163
384	13	30	30	7.5	828
384	14	40.617	40.617	8.123	2954
384	15	10.187	10.187	5.093	2404
384	16	19.437	19.437	6.479	6022
384	17	21.875	21.875	5.468	2039
384	18	4.625	4.625	4.625	3230
384	19	11.438	11.438	5.719	1341
384	20	12.75	12.75	6.375	803
384	21	36.044	36.044	6.007	10855
384	22	.	0	.	.
384	23	.	0	.	.
384	24	5.021	5.021	5.021	5099
384	25	8.438	8.438	8.438	420
384	26	6.312	6.312	6.312	710
384	27	12.562	12.562	6.281	1836
384	28	10.831	10.831	5.419	1580
384	29	.	0	.	.
384	30	.	0	.	.
384	31	28.212	28.212	5.642	3578
384	32	4.687	4.687	4.687	350
384	33	13.551769	11.010813	6.5877308	3073.1154

Var	Ap#	TotimeinSE2	MedurOfSE2	Total # SE1	Total # SE2
128	1	0	.	0	0
128	2	4045	1348	3	3
128	3	874	437	2	2
128	4	2546	2546	1	1
128	5	1344	672	2	2
128	6	332	332	1	1
128	7	3305	3305	1	1
128	8	13061	13061	1	1
128	9	2762	1381	2	2
128	10	7821	7821	1	1
128	11	1710	1710	1	1
128	12	2291	2291	1	1
128	13	5072	1690.7	3	3
128	14	4964	4964	1	1
128	15	3393	1696.5	2	2
128	16	0	.	0	0
128	17	1877	625.7	3	3
128	18	5205	5205	1	1
128	19	12189	12189	1	1
128	20	10449	10449	1	1
128	21	0	.	0	0
128	22	9738	9738	1	1
128	23	0	.	0	0
128	24	0	.	0	0
128	25	0	.	0	0
128	26	4046	2023	2	2
128	27	0	.	0	0
128	28	8848	8848	1	1
128	29	1961	1961	1	1
128	30	984	984	1	1
555	1	4560	4560	1	1
555	2	0	.	0	0
555	3	319	319	1	1
555	4	0	.	0	0
555	5	8581	8581	1	1
555	6	749	749	1	1
555	7	941	941	4	4
555	8	0	.	0	0
555	9	0	.	0	0
555	10	6045	1511.3	4	4
555	11	1125	1125	1	1
555	12	441	441	1	1
555	13	121	121	1	1
555	14	3821	1273.7	3	3
555	15	3800	3800	1	1
555	16	0	.	0	0

555	17	0	.	0	0
555	18	189	189	1	1
555	19	129	129	1	1
555	20	430	430	1	1
555	21	1298	649	2	2
555	22	3095	1031.7	3	3
555	23	4030	1007.5	4	4
555	24	647	161.8	4	4
555	25	0	.	0	0
555	26	1517	758	2	2
555	27	144	144	1	1
555	28	9451	9451	1	1
384	1	0	.	0	0
384	2	296	296	1	1
384	3	487	243.5	2	2
384	4	144	144	1	1
384	5	3607	3607	1	1
384	6	6440	6440	1	1
384	7	6230	6230	1	1
384	8	1194	1194	1	1
384	9	0	.	0	0
384	10	10443	10443	1	1
384	11	1848	1848	1	1
384	12	5163	2581.5	2	2
384	13	828	207	4	4
384	14	2954	592	5	5
384	15	2404	1202	2	2
384	16	6022	2007.2	3	3
384	17	2039	509	4	4
384	18	3230	3230	1	1
384	19	1341	670.5	2	2
384	20	803	401.5	2	2
384	21	10855	1809	6	6
384	22	0	.	0	0
384	23	0	.	0	0
384	24	5099	5099	1	1
384	25	420	420	1	1
384	26	710	710	1	1
384	27	1836	918	2	2
384	28	1580	790	2	2
384	29	0	.	0	0
384	30	0	.	0	0
384	31	3578	715.6	5	5
384	32	350	350	1	1
384	33	2496.9063	2025.3	1.6875	1.6875

Var	Ap#	E2 < 10 min	E2 > 10 min	Tot#Pds	PdstoSE
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128	1	0	0	54	.
128	2	2	1	52	19
128	3	2	0	52	28
128	4	0	1	59	21
128	5	1	1	18	6
128	6	1	0	41	26
128	7	0	1	86	58
128	8	0	1	26	26
128	9	0	2	59	27
128	10	0	1	15	13
128	11	0	1	22	19
128	12	0	1	36	10
128	13	1	2	45	12
128	14	0	1	46	32
128	15	1	1	45	21
128	16	0	0	19	.
128	17	2	1	33	17
128	18	0	1	5	4
128	19	0	1	30	30
128	20	0	1	4	.
128	21	0	0	32	.
128	22	0	1	26	26
128	23	0	0	50	.
128	24	0	0	43	.
128	25	0	0	22	.
128	26	0	2	22	14
128	27	0	0	7	.
128	28	0	1	25	25
128	29	0	1	61	27
128	30	0	1	64	31
555	1	0	1	35	35
555	2	0	0	51	.
555	3	1	0	13	12
555	4	0	0	38	.
555	5	0	1	47	47
555	6	0	1	22	22
555	7	3	1	91	21
555	8	0	0	9	.
555	9	0	0	16	.
555	10	2	2	89	10
555	11	0	1	30	19
555	12	1	0	103	53
555	13	1	0	8	8
555	14	2	1	82	21
555	15	0	1	81	36
555	16	0	0	5	.
555	17	0	0	4	.
555	18	1	0	81	28

555	19	1	0	32	32
555	20	1	0	29	25
555	21	0	2	34	14
555	22	1	2	20	6
555	23	1	3	52	8
555	24	4	0	65	15
555	25	0	0	14	.
555	26	1	1	90	24
555	27	1	0	43	26
555	28	0	1	26	17
384	1	0	0	8	.
384	2	1	0	49	9
384	3	2	0	30	9
384	4	1	0	15	10
384	5	0	1	38	30
384	6	0	1	50	50
384	7	0	1	12	12
384	8	0	1	13	10
384	9	0	0	8	.
384	10	0	1	11	11
384	11	0	1	22	22
384	12	1	1	28	14
384	13	4	0	11	3
384	14	3	2	70	16
384	15	1	1	35	22
384	16	0	2	58	21
384	17	3	1	93	16
384	18	0	1	40	35
384	19	1	1	40	8
384	20	2	0	55	19
384	21	0	6	34	7
384	22	0	0	10	.
384	23	0	0	24	.
384	24	0	1	30	30
384	25	1	0	19	16
384	26	0	1	27	22
384	27	1	1	21	7
384	28	1	1	21	12
384	29	0	0	3	.
384	30	0	0	42	.
384	31	1	4	44	8
384	32	1	0	17	8
384	33	0.75	0.90625	30.5625	16.423077

Var	Ap#	Tot#PW	To#Xy	To#SE	se w/o 0
128	1	8	5	0	.
128	2	6	2	3	3
128	3	7	3	2	2

128	4	8	1	1	1
128	5	5	0	2	2
128	6	2	0	1	1
128	7	5	0	1	1
128	8	1	0	1	1
128	9	7	0	2	2
128	10	2	0	1	1
128	11	3	0	1	1
128	12	3	1	1	1
128	13	9	1	3	3
128	14	3	0	1	1
128	15	5	0	2	2
128	16	4	4	0	.
128	17	3	0	3	3
128	18	2	1	1	1
128	19	2	0	1	1
128	20	1	0	1	1
128	21	9	5	0	
128	22	4	2	1	1
128	23	9	1	0	.
128	24	6	3	0	.
128	25	5	2	0	.
128	26	7	2	2	2
128	27	2	1	0	.
128	28	3	0	1	1
128	29	4	1	1	1
128	30	7	1	1	1
555	1	5	0	1	1
555	2	7	5	0	.
555	3	7	0	1	1
555	4	12	2	0	.
555	5	3	0	1	1
555	6	2	0	1	1
555	7	8	0	4	4
555	8	2	2	0	.
555	9	1	0	0	.
555	10	11	1	4	4
555	11	11	4	1	1
555	12	11	3	1	1
555	13	4	0	1	1
555	14	7	0	3	3
555	15	6	0	1	1
555	16	2	1	0	.
555	17	2	1	0	.
555	18	6	1	1	1
555	19	5	1	1	1
555	20	4	0	1	1
555	21	8	4	2	2

555	22	6	0	3	3
555	23	6	2	4	4
555	24	10	0	4	4
555	25	8	6	0	.
555	26	8	0	2	2
555	27	3	0	1	1
555	28	2	0	1	1
384	1	6	1	0	.
384	2	5	0	1	1
384	3	5	0	2	2
384	4	3	0	1	1
384	5	1	0	1	1
384	6	3	0	1	1
384	7	4	1	1	1
384	8	4	0	1	1
384	9	1	1	0	.
384	10	1	0	1	1
384	11	2	0	1	1
384	12	3	0	2	2
384	13	5	0	4	4
384	14	8	0	4	4
384	15	12	0	2	2
384	16	6	0	3	3
384	17	15	0	4	4
384	18	8	0	1	1
384	19	8	0	2	2
384	20	10	0	2	2
384	21	7	0	6	6
384	22	3	2	0	.
384	23	5	0	0	.
384	24	5	0	1	1
384	25	5	0	1	1
384	26	5	0	1	1
384	27	10	0	2	2
384	28	9	0	2	2
384	29	10	2	0	.
384	30	10	4	0	.
384	31	12	0	5	5
384	32	6	2	1	1
384	33	6.15625	0.40625	1.65625	2.0384615

Var	Ap#	Tot.ti in PW	Tot.ti in Xy	Tot.ti in Xy	Tot.ti. In SE	Tot.ti. In SE
128	1	4743	989	989	0	.
128	2	10415	971	971	4066	4066
128	3	10762	3446	3446	886	886
128	4	9260	2585	2585	2554	2554
128	5	13537	0	.	1358	1358

128	6	14977	0	.	340	340
128	7	11066	0	.	3311	3311
128	8	2928	0	.	13072	13072
128	9	8186	0	.	2776	2776
128	10	3322	0	.	7828	7828
128	11	9502	0	.	1719	1719
128	12	3857	2241	2241	2300	2300
128	13	6981	1447	1447	5089	5089
128	14	8515	0	.	4969	4969
128	15	8263	0	.	3405	3405
128	16	4587	8757	8757	0	.
128	17	5420	0	.	1895	1895
128	18	1509	472	472	5209	5209
128	19	3378	0	.	12196	12196
128	20	300	0	.	10457	10457
128	21	6726	3514	3514	0	.
128	22	2767	3648	3648	9747	9747
128	23	10986	1477	1477	0	.
128	24	5523	1592	1592	0	.
128	25	7730	739	739	0	.
128	26	6117	407	407	4061	4061
128	27	2441	882	882	0	.
128	28	7002	0	.	8885	8885
128	29	10876	2024	2024	1965	1965
128	30	13002	267	267	990	990
555	1	8574	0	.	4566	4566
555	2	11033	4458	4458	0	.
555	3	2894	0	.	326	326
555	4	9446	2253	2253	0	.
555	5	6452	0	.	8588	8588
555	6	14478	0	.	755	755
555	7	10831	0	.	974.68	974.68
555	8	1885	12989	12989	0	.
555	9	1456	0	.	0	.
555	10	8306	1359	1359	6069	6069
555	11	5804	6487	6487	1132	1132
555	12	12994	2083	2083	446	446
555	13	11744	0	.	129	129
555	14	11820	0	.	3843	3843
555	15	9703	0	.	3807	3807
555	16	725	14516	14516	0	.
555	17	634	7906	7906	0	.
555	18	12664	2161	2161	196	196
555	19	4166	3282	3282	137	137
555	20	4663	0	.	439	439
555	21	10323	3532	3532	1312	1312
555	22	11915	0	.	3113	3113
555	23	10279	1606	1606	4059	4059

555	24	13572	0	.	671	671
555	25	11896	2641	2641	0	.
555	26	12029	0	.	1532	1532
555	27	12379	0	.	152	152
555	28	6516	0	.	9459	9459
384	1	4995	3613	3613	0	.
384	2	13119	0	.	305	305
384	3	11858	0	.	500	500
384	4	8256	0	.	151	151
384	5	8572	0	.	3616	3616
384	6	4226	0	.	6445	6445
384	7	3167	993	993	6236	6236
384	8	2465	0	.	1201	1201
384	9	1315	1553	1553	0	.
384	10	3555	0	.	10453	10453
384	11	12288	0	.	1855	1855
384	12	5963	0	.	5181	5181
384	13	2608	0	.	858	858
384	14	12579	0	.	2994	2994
384	15	7705	0	.	2414	2414
384	16	6195	0	.	6041	6041
384	17	10938	0	.	2060	2060
384	18	9697	0	.	3234	3234
384	19	9587	0	.	1353	1353
384	20	11256	0	.	816	816
384	21	3508	0	.	10891	10891
384	22	8807	990	990	0	.
384	23	7026	0	.	0	.
384	24	6197	0	.	5104	5104
384	25	6983	0	.	429	429
384	26	10428	0	.	716	716
384	27	11644	0	.	1848	1848
384	28	11933	0	.	1591	1591
384	29	2888	1487	1487	0	.
384	30	8684	5156	5156	0	.
384	31	9630	0	.	3608	3608
384	32	5553	7732	7732	355	355
384	33	7613.2813	672.625	3074.8571	2507.9688	3086.730769

Chapter 4- Relative amount of FAAs in phloem sap samples- without transformation

Variety	Sample	Alanine	Aspartic	Cystine	Glutamic
128	1	13.86867	0	0	11.01041
128	2	19.99075	12.63452	0	19.16413
128	3	13.22758	20.8296	0	16.7859
128	4	31.45563	20.72813	0	29.29826
128	5	27.8214	25.66916	0	24.07299

128	6	28.34726	0	0	9.8057
128	7	23.25069	6.120919	0	7.681564
128	8	29.18181	10.38565	0	15.48475
128	9	35.82014	23.16904	0	17.29306
128	10	30.20891	21.35141	0	31.97612
128	11	33.32894	22.17507	0	24.52299
555	1	33.77349	17.94837	0	18.9831
555	2	20.57201	26.56755	0	22.84573
555	3	30.36109	19.68344	0	20.11635
555	4	32.998	24.10458	0	24.24352
555	5	35.26111	18.76836	0	27.29707
555	6	36.19589	20.57116	0	22.05552
555	7	35.39756	21.56643	0	26.24389
555	8	35.18412	21.26552	0	27.29063
555	9	31.49646	18.80799	0	29.36919
555	10	46.95926	12.36708	0	13.64257
555	11	31.5119	20.84534	0	22.06074
555	12	41.76529	14.54504	0	16.10165
555	13	41.08808	15.17717	0	19.11695
555	14	39.09308	0	0	23.0128

Variety	Sample	Glycine	Serine	Tyrosine	Proline
128	1	0	7.829933	0	0
128	2	0	8.049445	0	0
128	3	11.03292	10.03347	0	0
128	4	18.51798	0	0	0
128	5	22.43645	0	0	0
128	6	15.53637	33.89099	0	0
128	7	12.87501	24.87508	0	0
128	8	0	24.29498	0	0
128	9	0	23.71775	0	0
128	10	0	16.46356	0	0
128	11	0	19.973	0	0
555	1	8.083864	13.96218	0	0
555	2	0	15.73936	0	0
555	3	4.813038	11.75082	0	0
555	4	0	18.65391	0	0
555	5	0	18.67346	0	0
555	6	5.088495	10.49737	0	0
555	7	0	16.79213	0	0
555	8	0	16.25972	0	0
555	9	20.32636	0	0	0
555	10	7.880461	19.15063	0	0
555	11	10.06779	15.51423	0	0
555	12	11.67526	15.91277	0	0
555	13	10.11441	14.50339	0	0
555	14	17.30666	20.58746	0	0

Variety	Sample	Arginine	Histidine	Isoleucine	Leucine
128	1	14.88947	52.40152	0	0
128	2	5.462771	34.69839	0	0
128	3	7.301817	12.50676	0	0
128	4	0	0	0	0
128	5	0	0	0	0
128	6	0	0	0	0
128	7	7.539573	8.246861	0	0
128	8	8.743065	11.90974	0	0
128	9	0	0	0	0
128	10	0	0	0	0
128	11	0	0	0	0
555	1	0	0	0	0
555	2	0	0	0	0
555	3	0	0	0	0
555	4	0	0	0	0
555	5	0	0	0	0
555	6	0	0	0	0
555	7	0	0	0	0
555	8	0	0	0	0
555	9	0	0	0	0
555	10	0	0	0	0
555	11	0	0	0	0
555	12	0	0	0	0
555	13	0	0	0	0
555	14	0	0	0	0

Variety	Sample	Lysine	Methionine	Phenyl.	Threonine	Valine
128	1	0	0	0	0	0
128	2	0	0	0	0	0
128	3	0	0	0	8.281945	0
128	4	0	0	0	0	0
128	5	0	0	0	0	0
128	6	0	0	0	12.41968	0
128	7	0	0	0	9.410302	0
128	8	0	0	0	0	0
128	9	0	0	0	0	0
128	10	0	0	0	0	0
128	11	0	0	0	0	0
555	1	0	0	0	7.248991	0
555	2	0	0	0	5.765577	8.509779
555	3	0	0	0	6.113784	7.161474
555	4	0	0	0	0	0
555	5	0	0	0	0	0
555	6	0	0	0	5.591572	0
555	7	0	0	0	0	0
555	8	0	0	0	0	0
555	9	0	0	0	0	0

555	10	0	0	0	0	0
555	11	0	0	0	0	0
555	12	0	0	0	0	0
555	13	0	0	0	0	0
555	14	0	0	0	0	0

Chapter 4- Relative amounts of FAAs in honeydew samples- without transformation

Vari.	Alanine	Aspartic	Cystine	Glutamic	Glycine	Serine
128	14.48595	161.4007	0	178.4968	36.46528	72.81045
128	18.44188	33.63647	0	63.69031	0	31.45161
128	0	95.1104	0	80.11713	0	61.21224
128	8.215103	51.75312	0	41.48851	13.80185	16.896
128	19.94702	87.32074	0	82.41402	17.01502	83.48387
555	0	14.03967	0	25.27343	5.875581	10.0901
555	4.373307	12.70453	0	22.47474	5.222834	10.45475
555	0	13.46381	0	17.11661	6.471285	11.84784
555	5.938855	36.01885	0	38.42035	0	8.01949
555	8.692126	13.5754	0	32.95561	0	0

Vari.	Tyrosine	Proline	Arginine	Histidine	Isoleucine
128	9.050937	28.22061	43.42067	11.0491	8.172978
128	0	56.15763	26.35635	0	0
128	0	167.6111	0	0	0
128	6.49792	0	56.79552	47.8624	5.689362
128	4.461676	6.818073	48.89604	25.28242	5.652375
555	0	0	8.669564	0	0
555	0	0	10.13634	19.1749	0
555	0	0	3.004007	2.970622	0
555	0	0	5.38033	24.20185	0
555	0	0	0	17.4518	0

Vari.	Leucine	Lysine	Methionine	Phenyl.	Threonine	Valine
128	25.13058	5.650911	0	0	24.71214	0
128	0	0	0	0	0	0
128	0	0	0	0	0	0
128	7.499671	7.841769	0	7.236186	8.461836	23.51644
128	4.673194	10.87553	0	6.583758	28.83697	15.68184
555	0	0	0	0	4.443795	0
555	0	3.395437	0	3.448643	4.578422	2.146358
555	0	2.701811	0	3.23959	2.96971	0
555	0	12.84197	0	10.44692	22.01976	9.628097
555	0	8.547327	0	4.079707	0	0

Chapter 6- Number of aphids on different cultivars during 2007- Plant cane data

Year	Week	Var.	Rep	Ap/10 pl	SA/10 pl	YSA/10
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						plant
2007	1	128	1	2	1	1
2007	1	128	2	5.9	1.1	4.8
2007	1	128	3	1.9	1.7	0.2
2007	1	128	4	2	1.4	0.6
2007	1	128	5	1.3	1.2	0.1
2007	1	384	1	3.4	1.1	2.3
2007	1	384	2	2	1	1
2007	1	384	3	2.5	0.5	2
2007	1	384	4	2.9	1	1.9
2007	1	384	5	10	0.9	9.1
2007	1	540	1	2.2	0	2.2
2007	1	540	2	1.4	0	1.4
2007	1	540	3	1.5	0	1.5
2007	1	540	4	0.3	0	0.3
2007	1	540	5	1.2	0	1.2
2007	1	555	1	3.3	0	3.3
2007	1	555	2	0.8	0	0.8
2007	1	555	3	4.5	4.5	0.1
2007	1	555	4	2.3	2	0.2
2007	1	555	5	0	0	0
2007	1	988	1	1.2	0.1	1.1
2007	1	988	2	5.7	5.3	0.4
2007	1	988	3	4	2.8	1.2
2007	1	988	4	8.1	2	6.1
2007	1	988	5	22.3	21.7	0.6
2007	2	128	1	2	1	1
2007	2	128	2	1.9	1.2	0.7
2007	2	128	3	1.4	0.6	0.8
2007	2	128	4	2	1	1
2007	2	128	5	1	0	1
2007	2	384	1	1	1	0
2007	2	384	2	3.2	1.2	2
2007	2	384	3	2.4	1.3	1.1
2007	2	384	4	5.8	5.8	0.8
2007	2	384	5	2	0.5	1.5
2007	2	540	1	0.1	0	0.1
2007	2	540	2	0	0	0
2007	2	540	3	0.1	0	0.1
2007	2	540	4	0.1	0	0.1
2007	2	540	5	0.1	0	0.1
2007	2	555	1	0	0	0
2007	2	555	2	0.3	0	0.3
2007	2	555	3	0.9	0	0.9
2007	2	555	4	0	0	0

2007	2	555	5	0.2	0	0.2
2007	2	988	1	2.3	1	1.3
2007	2	988	2	1.3	1.3	0
2007	2	988	3	2.2	2	0.2
2007	2	988	4	1.1	1.1	0
2007	2	988	5	1.3	1	0.3
2007	3	128	1	5.6	0.6	5
2007	3	128	2	10.8	4	6.8
2007	3	128	3	5.5	5	0.5
2007	3	128	4	9.1	6.5	2.6
2007	3	128	5	11.4	4	7.4
2007	3	384	1	16	5	11
2007	3	384	2	9.6	2.3	7.3
2007	3	384	3	6.8	4.2	2.6
2007	3	384	4	9.3	2	7.3
2007	3	384	5	9	1.5	7.5
2007	3	540	1	8	1.2	6.8
2007	3	540	2	7.8	2.3	5.5
2007	3	540	3	6.1	1	5.1
2007	3	540	4	5.6	0.8	4.8
2007	3	540	5	8.1	1.1	7
2007	3	555	1	9.5	3.5	6
2007	3	555	2	7	1	6
2007	3	555	3	8.2	1	7.2
2007	3	555	4	5.1	0.5	4.6
2007	3	555	5	5.8	0.5	5.3
2007	3	988	1	7.2	1.5	5.7
2007	3	988	2	17	14.7	2.3
2007	3	988	3	6.7	4.8	1.9
2007	3	988	4	11.2	10	1.2
2007	3	988	5	12	3	9
2007	4	128	1	10.6	3	7.6
2007	4	128	2	50.7	49.5	1.2
2007	4	128	3	12.5	1.8	10.7
2007	4	128	4	11.9	10.3	1.6
2007	4	128	5	67.5	64.5	3
2007	4	384	1	33.1	25	8.1
2007	4	384	2	17.1	4	13.1
2007	4	384	3	34.3	23.4	10.9
2007	4	384	4	14.9	8	6.9
2007	4	384	5	11.6	9.5	2.1
2007	4	540	1	14.7	12.8	1.9
2007	4	540	2	10	9.5	0.5
2007	4	540	3	12.2	3	9.2
2007	4	540	4	13.2	1	12.2

2007	4	540	5	19.1	15.4	3.7
2007	4	555	1	10.6	1	9.6
2007	4	555	2	10.2	9	1.2
2007	4	555	3	12.4	1	11.4
2007	4	555	4	11.8	1	10.8
2007	4	555	5	12.2	1.5	10.7
2007	4	988	1	10	5	5
2007	4	988	2	17.6	15.1	2.5
2007	4	988	3	26	10	16
2007	4	988	4	37.4	26.4	11
2007	4	988	5	17.7	14.5	3.2
2007	5	128	1	20.5	10.3	10.2
2007	5	128	2	63.9	60	3.9
2007	5	128	3	20.8	20	0.8
2007	5	128	4	85.7	85.7	0
2007	5	128	5	16.3	11	5.3
2007	5	384	1	20.8	18.6	2.2
2007	5	384	2	22.2	8.7	13.5
2007	5	384	3	53.7	50	3.7
2007	5	384	4	22.5	18.5	4
2007	5	384	5	24.6	14.6	10
2007	5	540	1	10.2	10	0.2
2007	5	540	2	19.7	17.5	2.2
2007	5	540	3	17.7	15.5	2.2
2007	5	540	4	11.1	10.5	0.6
2007	5	540	5	15.8	5.4	10.4
2007	5	555	1	10.2	1	9.2
2007	5	555	2	10	5	5
2007	5	555	3	11.3	5	6.3
2007	5	555	4	10.1	1	9.1
2007	5	555	5	10.6	10	0.6
2007	5	988	1	23.5	19.2	4.3
2007	5	988	2	36.4	35	1.4
2007	5	988	3	39.6	38	1.6
2007	5	988	4	65.9	63.9	2
2007	5	988	5	22.9	20	2.9
2007	6	128	1	42.7	28.6	14.1
2007	6	128	2	66.8	65	1.8
2007	6	128	3	32	31	1
2007	6	128	4	61.5	59	2.5
2007	6	128	5	71.6	65	6.6
2007	6	384	1	24	19	5
2007	6	384	2	57	14.1	42.9
2007	6	384	3	36	14	22
2007	6	384	4	58.9	21.5	37.4

2007	6	384	5	23.3	21.5	1.8
2007	6	540	1	16.8	16.8	0
2007	6	540	2	11.3	10	1.3
2007	6	540	3	11.6	4	7.6
2007	6	540	4	16.5	7	9.5
2007	6	540	5	26.6	25	1.6
2007	6	555	1	10.2	0.5	9.7
2007	6	555	2	24.3	2	22.3
2007	6	555	3	11.2	4	7.2
2007	6	555	4	10.8	8.5	2.3
2007	6	555	5	11.2	5.6	5.6
2007	6	988	1	46.2	35	11.2
2007	6	988	2	124.9	101.5	23.4
2007	6	988	3	11.6	10	1.6
2007	6	988	4	26.7	25.5	1.2
2007	6	988	5	32.2	31.5	0.7
2007	7	128	1	45.4	42.5	2.9
2007	7	128	2	76.5	51	25.5
2007	7	128	3	22.5	20	2.5
2007	7	128	4	83	71	12
2007	7	128	5	46.4	45.5	0.9
2007	7	384	1	42.5	38.5	4
2007	7	384	2	74.5	73	1.5
2007	7	384	3	47.6	46	1.6
2007	7	384	4	10.9	10.9	0
2007	7	384	5	80.6	4	76.6
2007	7	540	1	11.4	4	7.4
2007	7	540	2	14.2	4.5	9.7
2007	7	540	3	13.5	12.5	1
2007	7	540	4	13	6	7
2007	7	540	5	31.7	29	2.7
2007	7	555	1	11.1	1	10.1
2007	7	555	2	11.9	9.4	2.5
2007	7	555	3	12.8	7.9	4.9
2007	7	555	4	11.4	9.8	1.6
2007	7	555	5	12.2	4.5	7.7
2007	7	988	1	49.9	47.5	2.4
2007	7	988	2	89.9	69	20.9
2007	7	988	3	45.9	44.5	1.4
2007	7	988	4	57.4	57.4	0
2007	7	988	5	42.3	41	1.3
2007	8	128	1	43.3	40	3.3
2007	8	128	2	141.9	138.8	3.1
2007	8	128	3	22.1	22.1	0
2007	8	128	4	63.2	61	2.2

2007	8	128	5	53.2	53.2	0
2007	8	384	1	6.6	6.6	0
2007	8	384	2	12.4	8.5	3.9
2007	8	384	3	27.4	27.4	0
2007	8	384	4	10.5	10.5	0
2007	8	384	5	58.7	47.8	10.9
2007	8	540	1	11	8.7	2.3
2007	8	540	2	14.2	12.5	1.7
2007	8	540	3	12.5	12.5	0
2007	8	540	4	10.4	10.4	0
2007	8	540	5	11.1	8.8	2.3
2007	8	555	1	7.1	2.8	4.3
2007	8	555	2	5.3	2.1	3.2
2007	8	555	3	25.1	20	5.1
2007	8	555	4	2	1	1
2007	8	555	5	2.5	1.6	0.9
2007	8	988	1	43.8	30	13.8
2007	8	988	2	12	12	0
2007	8	988	3	25	21	4
2007	8	988	4	22	21.5	0.5
2007	8	988	5	5.7	5.7	0
2007	9	128	1	10	10	0
2007	9	128	2	22	11.2	10.8
2007	9	128	3	87.5	72.5	15
2007	9	128	4	4.2	3.7	0.5
2007	9	128	5	54.4	44	10.4
2007	9	384	1	0.1	0	0.1
2007	9	384	2	1	1	0
2007	9	384	3	0.1	0.1	0
2007	9	384	4	3.9	3.9	0
2007	9	384	5	1.3	1.3	0
2007	9	540	1	14	14	0
2007	9	540	2	2.7	2.2	0.5
2007	9	540	3	1.4	1.4	0
2007	9	540	4	0.7	0.7	0
2007	9	540	5	0.8	0.8	0
2007	9	555	1	0.2	0.2	0
2007	9	555	2	2	2	0
2007	9	555	3	5.5	5.4	0.1
2007	9	555	4	2.2	2.2	0
2007	9	555	5	0	0	0
2007	9	988	1	37.6	37.6	0
2007	9	988	2	10.8	10.8	0
2007	9	988	3	53	53	0
2007	9	988	4	18	18	0

2007	9	988	5	6.8	6.8	0
2007	10	128	1	21	21	0
2007	10	128	2	5	5	0
2007	10	128	3	42	42	0
2007	10	128	4	7.6	7.5	0.1
2007	10	128	5	7.5	7.5	0
2007	10	384	1	2.2	2.2	0.1
2007	10	384	2	0.5	0.5	0
2007	10	384	3	0	0	0
2007	10	384	4	0.6	0.6	0
2007	10	384	5	0	0	0
2007	10	540	1	0	0	0
2007	10	540	2	0	0	0.5
2007	10	540	3	0	0	0
2007	10	540	4	0	0	0
2007	10	540	5	0	0	0
2007	10	555	1	0.2	0.2	0
2007	10	555	2	2	2	0
2007	10	555	3	0.1	0	0.1
2007	10	555	4	2.2	2.2	0
2007	10	555	5	0	0	0
2007	10	988	1	17.3	17.3	0
2007	10	988	2	21	21	0
2007	10	988	3	12	12	0
2007	10	988	4	4.2	4.2	0
2007	10	988	5	0	0	0

Chapter 6- Number of aphids on different cultivars during 2008- ratoon cane data

Year	Week	Variety	Rep	TOT/10 PL	Tot SA/10 pl	Tot YSA/10 pl
2008	1	128	1	9	0	9
2008	1	128	2	9.4	3	6.4
2008	1	128	3	10	2.8	7.2
2008	1	128	4	7.2	0	7.2
2008	1	128	5	5	3.6	1.4
2008	1	384	1	3.9	0.3	3.6
2008	1	384	2	8.1	0	8.1
2008	1	384	3	8.4	0	8.4
2008	1	384	4	11.5	11.5	1.5
2008	1	384	5	1.5	0	1.5
2008	1	540	1	8.2	8.2	4.1
2008	1	540	2	4.3	0	4.3
2008	1	540	3	12.3	3.2	8.8
2008	1	540	4	4.6	0	4.6
2008	1	540	5	10.2	0	10.2

2008	1	555	1	13.6	10	3.6
2008	1	555	2	5.4	0	5.4
2008	1	555	3	9.6	0	9.6
2008	1	555	4	3.7	0	3.7
2008	1	555	5	4.5	0	4.5
2008	1	988	1	3.6	0	3.6
2008	1	988	2	20.2	8.5	11.7
2008	1	988	3	7.7	1.2	6.5
2008	1	988	4	14.5	11	3.5
2008	1	988	5	5.6	1.8	3.8
2008	2	128	1	13	13	0.1
2008	2	128	2	11.3	0	1.3
2008	2	128	3	7.6	0	7.6
2008	2	128	4	1.4	1	0.4
2008	2	128	5	13.9	12.6	1.3
2008	2	384	1	4	4	0
2008	2	384	2	1.1	0.7	0.4
2008	2	384	3	0	0	0
2008	2	384	4	1.4	0	1.4
2008	2	384	5	0.6	0	0.6
2008	2	540	1	0	0	0
2008	2	540	2	1.7	0	1.7
2008	2	540	3	2.7	2.7	0.2
2008	2	540	4	0	0	0
2008	2	540	5	1.8	0	1.8
2008	2	555	1	1.3	0	1.3
2008	2	555	2	0.7	0.1	0.6
2008	2	555	3	2.7	0	2.7
2008	2	555	4	0	0	0
2008	2	555	5	2.1	0	2.1
2008	2	988	1	80.4	77.4	3
2008	2	988	2	49.6	49.5	0.1
2008	2	988	3	15.1	14.5	0.6
2008	2	988	4	25.3	25.2	0.1
2008	2	988	5	29	27.9	1.1
2008	3	128	1	16.5	10	6.5
2008	3	128	2	6.5	6.1	0.4
2008	3	128	3	7.5	6.9	0.6
2008	3	128	4	22.5	19.2	0.2
2008	3	128	5	46.3	46.2	0.1
2008	3	384	1	7.4	7.4	0.4
2008	3	384	2	8.8	8.8	0
2008	3	384	3	1.4	1.2	0.2
2008	3	384	4	4.1	4.1	0
2008	3	384	5	0	0	0

2008	3	540	1	12	12	0.1
2008	3	540	2	0.7	0	0.7
2008	3	540	3	14.6	12.9	1.7
2008	3	540	4	0.1	0	0.1
2008	3	540	5	0.9	0	0.9
2008	3	555	1	0.5	0.2	0.3
2008	3	555	2	1.5	0.3	1.2
2008	3	555	3	2.4	0.5	1.9
2008	3	555	4	2.2	0	2.2
2008	3	555	5	13.3	13.3	0.2
2008	3	988	1	48.2	47	1.2
2008	3	988	2	33.3	33	0.3
2008	3	988	3	21.3	20.9	0.4
2008	3	988	4	84.8	84.8	0.6
2008	3	988	5	64.8	64.8	1.1
2008	4	128	1	26	24	2
2008	4	128	2	42	42	0
2008	4	128	3	38.7	34.3	4.4
2008	4	128	4	17.4	17.4	0
2008	4	128	5	39.6	39.6	0
2008	4	384	1	9.8	9.7	0.1
2008	4	384	2	11.7	11.7	1.2
2008	4	384	3	15.3	15	0.3
2008	4	384	4	5	5	2.8
2008	4	384	5	3.2	3.2	0
2008	4	540	1	0	0	0
2008	4	540	2	8.8	6.6	2.3
2008	4	540	3	2.8	0.4	2.4
2008	4	540	4	6.2	6.2	0
2008	4	540	5	12.2	12.2	0
2008	4	555	1	1.4	1.4	0
2008	4	555	2	5.8	0.9	4.9
2008	4	555	3	10	0.1	9.9
2008	4	555	4	1	0	1
2008	4	555	5	16.6	16.6	0.1
2008	4	988	1	316.3	316.2	0.1
2008	4	988	2	42	41.4	0.5
2008	4	988	3	156	156	0.1
2008	4	988	4	13.2	13.2	0
2008	4	988	5	107	107	0
2008	4			126.9	126.76	0.14
2008	5	128	1	44.3	40.4	3.9
2008	5	128	2	35.8	35.8	0
2008	5	128	3	56.5	47.5	9.2
2008	5	128	4	12.5	10.7	0

2008	5	128	5	62	52	0
2008	5	384	1	39.6	38.8	0.8
2008	5	384	2	8.3	8.2	0.1
2008	5	384	3	13.5	12.7	0.8
2008	5	384	4	12.3	11.4	5.9
2008	5	384	5	33.1	10	23
2008	5	540	1	5.6	5.6	0
2008	5	540	2	19.3	19.3	0
2008	5	540	3	11.3	11.3	0.2
2008	5	540	4	7.8	7.8	0
2008	5	540	5	0.4	0.4	0
2008	5	555	1	29.7	29.7	0
2008	5	555	2	7	5.8	1.2
2008	5	555	3	0.7	0	0.7
2008	5	555	4	0.2	0.1	0.1
2008	5	555	5	0.2	0.1	0.1
2008	5	988	1	184.2	224.8	0
2008	5	988	2	151.1	151.1	0
2008	5	988	3	15	15	0
2008	5	988	4	91.8	91.8	0
2008	5	988	5	93.7	93.7	0
2008	6	128	1	96	97	0
2008	6	128	2	55.1	49	6.1
2008	6	128	3	80.4	75.2	5.3
2008	6	128	4	84.3	72.9	0
2008	6	128	5	28.9	25.9	3
2008	6	384	1	56.9	59.6	0
2008	6	384	2	5.3	5.3	0
2008	6	384	3	102.4	102.4	0
2008	6	384	4	4.3	4.3	0
2008	6	384	5	2.4	0.2	2.2
2008	6	540	1	32.2	32.2	0
2008	6	540	2	88.3	86.8	1.5
2008	6	540	3	31.8	21.2	10.6
2008	6	540	4	5.8	2.2	3.6
2008	6	540	5	0.9	0.9	0
2008	6	555	1	46.9	46.9	0
2008	6	555	2	3.9	2	1.9
2008	6	555	3	23.3	23.3	3.1
2008	6	555	4	3.2	2.9	0.3
2008	6	555	5	1.7	1	0.7
2008	6	988	1	86.6	86.6	0
2008	6	988	2	246.9	246.9	0
2008	6	988	3	121.6	121.6	0
2008	6	988	4	90.8	90.8	0

2008	6	988	5	105.6	108.6	0
2008	7	128	1	56	56	0
2008	7	128	2	59.2	59.2	0
2008	7	128	3	76.3	76.3	5.6
2008	7	128	4	142.2	142.2	0
2008	7	128	5	183	152	7.1
2008	7	384	1	13.5	13.5	0
2008	7	384	2	32.6	32.6	0.1
2008	7	384	3	30	30	0
2008	7	384	4	7	7	0
2008	7	384	5	21	21	0
2008	7	540	1	46.2	46.2	0
2008	7	540	2	15.8	14.6	1.2
2008	7	540	3	24.8	24.8	1.7
2008	7	540	4	1.2	1.2	0
2008	7	540	5	2.4	2.4	0
2008	7	555	1	8.4	6.2	2.2
2008	7	555	2	14.9	14.9	0.2
2008	7	555	3	4.4	0.4	4
2008	7	555	4	0	0	0
2008	7	555	5	0.6	0.6	0
2008	7	988	1	78.6	78.6	0
2008	7	988	2	57.7	57.7	0
2008	7	988	3	46	46	0
2008	7	988	4	85	85	0
2008	7	988	5	63.6	63.6	0
2008	8	128	1	72.4	72.4	0
2008	8	128	2	40	30.4	10.1
2008	8	128	3	44.3	33.2	11.1
2008	8	128	4	48.8	48.8	0
2008	8	128	5	85	59.2	81.5
2008	8	384	1	7	7	0
2008	8	384	2	14	14	0
2008	8	384	3	2.5	2.5	0
2008	8	384	4	0	0	0
2008	8	384	5	9.5	9.5	0
2008	8	540	1	2.3	2.3	0
2008	8	540	2	15.8	11.4	4.4
2008	8	540	3	4.6	1.7	2.9
2008	8	540	4	6.7	6.5	0.2
2008	8	540	5	23.4	23.4	0
2008	8	555	1	14	14	1.4
2008	8	555	2	0.3	0.2	3.2
2008	8	555	3	5.2	2	4
2008	8	555	4	7.5	7.5	0

2008	8	555	5	0.4	0.4	0
2008	8	988	1	51	51	0
2008	8	988	2	125	125	0
2008	8	988	3	81.4	81.4	0
2008	8	988	4	67.6	67.6	0
2008	8	988	5	104.5	104.5	0
2008	9	128	1	67.8	67.8	0
2008	9	128	2	45.5	45.5	0
2008	9	128	3	21	18.5	2.5
2008	9	128	4	3.5	3.5	0
2008	9	128	5	29.8	18.8	11
2008	9	384	1	3.2	3.2	0
2008	9	384	2	10	10	0
2008	9	384	3	5.5	5.5	0
2008	9	384	4	15	15	0
2008	9	384	5	2.6	2.6	0
2008	9	540	1		0	0
2008	9	540	2	9.2	7.6	2.6
2008	9	540	3	6	6	0
2008	9	540	4	23.4	23.4	0
2008	9	540	5	7	7	0
2008	9	555	1	0	0	0
2008	9	555	2	2.8	2.8	0
2008	9	555	3	7.5	7.5	0
2008	9	555	4	4	4	0
2008	9	555	5	0	0	0
2008	9	988	1	125	125	0
2008	9	988	2	43.2	43.2	0
2008	9	988	3	5.6	5.6	0
2008	9	988	4	111	111	0
2008	9	988	5	65	65	0
2008	10	128	1	15	15	0
2008	10	128	2	7.5	7.5	0
2008	10	128	3	32.2	32.2	0
2008	10	128	4	58.7	58.7	0
2008	10	128	5	10	10	11
2008	10	384	1	0	0	0
2008	10	384	2	3.5	3.5	0
2008	10	384	3	1	1	0
2008	10	384	4	4	4	0
2008	10	384	5	3.5	3.5	0
2008	10	540	1	0	0	0
2008	10	540	2	15	15	0
2008	10	540	3	1.8	1.8	0
2008	10	540	4	0	0	0

2008	10	540	5	4.5	4.5	0
2008	10	555	1	0	0	0
2008	10	555	2	2.8	2.8	0
2008	10	555	3	4.5	4.5	0
2008	10	555	4	1.2	1.2	0
2008	10	555	5	0	0	0
2008	10	988	1	38.8	38.8	0
2008	10	988	2	69	69	0
2008	10	988	3	31	31	0
2008	10	988	4	67.6	67.6	0
2008	10	988	5	87	87	0

APPENDIX G: LETTER OF PERMISSION FOR CHAPTER 7

Page 1 of 2

Akbar, Waseem

From: Alan Kahan [akahan@entsoc.org]
Sent: Monday, September 21, 2009 7:00 AM
To: Akbar, Waseem
Subject: Re: Permission Request
September 21, 2009

Waseem Akbar
Pest Management and Ecology, Sugarcane Insects
Dept. of Entomology
Louisiana State University
E-Mail : wakbar@agcenter.lsu.edu

Dear Mr. Akbar,

The Entomological Society of America grants you permission to include the article cited below as a chapter in your Ph.D. dissertation for Louisiana State University.

Akbar, W., C. Carlton, and T.E. Reagan. 2009. Life Cycle and Larval Morphology of *Diomus terminatus* (Coleoptera: Coccinellidae) and Its Potential as a Biological Control Agent of *Melanaphis sacchari* (Hemiptera: Aphididae). *Annals of the Entomological Society of America*. 102(1): 96-103.

Please provide proper attribution.

Sincerely,

Alan Kahan
Director of Communications/Managing Editor
Entomological Society of America
10001 Derekwood Lane, Suite 100
Lanham, MD 20706-4876
Phone: 301-731-4535 ext. 3020
Fax: 301-731-4538
akahan@entsoc.org
www.entsoc.org

VITA

Waseem Akbar was born in 1976 in Rawalpindi, Pakistan. He attended F.G. BRC School Abbotabad for his primary education and then F.G. Adamjee Road School Rawalpindi for his high school education. Waseem went to F.G. Sirsyed College Rawalpindi to complete his higher secondary school education. He joined University of Arid Agriculture Rawalpindi in 1993 and received his bachelor degree in agricultural entomology and zoology in 1997. He continued at U.A.A.R. to pursue a master degree in entomology, which was completed in 2000. Waseem came to the United States in 2001 to Kansas State University, Manhattan, Kansas, where he earned another master degree in entomology in 2003. Waseem joined Louisiana State University AgCenter in 2003 as a Research Associate in sugarcane entomology. While working there, Waseem decided to pursue a doctoral degree and was admitted into the doctorate program in the Spring of 2006 under the guidance of Dr. Thomas E. (Gene) Reagan. He is currently completing the requirements for his doctoral degree and plans to pursue his career as a researcher/academician.

From early on Waseem has received several accolades. He secured third position in Peshawar Cantt. Garrison schools at primary school level and received a merit scholarship for three years. He received another merit scholarship at middle school level from Federal Board of Intermediate and Secondary Education Islamabad for two years to complete his high school education. During his undergraduate program at U.A.A.R., Waseem received a merit scholarship as well as first prize in a university-wide debate contest. At K.S.U., Waseem was the recipient of R.H. Painter and R.C. Smith awards. During 2009, Waseem won the L.D. Newsom Outstanding Ph.D. Student Award from the Department of Entomology, L.S.U., and the John Henry Comstock Award of the Southeastern Branch, the Entomological Society of America's top student award.

Waseem is married to Shamsa and blessed with a baby girl, Hadia.