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Impact of Sorghum Cultivar, Phenology, Nitrogen, and Silicon Fertilization on Melanaphis sacchari Biology

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IMPACT OF SORGHUM CULTIVAR, PHENOLOGY, NITROGEN, AND SILICON FERTILIZATION ON *MELANAPHIS SACCHARI* BIOLOGY

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ABSTRACT

The sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae), is an invasive pest of grain sorghum, *Sorghum bicolor* (L.) Moench. Since its first outbreak in sorghum in 2013, severe infestations have spread throughout the southern U.S. causing high economic losses. While insecticidal control has mitigated some of the impacts of this pest, a sustainable ecology-based management program is needed to reduce reliance on chemical control. We studied the effects of silicon (rates equivalent to 0 and 3360 kg Si/ha) and nitrogen (rates equivalent to 0, 110, and 220 kg N/ha) on *M. sacchari* growth and reproduction on resistant (DKS 37-07) and susceptible cultivars (DK 38-88) of grain sorghum in a completely randomized factorial arrangement of treatments with five replications in a greenhouse. We calculated life table parameters including growth rate, fecundity, and the intrinsic rate of increase (r_m) of *M. sacchari* for each treatment. A field study with a complete factorial design consisting of 24 treatment combinations (2 cultivars x 4 infestation levels x 3 N fertilization levels) was used. The high rate of nitrogen fertilization increased *M. sacchari* fecundity, and r_m in the greenhouse study, but not in the field study. Sorghum plants with a high rate of N (220kg/ha) had a higher yield. In addition to this, DKS 37-07 resistant cultivar showed a high level of resistance to *M. sacchari* in both of the studies, and also gave substantial yield. Furthermore, insecticide application at infestation level of 50 aphids/leaf reduced aphid population effectively when compared to the application at bi-weekly and unprotected plots. Finally, Si had no effect on *M. sacchari* growth and reproduction in the greenhouse study. Results suggest that resistant cultivar if grown with a high level of N (224 kg/ha), and application of an insecticide at low infestation level (50 aphids/leaf) will manage an *M. sacchari* population thereby maintaining sorghum yield.

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Grain sorghum, *Sorghum bicolor* (L.) Moench, is one of the top five cereal crops in the world and an important cereal crop of the United States (United Sorghum Checkoff 2016). Sorghum is attacked by 150 insect species worldwide (Sharma 1993). It has been infested by the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Hemiptera: Aphididae) in North America since 2013 (Bowling *et al.* 2016). Since the 2013 outbreak, *M. sacchari* has now become an economically important pest of sorghum in more than 17 states of the United States and Mexico (Bowling *et al.* 2016). *Melanaphis sacchari* feeds on the phloem sap of sorghum reducing the nutrients that sorghum requires for optimal growth and yield (Singh *et al.* 2004). Yield losses of up to 50–70% resulting from *M. sacchari* infestation have been reported (Villanueva *et al.* 2014). While insecticidal control has mitigated some of the impacts of this pest (Seiter 2016), a sustainable ecology-based management program is needed to reduce reliance on chemical control. Aphid growth and reproduction are affected by host plant nutritional quality in many other systems. Examining the effects of host phenology and nutrient content on *M. sacchari* population dynamics will assist in the development of effective integrated pest management (IPM) program for this damaging pest.

Objectives of this study are:

1. Quantify the life table parameters for *M. sacchari* populations on a susceptible cultivar of sorghum as affected by sorghum phenology, nitrogen (N), and silicon (Si) fertilization.
2. Quantify the life table parameters for *M. sacchari* populations as affected by sorghum cultivar, nitrogen (N), and Silicon (Si) fertilization.

3. Examine the effects of cultivar, N fertilization, and infestation level on *M. sacchari* population dynamics in the field.
4. Determine yield response of sorghum to *M. sacchari* under variable field conditions.

1.2 Literature review

1.2.1 Sorghum production

Sorghum is a major grain and forage crop in the United States. After corn and wheat, sorghum stands third regarding grain harvested acreage (>2.5 million hectares), production (>12 million metric ton) and exports (>6 million metric ton) among grain crops in the United States in 2017 (USDA 2018). The top three-grain sorghum producing states are Kansas, Texas, and Arkansas (USDA 2017). Moreover, sorghum production in Louisiana was 4,692,000 bushels in 2016, which makes Louisiana the 8th largest sorghum-producing state. The economic impact of grain sorghum in Louisiana was $15.9 million in 2016 (USDA-NASS 2016). Additionally, in the United States, the sorghum area harvested has increased by 22% from 2015 (2.6 mill ha) to 2016 (31.8 mill ha), and production also increased by 37% (10 to 15.16 million metric ton) (USDA 2018). The economic value of sorghum in 2015 was approximately 2 billion, which was highest in recent years (USDA NASS 2018). However, both area and production of sorghum in the United States have declined from 2015/2016 to 2016/2017 by 21.6 % and 19.5% respectively (USDA 2017). This production decline is frequently attributed to increased input costs associated with *M. sacchari* management.

1.2.2 *Melanaphis sacchari* biology

Melanaphis sacchari is a small (1–2 mm) soft-bodied, ovate-shaped insect found in colors ranging from yellow, brown, or pinkish depending mainly on the pest's host plant and environment (Blackman and Eastop 2000, Villanueva *et al.* 2014). Towards the end of the abdomen, they have a pair of backward facing, tube-like structures called cornicles which secrete

wax for their defense (Dixon 1977). Wingless *M. sacchari* is recognized by its dark-colored antennal tips, cornicles, and feet tips, which are distinguishing characters from other aphids (Villanueva *et al.* 2014). Alate *M. sacchari* head is dark brown with small dots on dorsal part of the head, with well-developed mesothorax, normal wing venations with thick brown veins, and front half part of genital plate has five to nine hairs (Raychaudhuri and Banerjee 1974).

Melanaphis sacchari primarily reproduces asexually (Blackman and Eastop 2000); however, sexual reproduction has also been reported on sorghum in China (David and Sandhu 1976, Zhang and Zhong 1983). The female aphid produces live young, wingless female (apterae) nymphs, parthenogenetically. These offspring will reach adulthood after four nymphal stages. This process takes 4–12 days to complete (Singh *et al.* 2004). Females of *M. sacchari* are reproductively proficient and produce an average of 60–100 nymphs within one reproductive period of 13–20 days, which often generates overcrowded conditions (Teetes *et al.* 1983). Winged females (alates) are produced when the infested plants become unable to sustain the aphid population, and alates migrate in search of a more suitable host (Dixon 1977). Four nymphal stages are required to produce wingless adults, while five molts are required for alates (Sharma *et al.* 2013).

The sexual cycle begins with the production of winged male aphids, usually occurring before the winter, which mate with wingless females (Endicott and Rice 2016). The wingless female produces eggs to overwinter in an inactive form on a host like *Miscanthus sacchariflorus* (Blackman and Eastop 2000). Overwintering in the asexual cycle is done as apterae or nymphs on wild hosts (Bowling *et al.* 2015). The host range includes the grass crops: sugarcane, *Saccharum* spp.; sorghum, *Sorghum bicolor* (L.); rice, *Oryza sativa* (L.); and corn, *Zea mays* (L.)

as well as weedy grasses including: Johnsongrass, *Sorghum halepense* (L); Barnyardgrass, *Panicum colonum (L*); *Pennisetum* spp., and Bermudagrass, *Cynodon dactylon* (L) (Singh *et al.* 2004).

1.2.3 *Melanaphis sacchari* distribution and pest status

Melanaphis sacchari is widely distributed among tropical and sub-tropical regions of the world including Asia, Africa, Australia, North America, and South America (Sharma *et al.* 2013, Zapata *et al.* 2016). In the United States, it was first reported infesting sugarcane in Hawaii during 1896 (Singh *et al.* 2004), and then later in Florida in 1977, and in Louisiana in 1999 (White *et al.* 2001). *Melanaphis sacchari* remained a sporadic pest of sugarcane but was not reported as a pest of sorghum in the United States until 2013. In that year, a major outbreak in sorghum was first reported in Texas and then spread to the surrounding states of Louisiana, Oklahoma, and Mississippi (Villanueva *et al.* 2014, Bowling *et al.* 2016). By 2015, it had been reported attacking sorghum in 17 states and is now considered as an important economic pest of sorghum throughout the southern United States and Mexico (Bowling *et al.* 2016). The utilization of a wide range of overwintering hosts, its survival in elevated temperatures (>20°C), its high reproduction rate, and wind-aided dispersal might be few of the factors that affect the pest's status and its spread throughout the United States (Colares *et al.* 2015b, Bowling *et al.* 2016).

Melanaphis sacchari injures sorghum by piercing plant tissue with its stylet and sucking the phloem sap from host leaves (Singh *et al.* 2004). At infestation initiation, they colonize the lower leaves of sorghum (1st to third leaves from the bottom) before moving towards the upper leaves later in the growing season (Wang 1961). The injury caused by *M. sacchari* leads to chlorosis and necrosis resulting in reduced plant growth and grain yield (Singh *et al.* 2004, Sharma *et al.* 2013, Brown *et al.* 2015). The phloem sap which aphids feed on is rich in sugar

and water, requiring large quantities to be consumed to obtain sufficient nitrogen. The excess carbohydrates are secreted as a sugary substance known as honeydew (Blackman and Eastop 2000). The honeydew serves as a source of food for fungi, which results in a black sooty mold covering the leaf, thereby reducing the photosynthetic area of the plant (Blackman and Eastop 2000, Singh *et al.* 2004). Infestations of *M. sacchari* are typically seen from the initial stages of growth on sorghum plants, which could kill the young seedlings (Zapata *et al.* 2016). However, populations of *M. sacchari* are highest in the field beginning with the emergence of the panicle and flowering stage, up to the maturation of seed (Setokuchi 1977, Fang 1990, Singh *et al.* 2004, Villanueva *et al.* 2014, Armstrong *et al.* 2015). Heavy infestation during the pre-flowering stage and grain filling stage lead to the reduced numbers of heads and low seed weights while infestation after the flowering stage affects grain quality and harvesting efficiency (Bowling *et al.* 2016). In addition to grain yield reductions, harvesting efficiency is reduced under severe infestations by the honeydew-covered leaves clogging the harvesting machinery (Sharma *et al.* 2013, Armstrong *et al.* 2015). Taken together, these activities of aphids can reduce the yield of sorghum from 46–78% in the absence of insecticides (Van den Berg 2002), and in some instances, have resulted in complete crop loss (Sharma *et al.* 1997, Bowling *et al.* 2016).

1.2.4 *Melanaphis sacchari* integrated pest management

Management practices such as resistant cultivar, biological control, and insecticides have the potential to manage *M. sacchari* (Bowling *et al.* 2016). Much of the research on management of *M. sacchari* has been conducted in Asia and Africa, which may not apply to the United States production system. Because the emergence of *M. sacchari* as a major pest of sorghum in the United States was in 2013, there has been limited time and opportunity to conduct research and develop science-based control strategies.

Insecticide applications are timed by frequent monitoring of infestations (scouting) in early crop growth stages, as aphid populations can increase rapidly. Scouting should be initiated at 20 days after planting and should be increased to two times a week after aphids are seen in the field (Bowling *et al.* 2015, Knutson *et al.* 2016). Treatment should be started as soon as infestations reach the currently recommended economic threshold level of 50 aphids per leaf with 25% of the plants in the field infested (Buntin 2016, Knutson *et al.* 2016). Insecticides are the primary means used to control populations of *M. sacchari* quickly in sorghum, though there are chances of having pest resurgence and economic benefits are not certain.

Transform (sulfoxaflor) and Sivanto (flupyradifurone) have received Section 18 emergency exemption labels for use in sorghum in 2014, 2015, 2016 (Seiter 2016). Both are effective in controlling *M. sacchari* (> 98% mortality) up to 21 days after application in the field (Brown *et al.* 2015, Bowling *et al.* 2016, Seiter 2016, and Knutson *et al.* 2016). Transform is not suitable for suppressing late season infestations because it is restricted to use before bloom by the Section 18 emergency level (Seiter 2016). Transform and Sivanto are systemic, relatively more specific to targeted insects, and less toxic to beneficial insects, thereby decreasing the chance of pest resurgence or secondary pest outbreaks (Bowling *et al.* 2016, Brown *et al.* 2015). Whereas broad-spectrum insecticides, such as pyrethroids, should be avoided as they have potential to increase aphid populations by killing beneficial insects (Bessin *et al.* 1998). Neonicotinoid seed treatments including thiamethoxam, clothianidin, and imidacloprid are also effective and can suppress aphid infestations for about 20–40 days after planting (Brown *et al.* 2015). A better understanding of *M. sacchari* population dynamics in the field can improve scouting strategies and timing of insecticide applications. Therefore, it is important to study several factors affecting growth and reproduction of the aphid.

The use of host plant resistance in IPM is very beneficial, as it is easy to use, has low input costs, and is compatible with other control tactics (Sharma *et al.* 1997). Sorghum lines, resistant to green bug, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), have been developed (Johnson *et al.* 1982, Peterson *et al.* 1984), and these genotypes have recently been screened for resistance to *M. sacchari* (Teetes *et al.* 1995, Armstrong *et al.* 2015). In the study conducted by Armstrong *et al.* (2015), RTx2783 stood out as the most resistant and tolerant (showed no injury symptoms in the presence of the *M. sacchari*) sorghum genotype. B11055, B11070, Ent62/SADC, SC110, SC170, and (SV1*Sima/IS23250)–LG15 sorghum genotypes also had a high degree of resistance towards *M. sacchari* (Armstrong *et al.* 2015). Whereas, PI 55610 and Tam 428 were moderately resistant, and PI 264453 and PI 55607 were highly susceptible to *M. sacchari* (Armstrong *et al.* 2015). In a separate study, Tx3408 and Tx3409 sorghum germplasm lines showed a high level of tolerance to *M. sacchari* infestation (Mbulwe *et al.* 2016). However, the mechanism behind this resistance and its effect on *M. sacchari* biology is unknown. In a screening study conducted on 462 sorghum lines, IS12664C, IS2609C, IS12158C, and IS12661C were highly resistant to *M. sacchari*; reducing aphid longevity, reproducing days and number of progeny (Teetes *et al.* 1995).

Physiological traits related to resistant sorghum are tall plant height, the presence of waxy lamina, greater distance between the leaves, and slender shaped leaves (Singh *et al.* 2004). Bio-chemicals present in the plant also play a key role in determining the extent of aphid resistance (Singh *et al.* 2004). Available nitrogen content is an important aspect determining aphid development and reproduction, which is often associated with host plant resistance to aphids (Mattson 1980, Akbar *et al.* 2013). Sorghum cultivars with high potassium, polyphenols, and phosphorus are less susceptible to aphid infestation; however, the presence of high nitrogen,

sugar, and chlorophyll content results in greater levels of infestation (Singh *et al.* 2004). Continued research into cultivar resistance to *M. sacchari* will be critical to the development of an effective IPM program for this damaging pest.

Previous studies have suggested that the wide range of predators and parasitoids can act as effective bio-control agents in IPM programs for *M. sacchari*. Major predators of aphids include several species of lady beetles (Coleoptera: Coccinellidae), syrphid flies (Diptera: Syrphidae), and lacewings (Neuroptera: Chrysopidae and Hemerobiidae) (Singh *et al.* 2004, Colares *et al.* 2015a, b). Species which have been recorded predating *M. sacchari* in the United States include several Coccinellid beetles: *Menochilus sexmaculata* (Young and Teetes 1977), *Diomus terminates* (Hall 1987, Akbar 2009), *Hippodamia convergens*, and *Coleomegilla maculata* (Colares *et al.* 2015a, b). Syrphid species include *Allograpta exotica* (Hall 1987, White *et al.* 2001), *Xanthogramma aegyptium* (Singh *et al.* 2004), and *Allograpta obliqua* (Colares *et al.* 2015a). Other indigenous aphid predators known to feed on *M. sacchari* include *Chrysoperla carnea* (Neuroptera: Chrysopidae) and *Orius insidiosus* (Hemiptera: Anthocoridae) (Colares *et al.* 2015a, b). Parasitic wasps (Hymenoptera: Aphelinidae) are also important natural enemies which lay their eggs inside aphids. Parasitic wasps including *Lysiphlebus testaceipes* (Hall 1987) and *Aphelinus maidis* have been reported to attack *M. sacchari* (Zimmerman 1948). In addition to arthropod natural enemies, pathogens such as *Verticillium lecanii (A, O, FM)* were also found to be effective for controlling *M. sacchari* in Florida (Hall 1987). Although these natural enemies are effective in reducing aphid infestations, their role in *M. sacchari* population dynamics has not been thoroughly examined. Further, infestations of *M. sacchari* frequently reach damaging levels despite the presence of natural enemy populations and additional controls are often needed to reduce yield losses (Knutson *et al.* 2016).

Cultural control is an intentional modification of crop growing environment or the production practices to minimize pest populations or avoid pest damage to crops (Ashdown 1977). During fall and winter, host plants that *M. sacchari* uses for overwintering should be destroyed as they can enhance the source of aphids to infest crops in the following spring (Singh *et al.* 2004). Early planting helps to avoid damaging infestations of *M. sacchari* as well as other pests including sorghum midge and head worm problems (Brown *et al.* 2015). Crop rotation and high-density planting are few tactics that can be used for managing *M. sacchari* (Singh *et al.* 2004). However, in fully irrigated fields, high populations of *M. sacchari* are seen due to the high vigor of the plant; thus, avoiding over-watering could reduce high infestation of the aphid (Singh *et al.* 2004). Cultural controls which reduce plant vigor are likely not compatible with sorghum production in the United States. More research into possible cultural controls which are economical and compatible with other production practices is needed to reduce reliance on insecticides.

1.2.5 Nitrogen and silicon host plant resistance

Aphid feeding occurs by sucking phloem sap, but the presence of certain chemicals in the leaf tissue might affect stylet penetration and access to the phloem tissue (Akbar *et al.* 2013). Numerous studies have documented the importance of silicon (Si) to plants subjected to various biotic and abiotic stresses (Rodrigues and Datnoff 2015). The role of Si in inducing insect resistance in a crop was first reported by McColloch and Salmon (1923) in their research on maize plant resistance to Hessian fly (*Mayetiola destructor*) (Say) (Diptera: Cecidomyiidae). Silicon is found to induce defense mechanisms in the plants by enhancing physical resistance to feeding or increasing plant chemical defenses (Gomes *et al.* 2005, Moraes *et al.* 2005, Goussain *et al.* 2005, and Reynolds *et al.* 2009). Physical resistance can occur as a mechanical barrier, resulting from the deposition of Si in the form of amorphous silica in the leaf tissue, which may

impede stylet penetration (Moraes *et al.* 2005, Gomes *et al.* 2005). Stylet withdrawal during *S. graminum* feeding was seen more frequently in plants treated with Si (Goussain *et al.* 2005). This indicates that some change in leaf tissue (physical or chemical) was restricting the stylet penetration. Si absorption augments phenolic compounds like lignin, quinones, chitinases, peroxidases, polyphenol oxidase, phenylalanine ammonia-lyase, and active oxygen, which are responsible for antibiotic action (Gomes *et al.* 2005, Goussain *et al.* 2005). Several works reporting the role of Si in resistance to herbivory have been done in various crops including sugarcane (Keeping *et al.* 2009), rice (Moraes *et al.* 2005), sorghum (Carvalho *et al.* 1999), corn (Moraes *et al.* 2005), and wheat (Goussain *et al.* 2005, Gomes *et al.* 2005). *Schizaphis graminum* preference and fecundity were decreased with Si fertilization in sorghum (Carvalho *et al.* 1999), and a similar result was seen in wheat (Gomes *et al.* 2005). Similarly, Si has been previously reported to reduce the preference of corn leaf aphid (*Rhopalosiphum maidis*) in corn (Moraes *et al.* 2005), reduce the reproductive days and longevity of greenbug in wheat (Goussain et al. 2005), reduced cumulative fecundity and reduce feeding by the planthopper, *Sogatella fucifera* (Hemiptera: Delphacidae) in rice seedlings (Kin and Heinrichs 1982). Despite having considerable research on the effects of Si in plant resistance in various crops, including sorghum, there has been no study of its effect on *M. sacchari*. Therefore, studying the level of resistance provided by the addition of Si could aid in developing a sustainable IPM program for *M. sacchari*.

Nitrogen (N) is applied as an important fertilizer for many crops, including sorghum for better vegetative and reproductive growth of the plant. However, high N levels can also increase insect infestations by improving the availability of nutrients (Morales *et al.* 1999). The effect of N fertilization on insect pests has been widely studied, and both positive and negative impacts on

pest biology have been reported. Nitrogen fertilization has shown to increase insect growth, reproduction and population density (Lu *et al.* 2007). Examples include the Comstock mealybug (*Pseudococcus comstocki*) (Hemiptera: Coccoidea) on apple (*Malus* sp.) (Luna 1988), European corn borer (*Ostrinia nubilalis*) (Lepidoptera: Crambidae) on field corn (Luna 1988), and thrips (*Frankliniella occidentalis*) (Thysanoptera: Thripidae) on tomatoes (Brodbeck *et al.* 2001).

Sucking insects are attracted towards the plants with high N fertilization because N is often the limiting nutrient for phloem feeders; however, chewing insects (e.g., Lepidoptera) are often negatively affected by high levels of N (Altieri and Nicholls 2003). Research conducted in sorghum documented an increase in *S. graminum* populations with increased N content in plants (Archer *et al.* 1982). A similar experiment was performed in the greenhouse by Schweissing and Wilde (1979) in which *S. graminum* infestations were higher in both susceptible and resistant sorghum varieties when N was incorporated. Reproduction and growth rate of the green peach aphid (*Myzus persicae*) was also increased with a high content of soluble N in leaf tissues in Brussel sprouts (*Brassica oleracea*) (Van Emden *et al.* 1969). While there are several studies demonstrating effects of N under controlled conditions, these results are often difficult to reproduce under field conditions (Alteiri and Nicholls 2003).

While Si and N fertilization are known to be important influences of insect-plant interactions in other systems, their effect on *M. sacchari* has not been documented. The objective of this study is to determine the effects of variable rates of N and Si fertilization as well as host phenology and cultivar on *M. sacchari* development and reproduction on sorghum.

CHAPTER 2. IMPACT OF SORGHUM CULTIVAR, PHENOLOGY, NITROGEN AND SILICON FERTILIZER ON *M. SACCHARI* BIOLOGY

2.1 Introduction

Sugarcane aphid (*Melanaphis sacchari*) has been an important economic pest of sorghum throughout the southern United States and Mexico, since its first outbreak in sorghum (*Sorghum bicolor* (L.) Moench) in 2013 (Bowling *et al.* 2016). Previous research examining *M. sacchari* in sorghum has focused largely on chemical control and resistant cultivars (Singh *et al.* 2004, Armstrong *et al.* 2015), with little emphasis on the effects of other agronomic practices on aphid biology. Application of nitrogen fertilizer is common in sorghum production to increase growth and yield, thus understanding the impact of nitrogen on *M. sacchari* biology will improve our understanding of how infestations respond to various fertilization practices. Previous studies have documented positive effects on growth and reproduction of insects such as aphids with increasing plant available nitrogen (Moon *et al.* 1995, Hosseini *et al.* 2010, and Eini *et al.* 2017).

Silicon is another important nutrient which can affect plant physiology and influence herbivore populations. Silicon is known to induce plant defense mechanisms by enhancing physical resistance to feeding or increasing plant chemical defenses (Gomes *et al.* 2005, Goussain *et al.* 2005, Reynolds *et al.* 2009, and Rodrigues and Datnoff 2015). Despite considerable research being done on the effects of Si on plant resistance in various crops, including sorghum, there has been no study of its effect on *M. sacchari*. Therefore, studying the level of resistance provided by the addition of Si could aid in developing a sustainable IPM program for *M. sacchari*.

In this study, a resistant cultivar (DK 37-07) and a susceptible cultivar (DK 38-88) are included to assess the influence of host plant resistance on *M. sacchari* population dynamics. The objectives of this study were to examine the influence of host plant characteristics including

sorghum cultivar, phenology, nitrogen content, and silicon content on *M. sacchari* biology and population dynamics.

2.2 Materials and methods

2.2.1 *Melanaphis sacchari* colony

The aphids used in the following experiments were taken from a laboratory colony reared on sorghum line SP 7868. The clonal colony was obtained from one single apterous adult that was collected from a sorghum field at the Dean Lee research station, Alexandria, LA in 2014.

2.2.2 Greenhouse plants

Plants for the experiments were grown from seeds of a susceptible (SP 7868) cultivar in the greenhouse on the campus of Louisiana State University (Baton Rouge, LA) in plastic pots (6.06 liter) with a soil mixture (2-parts autoclaved river silt + 1-part peat +1-part sand). Silicon was incorporated into the soil of half of the pots at planting. Wollastonite ($CaSiO_3$ 23% Si) at a rate of 17g /pot was applied to half of the plants. This rate corresponds to 3360 kg Si/hectare at a plant density of 197000 seeds/hectare (Lanclos 2007). Wollastonite contains calcium in addition to Si, so 9.58g/pot of calcium source (CaO) was incorporated in all the control pots to balance the calcium effect. Five seeds were planted initially (1-inch-deep in the soil) to ensure sufficient plant establishment, and four of the seedlings were removed after emergence. Soil analysis was done for N and Si content of the soil mixture in the Soil Testing and Plant Analysis Lab at Sturgis Hall, LSU Ag Center. Nitrogen was applied to pots four weeks later at rates of 0.0, 0.75, and 1.51 g /plant which corresponds to 0, 110, and 224 kg N/ ha at a plant density of 150000 plants/hectare. Urea was applied on wet soil around the plants by making a round furrow, and then covered by soil to avoid N loss by volatization. The experiment included sorghum at the five-leaf stage and boot stage. Seeds were planted on 25 Feb 2017 (for boot stage study) and 26 March 2017 (for five-leaf stage study). Life table study of the aphid for a five-leaf study started

from April 26 and ended on June 27. Thus, two Si treatments, three N treatments, and two sorghum phenology conditions resulted in twelve factorial (Si × N × phenology) treatment combinations. The experiment was conducted using a completely randomized design with five replications.

The second study was conducted with two Si treatments, three N treatments, and two sorghum cultivars resulting in twelve factorial (Si × N × cultivar) treatment combinations. All the treatments rates and methodologies were similar to the first study, except that only one stage of sorghum (flag leaf stage) was used, and two cultivars of sorghum were planted for the study. In this study, a resistant cultivar (DK 37-07) and a susceptible cultivar (DK 38-88) were planted on 13 July 2017. Furthermore, aphid life study started from August 13 to September 15. The temperatures for both studies were maintained at 26 °C during the day and 21°C during the night.

2.2.3 *Melanaphis sacchari* life table assays

To determine the effect of treatments on population growth parameters of *M. sacchari*, no-choice life table studies were conducted following the methods defined by Carey (1993) and Davis *et al.* (2006). A single adult wingless aphid was enclosed in a 1.2-cm-diameter clip cage on the upper surface of an upper third leaf of each plant and was left to larviposit for 24 hrs. After nymphs were deposited, the adult and all but a single first instar were removed from each cage. Aphid lifetable parameters were calculated age (x) in days, age-specific survival (l_x), pre-reproductive days, fecundity (m_x), and age-specific fecundity ($l_x m_x$) was calculated. Life table parameters such as intrinsic rate of increase (rm), net reproductive rate (R_0), mean generation time (T_G), finite rate of increase (λ_F), and doubling time (DT) were calculated using the equations (1.1–1.5) of Birch (1948) and Carey (1993).

Intrinsic rate of increase (r_m) was calculated by using the equation:

(1.1) $\Sigma\, e^{-rm}\, l_x\, m_x = 1$

Net reproductive rate (R_0) was calculated as:

(1.2) $R_0 = \Sigma\, l_x\, m_x$

Generation time (T) was calculated as:

(1.3) $T_G = ln\ (R_0)/r_m$

Finite rate of increase (λ) was calculated as:

(1.4) $\lambda = e^{\,rm}$

Doubling time (DT) was calculated as:

(1.5) $DT = ln\ (2)/rm$

Standard errors for all life table parameters and 95% CI (Confidence interval) were calculated using the Jackknife procedure described by Meyer *et al.* (1986). This method was used by Zamani *et al.* (2006), Hosseini *et al.* (2010), Rostami *et al.* (2012), and Eini *et al.* (2017) in their studies.

2.2.4 Leaf tissue analysis

Since N and Si treatments were similar in both studies, leaf tissue analysis was conducted only in the spring study. One leaf closest to the leaf with the caged aphid was excised from each plant for analysis of nutrient composition. Nitrogen content analysis of leaf tissues was conducted on 30 Aug 2017 at Soil and Plant Analysis Lab, Sturgis Hall, LSU AgCenter using the Dumas Dry-Combustion method (Horneck and Miller 1998).

Silicon content analysis was conducted in Dr. Brenda Tubana's lab, Sturgis Hall, LSU AgCenter. Firstly, leaves were dried in an oven for 24 hours then ground (using grinding machine) for the further process using Oven-Induced Digestion (OID) (Kraska and Breitenbeck 2010). Ground plant tissue samples were weighed (S_{wt}), transferred to 50 ml polyethylene screw-

cap centrifuge tubes, and then oven dried (in the tubes with a loose cap) for 15 min at 60°C. After removing samples from the oven, five drops (approx. 0.21 ml) of octyl alcohol and two ml of 30% hydrogen peroxide were added to each tube. Furthermore, all the tubes (with a loose cap) were incubated in the oven at 95°C for 30 min. After incubation, 4 ml of 50% NaOH were added to each sample which were then placed in the oven for further incubation at 95°C for 4 hours. Throughout the four-hour digestion, the samples were mixed every 15 minutes using a vortex mixer. After the digestion process, 1 ml of 5mM ammonium fluoride was added to each sample, and tubes were shaken using the vortex mixer. Finally, deionized water was added to all tubes to make a final volume of 50 ml (V_d). In addition to this, seven blank samples tubes (tubes without plant samples only with the reagents) were prepared and digested along with the plant samples following the same procedure.

Two ml (V_a) of all the digested plant sample solutions were used for the plant Si colorimetric procedure (Kraska and Breitenbeck 2010). Furthermore, 10 ml of 20% acetic acid was added to each tube (plant sample tubes + standard series), and they were shaken for 10 sec. In the next step, 4 ml of 0.26 M ammonium molybdate was added to the tubes and allowed to cool for 5 minutes. Aftercooling, 2 ml of 20% tartaric acid was added to the tubes, and they were swirled for 10 sec. Finally, 2 ml of ANSA (1-amino-2-napthol4-sulfonic acid) and 20% acetic acid were added to the tubes to prepare a final volume of 30 ml (V_c).

In addition to plant samples, seven extra standard series of 0, 0.4, 0.8, 1.6, 3.2, and 4.8, 6.4 ppm were prepared by pipetting 0, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 ml of 24 ppm of Si, respectively. Two ml of each extra seven digested blank solution were added to the seven-standard series. The Si colorimetric procedure was repeated in these seven tubes.

All the tubes were sealed with parafilm and shaken. After 30 minutes the absorbance readings of the samples (plant + standard blank) at 630 nm were taken with a spectrophotometer. With the use of Si ppm and absorbance data from the standard blank samples, we formed a regression line and equation. The slope and intercept of this equation were used for Si content assayed.

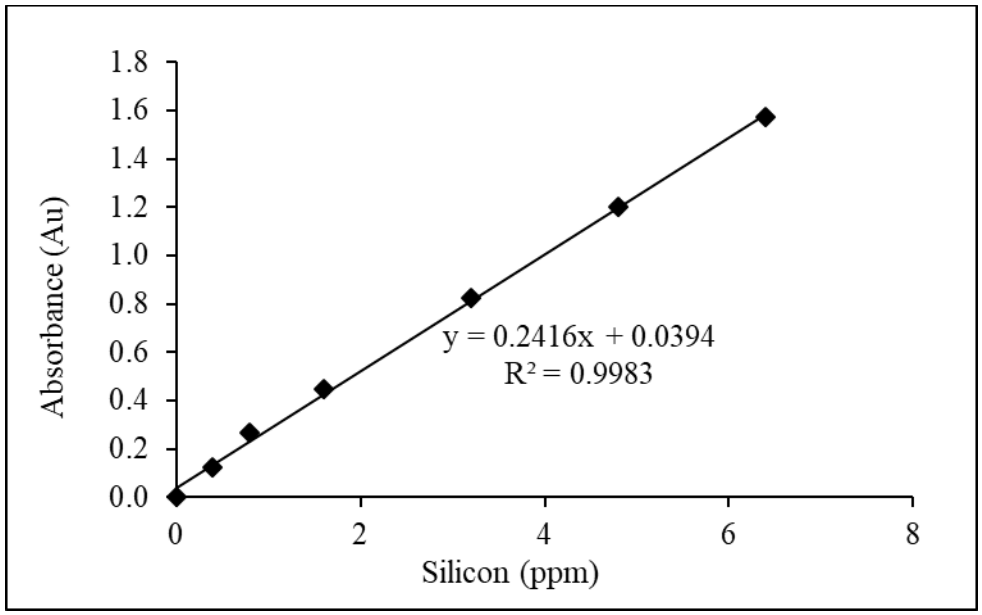

Figure 2.1: Absorbance reading relative to increasing silicon content (ppm).

$$Si\ content = \frac{\left(\ Abs_{samp} - \ Abs_{blk}\right) - Cfi}{C_{fs}} * \left[\frac{V_d}{S_{wt}} * \frac{V_c}{V_a}\right]$$

Where:

Abs_{samp} = absorbance reading of plant sample

Abs_{blk} = absorbance reading of reagent blank

C_{fi} = µg Si g^{-1} when absorbance is zero (or intercept derived from standard curve)

C_{fs}= µg Si g^{-1} per unit of absorbance (or intercept derived from standard curve)

V_d = final digest volume (mL)

S_{wt} = oven - dry equivalent weight of the digested sample (g)

V_c = final colorimetric volume (mL)

V_a = volume of aliquot used for colorimetric analysis (mL)

The silicon content data from the formula was divided by 1000 for the analysis of leaf silicon content percentage.

2.2.5 Data analysis

Nitrogen and Silicon content data were analyzed using mixed model analysis procedure (Proc Mixed, SAS Institute 2011), and Tukey HSD was used for mean separation.

Data were analyzed using mixed model analysis procedure (Proc Mixed, SAS Institute) for the phenology study which included N fertilization, Si amendment, sorghum phenology, and the interactions as fixed effects. Furthermore, cultivar study included N fertilization, Si amendment, and the interaction as fixed effects for parameters such as days to reproductive adult, fecundity, and lifespan, and Tukey's HSD was used for mean separation. The jackknifing technique was employed to estimate variance in life table parameters (Mayer *et al.* 1986). This technique is usually done by repeated calculation of the parameter estimates by excluding one sample in turn (Maia *et al.* 2000). None of the aphids on DK 37-07 cultivar survived long enough to collect data for any of the parameters. Thus, only data from the susceptible cultivar of sorghum were analyzed.

2.3 Results

2.3.1 Leaf tissue analysis

Nitrogen fertilizer application had an effect on the leaf N content (F = 8.17; df =2, 44; P = 0.001). Furthermore, both cultivar (F = 4.65; df = 1, 44; P = 0.03) and silicon (F = 15.25; df = 1, 44; P = 0.03) had an effect on leaf nitrogen content; however, no effect of interactions was seen on Nitrogen content. Leaf Si content of plants was affected by Silicon fertilization (F =

149.22; df = 1, 47; $P < 0.0001$), nitrogen fertilization ($F = 10.07$; df = 2, 47; $P < 0.0002$), but not by sorghum cultivars ($F = 0.05$; df = 1, 47; $P = 0.8$), and neither their interactions.

Table 2.1: Leaf nitrogen content percentage and silicon content percentage as affected by the treatments.

Treatments	Nitrogen Content	Silicon Content
Nitrogen fertilization (g/pot)		
0	0.714 b	2.909 a
0.75	0.959 a	1.971 b
1.75	0.986 a	2.075 b
Silicon (g/pot)		
0	1.005 a	1.186 b
17	0.768 b	3.451 a
Cultivar		
DK 37-07 (Resistant)	0.821 b	2.339 a
DK 38-88 (Susceptible)	0.952 a	2.297 a

Means with same letters are not significantly different ($P < 0.05$, Tukey's HSD).

2.3.2 Life table assays of phenology study

The pre-reproductive period of *M. sacchari* was not affected by either N fertilization, Si, phenology, or their interactions (Table 2.2). The fecundity of *M. sacchari* was affected by N fertilization (0 g = 45, 0.75 g = 62, and 1.5 g = 63), but not by Si, phenology, or any of the interactions. The lifespan of *M. sacchari* was affected by N fertilization (0 g = 22, 0.75 g = 28, and 1.51 g = 31), but not by Si, phenology, or any of the interactions. Nitrogen affected the

Table 2.2: Pre- reproductive days, total fecundity and lifespan of *M. sacchari* as affected by sorghum phenology (Phen), N, Si content, and their interactions, ($\alpha = 0.05$).

Effects	Pre-reproductive days				Total fecundity				Lifespan			
	Num DF	Den DF	*F*	Pr > F	Num DF	Den DF	*F*	Pr > F	Num DF	Den DF	*F*	Pr > F
N	2	48	1.27	0.291	2	48	7.6	0.001	2	48	11.77	<0.001
Si	1	48	0.12	0.727	1	48	0.01	0.909	1	48	0.16	0.688
N*Si	2	48	0.78	0.466	2	48	1.55	0.222	2	48	0.15	0.857
Phen	1	48	3.93	0.053	1	48	0.22	0.637	1	48	3.41	0.07
N*Phen	2	48	1.24	0.299	2	48	1.28	0.287	2	48	1.13	0.331
Si*Phen	1	48	1.65	0.205	1	48	1.46	0.232	1	48	0	0.949
N*Si*Phen	2	48	2.87	0.066	2	48	1.27	0.291	2	48	0	0.996

Table 2.3: Effect of different treatments on life table parameters estimates of *M. sacchari.* Jackknife estimates (means) and associated 95% CI. Non-overlapping 95% CI corresponds to a significant treatment effect ($\alpha = 0.05$) which are denoted by separate letters (Maia *et al.* 2000).

Phenological Stage	Treatments	r_m	λ	DT	T_G
Boot stage	Nitrogen fertilization (g)				
	0	0.25 ± 0.06 ab	1.29 ± 0.02	2.75	10.53
	0.75	0.18 ± 0.06 b	1.20 ± 0.02	3.81	13.37
	1.51	0.40 ± 0.09 a	1.43 ± 2.61	1.96	9.16
	Silicon fertilization (g)				
	0	0.25 ± 0.06	1.29 ± 0.02	2.75	10.53
	17	0.31 ± 0.09	1.36 ± 0.03	2.24	8.98
Five leaf stage	Nitrogen fertilization (g)				
	0	0.31 ± 0.04	1.37 ± 0.02	3.25	13.77
	0.75	0.20 ± 0.60	1.24 ± 0.27	3.24	10.62
	1.51	0.25 ± 0.08	1.28 ± 0.03	2.88	7.43
	Silicon fertilization (g)				
	0	0.31 ± 0.04 a	1.37 ± 0.02 a	3.25	13.77
	17	0.21 ± 0.04 b	1.24 ± 0.01 b	3.41	9.96

intrinsic rate of increase (r_m) of *M. sacchari* only in boot stage of sorghum with high r_m in the treatment with the highest rate of N (Table 2.3). In addition to this, higher N rate also decreased *DT* and T_G; however, no effect of N was seen on λ. Whereas, Silicon had a variable effect on parameters during boot and the five-leaf stage (Table 2.3).

Sorghum phenology affected λ, as higher λ was seen on five-leaf stage. However, *DT* and *TG* were shorter in the boot stage, and no effect of phenology was seen on r_m (Table 2.4).

Table 2.4: Effect of sorghum phenology on life table parameter estimates of *M. sacchari*. Jackknife estimates (means) and associated 95% CI. Non-overlapping 95% CI corresponds to a significant treatment effect ($\alpha = 0.05$) which are denoted by separate letters (Maia *et al.* 2000).

Phenology	r_m	λ	*DT*	T_G
Boot stage	0.25 ± 0.06	1.29 ± 0.02 b	2.75	10.53
Five leaf stage	0.31 ± 0.04	1.37 ± 0.02 a	3.25	13.77

2.3.3 Life table assays of cultivar study

High level of resistance was shown by the resistant cultivar DKS 37-07. Aphids were replaced each time when they were dead before reproduction till five times. Aphids on resistant cultivar DKS 37-07 survived on an average of two days before they could reproduce nymphs. Therefore, we were not able to take any data for analysis from the resistant cultivar DKS 37-07. The results presented below are from aphids which were fed on susceptible cultivar DKS 38-88.

Aphids caged on plants with no N fertilization took longer ($F = 3.6$; df = 2, 24; $P = 0.043$) to reach the reproductive stage than aphids caged on plants which received higher rates of N fertilization (Fig. 2.2A). No effect of Si ($F = 0.46$; df = 1, 24; $P = 0.5047$) or the interaction ($F = 0.51$, df = 2, 24; $P = 0.6047$) was seen on pre-reproductive days (Fig. 2.2 B, C). The fecundity of *M. sacchari* was affected by N treatments ($F = 19.8$; df = 2, 24; $P < 0.0001$), but not by Si ($F = 0.88$; df = 1, 24; $P = 0.3565$) or the interaction ($F = 0.27$; df = 2, 24; $P = 0.767$) (Fig. 2.3). Life span of *M. sacchari* was not affected by either N fertilization ($F = 0.23$; df = 2, 24; $P = 0.7981$), silicon ($F = 0.21$; df = 1, 24; $P = 0.6483$), or the interaction ($F = 0.76$, df = 2, 24; $P = 0.4803$) (Fig 2.4).

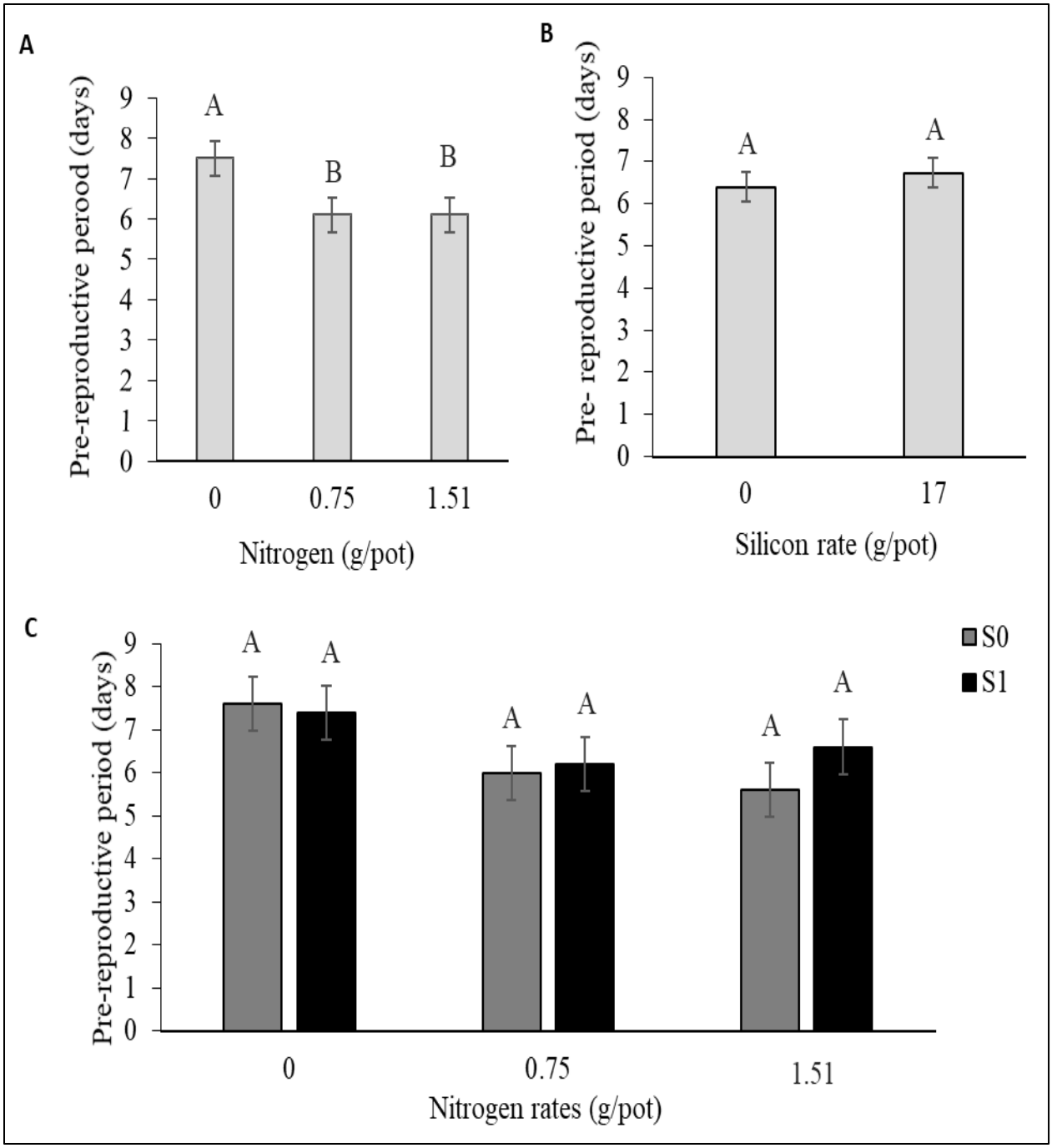

Figure 2.2: LS mean (±SE) of pre- reproductive days of *M. sacchari*, (A) under three N rates, (B) under two Si rates, (C) under three nitrogen rates and two silicon rates (S0 = 0g, S1 = 17g). Bars within each chart followed by the same letter are not significantly different ($P <$ 0.08, Tukey's HSD).

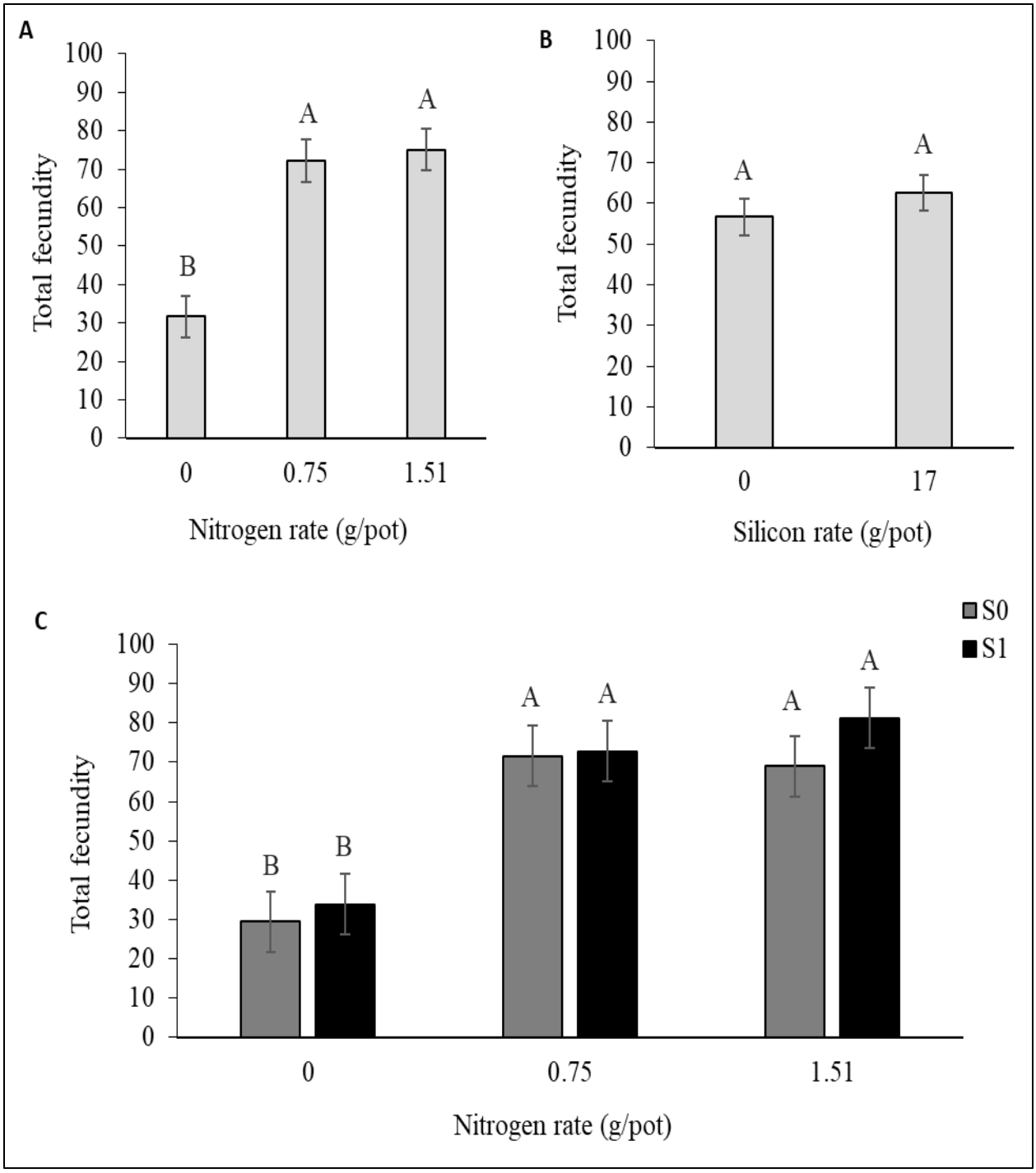

Figure 2.3: LS mean (±SE) of total fecundity (offspring per female) of *M. sacchari* (A) under three N rates, (B) under two Si rates and (C) under three N and two Si rates (S0 = 0g, S1 = 17g). Bars within each chart followed by the same letter are not significantly different ($P < 0.05$, Tukey's HSD)

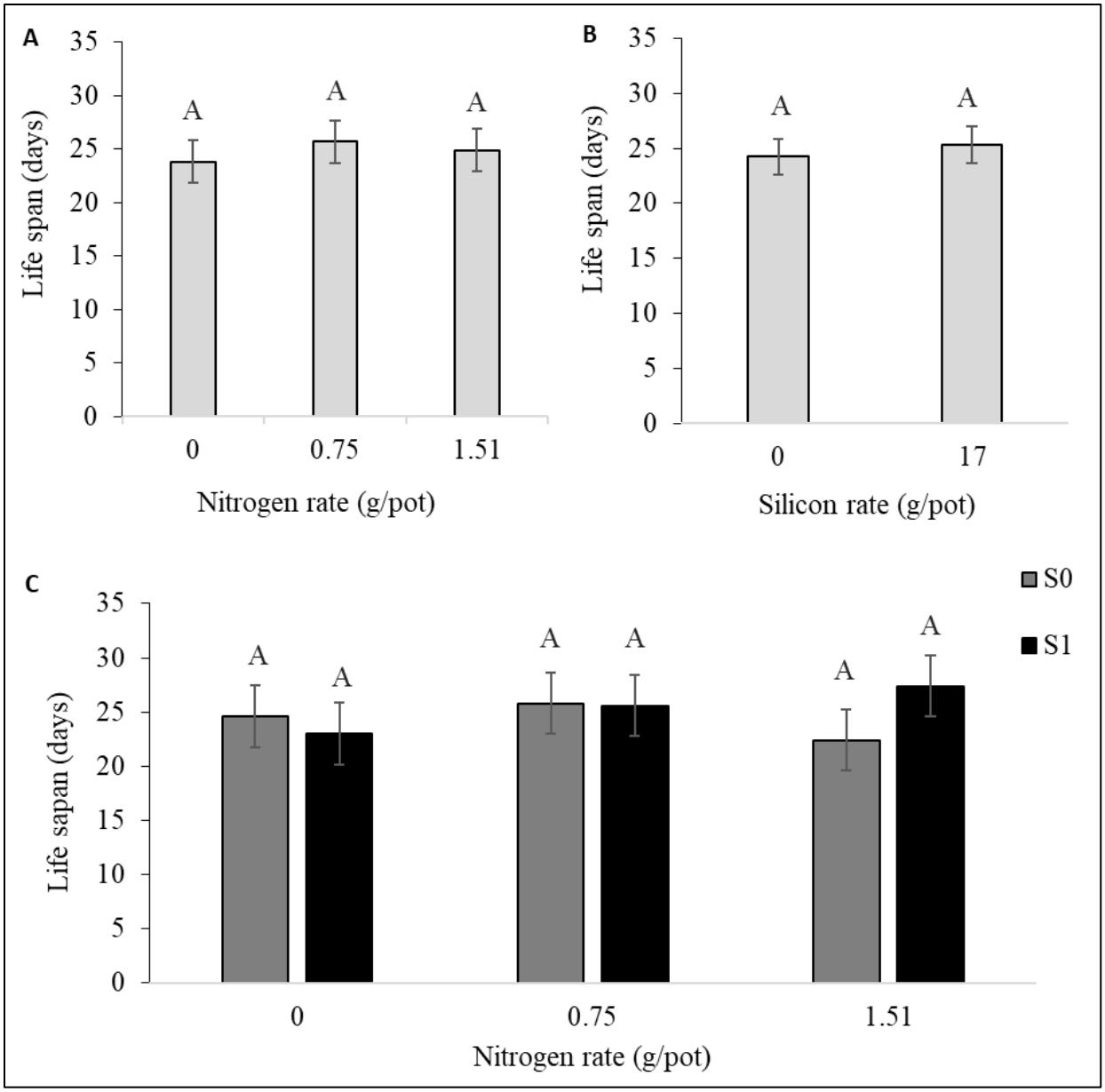

Figure 2.4: LS means (±SE) of lifespan of *M. sacchari* (A) under three N rates, (B) under two Si rates and (C) under three N and two Si rates (S0 = 0g, S1 = 17g). Bars within each chart followed by the same letter are not significantly different ($P < 0.05$, Tukey's HSD).

Intrinsic rate of increase (*rm*) was affected by N fertilization as was it was higher in plants which received the highest rate of N (Table 2.5). However, no effect of N on λF and *DT* was seen. The longest average generation time (*TG*) of 10.36 days was seen in *M. sacchari* with no N fertilization. Furthermore, Si did not affect any of the parameters (Table 2.5).

Table 2.5: Effects of Si and N treatments on lifetable parameter estimates of *M. sacchari* in the cultivar study. Jackknife estimates (means) and associated 95% CI. Non-overlapping 95% CI corresponds to a significant treatment effect ($\alpha = 0.05$) which are denoted by separate letters (Maia *et al.* 2000).

Source	r_m	λ	DT	T_G
Nitrogen fertilization (g)				
0	0.29 ± 0.04 b	1.34 ± 0.01	2.36	10.36
0.75	0.30 ± 0.18 ab	1.35 ± 0.05	1.39	1.39
1.51	0.50 ± 0.15 a	1.29 ± 0.06	2.69	7.26
Silicon fertilization (g)				
0	0.29 ± 0.04	1.34 ± 0.01	2.36	10.36
17	0.29 ± 0.05	1.34 ± 0.02	2.31	11.34

2.4 Discussion

The nutritional content of a host plant has an important influence on growth and reproduction of herbivores (Dixon 1987). In our experiment, we illustrated the effects of host plant cultivar, phenology, N, and Si content on herbivore biology. Nitrogen fertilization is an important part of sorghum production. Therefore, the understanding effect of N on *M. sacchari* biology is also essential. According to Lu *et al.* (2007), increase in N fertilizer enhances insect growth and reproduction. In the phenology experiment, there was an effect of N fertilizer on *M. sacchari* fecundity and lifespan but not on days to reproductive adult. The effect of N on *M. sacchari* on fecundity and lifespan observed in the phenology study is consistent with similar observation from other aphid species including *Aphis gossypii* Glover (Hosseini *et al.* 2010), and *Rhopalosiphum padi (L.)* (Aqueel and Leather 2011) which showed a positive effect of N on fecundity and lifespan. Similarly, effect of N were not seen on the rate of nymphal development

of other aphid species such as *R. padi* (Aqueel and Leather 2011) and *Diuraphis noxia* (Mordvilko), this result is consistent with our phenology study, where no effect of N on *M. sacchari* pre- reproductive days was seen (Moon *et al.* 1995).

Interestingly in our cultivar study, the main effect of N was positive for days to reproductive adult and fecundity but not for lifespan. A similar finding has been reported from studies of several other aphid species including *D. noxia* (Mordvilko) (Moon *et al.* 1995), *Brevicoryne brassicae L*. (Zarghami *et al.* 2010) and *Metopolophium dirhodum* (Gash 2012) which all failed to demonstrate an effect of N content on aphid lifespan. The positive effects of N on *M. sacchari* fecundity observed in our cultivar study was also reported for other aphid species such as *A. gossypii* (Nevo and Coll 2001) and *Hysteroneura setariae* (Thomas) (Jahn *et al.* 2005). However, a contradictory result for N effects on fecundity was reported by Eini *et al.* (2017), where fecundity of *A. gossypii* was lowered by a higher rate of N. Their results were likely influenced by the simultaneous application of Phosphorus (P) along with N and Si (Eini *et al.* 2017). These studies and results reported herein suggest that the positive effect of host plant N content on aphid fecundity is less variable then the effects of N on lifespan and nymphal development. In our studies, different cultivars of sorghum were used in each experiment which may explain the different effect of N on *M. sacchari* lifespan and nymphal development. Like our study, caged *S. graminum* population on sorghum increased with higher nitrogen rate (Archer *et al.* 1982); however, research on the effect of N on *M. sacchari* population on sorghum has not been conducted before. Therefore, our research provides an important overview of N effect on *M. sacchari* biology. However, a field study will give a better understanding of the impact of N fertilization on aphid population.

In both of our studies, treatments with a higher rate of N had a positive effect on the lifetable parameters intrinsic rate of increase (r_m). However, no effect on λ was seen. In addition to this, N also affected doubling time (*DT*) and generation time (T_G) in phenology study. However, in our cultivar study, there was no effect of N on λ and *DT*. Variation within the effect of N on these parameters might be because of lower replication of each treatment combination in our study, variation in sorghum cultivar, and change in daylight period between the two seasons. The positive relation of N fertilization with aphid life table parameters was also seen in several aphid species such as *H. setariae* (Thomas) in rice (Jahn *et al.* 2005), *B. brassicae* in oilseed rape (Zarghami *et al.* 2010), *A. gossypii* in cucumber and *Chrysanthemum lindicum* (Asteraceae) (Hosseini *et al.* 2010, Rostami *et al.* 2012) and *M. dirhodum* in wheat (Gash 2012). The study of *B. brassicae* on wild and cultivated Brassica species showed that about 43% of the variation in the intrinsic rate of increase is due to amino acid concentrations which are mostly dependent on N content in plants (Cole 1997). Increased N content is reported to increase total amino acids content in plants, which is the main nutrient for aphid growth and reproduction (Sauge *et al.* 2010). In our study, nitrogen content in sorghum leaves was positively correlated with the N fertilization as highest N fertilization resulted in highest leaf N content. Therefore, the positive effect of N fertilization on *M. sacchari* biology might be due to an increase in N content in the plant, which increased total amino acids nutrient for *M. sacchari*.

In both of our studies, Si had no detectable effect on *M. sacchari* fecundity, days to reproductive adult, lifespan, and life table parameters. However, during our phenology experiment, the effect of Si was variable among the life table parameters. These results are in contrast to other studies where Si significantly reduced the fecundity of *Schizaphis graminum* in wheat (Goussain *et al.* 2005), *Rhopalosiphum maidis* in corn (Moraes *et al.* 2005), and *Sitobion*

avenae in wheat (Dias *et al.* 2014). Furthermore, in the studies conducted by Dias *et al.* (2014) and Ranger *et al.* (2009), Si also affected the life table parameters of *S. avenae* in wheat and *M. persicae* in *Zinnia elegans*, respectively. This contradictory result might be because of the different source of Si used and the method of application conducted in these studies. The studies conducted by Goussain *et al.* (2005), Moraes *et al.* (2005) and Dias *et al.* (2014) used furnace slag, sodium silicate solution, and silica gel solution as a source of Si, respectively. Although the Wollastonite amendments in our study effectively increased leaf tissue Si content, the distribution of Si within plant tissue may vary between the Si source and way of application.

Furthermore, in studies conducted by Goussain *et al.* (2005) and Moraes *et al.* (2005) aphid fecundity was significant only in the treatment with soil plus foliar application of Si. However, leaf Si content in this treatment was not significantly different to the treatment that received only one soil application of Si. According to Moraes *et al.* (2005), foliar application of silica solution improves the distribution of Si in all plant parts, and proper distribution of Si in all plant parts is as important as its content in the plant regarding an effective result. On the contrary, we treated plants with soil application of Si (no additional foliar application). Therefore the Si might not have been well distributed within the plant, thus leading to the non-significant effect of Si on *M. sacchari* fecundity. Moreover, all these studies regarding silicon effect on aphid biology were conducted on different aphid species other than *M. sacchari*, whose biology is unique from other aphids. *S. graminum* produces toxins while feeding on the crop; whereas, *M. sacchari* sucks the plant sap and doesn't secrete any toxin. This might be one of the reasons for no silicon-induced resistance on *M. sacchari* fed sorghum plants.

In our greenhouse cultivar study, the resistant cultivar DK 37-07 showed a high level of resistance towards *M. sacchari*. DK 37-07 is a greenbug resistant cultivar, which has been used

in few field studies before where it showed an intermediate level of resistance to *M. sacchari* (Trostle 2015, Michaud and Zukoff 2017). Furthermore, in the previous study conducted by Armstrong *et al.* (2016), greenbug resistant cultivar such as RTx2783 and PI 55610 showed resistance towards *M. sacchari.* Therefore, these resistant cultivars have potential to manage *M. sacchari*, and should be included in sorghum production practices for *M. sacchari* management. However, research including these treatments in the field is important to understand its actual implication in field condition, where different variables act together.

CHAPTER 3. THE EFFECTS OF CULTIVAR, N FERTILIZATION, AND INFESTATION LEVEL ON *M. SACCHARI* POPULATION DYNAMICS AND SORGHUM YIELD

3.1 Introduction

Numerous factors influence pest infestations and resulting yield losses in the field, and much about how these influences interact remains poorly understood. A successful IPM program requires a thorough understanding of how environmental conditions affect pest ecology. Because *M. sacchari* has only recently emerged as an economic pest of sorghum in the U. S., little is known about what influences the pest's population dynamics in the field.

Sorghum cultivars resistant to aphids have been widely used to manage *S. graminum* (Teetes *et al.* 1995), and a few of them have also shown resistance towards *M. sacchari* (Armstrong *et al.* 2015, Brewer *et al.* 2017). However, use of resistant sorghum cultivars in commercial settings is low because the current level of resistance expressed is not sufficient to mitigate damaging *M. sacchari* infestations and chemical controls may still be needed. Further, resistant cultivars often have lower yield potential and are expected to have a similar yield as the susceptible cultivars despite having reduced levels of insect infestation (Sharma 1993). In addition to the effects of cultivars, *M. sacchari* infestations are likely to be influenced by the nutritional content of plants. Nitrogen fertilization is important for crop growth and grain production, but the application of high rates of N has potential to increase aphid infestations. High levels of soluble N in leaf tissue and soil increase nutrient availability in plants, allowing for greater aphid growth and reproduction (Altieri and Nicholls 2003).

While N content and cultivar resistance influence pest population dynamics, additional factors must be considered when examining pest impacts on yield. Pest density and infestation timing are key variables which determine crop damage. The objectives of this study are to

determine the effects of sorghum cultivar, N fertilization, and pest density on *M. sacchari* population dynamics and subsequent yield loss under field conditions.

3.2 Materials and methods

A field experiment was conducted in 2017 at the LSU AgCenter's Central Research Station (Ben Hur Rd, Baton Rouge, LA) to examine the influence of aphid density on yield loss for resistant (DKS 37-07) and susceptible (DKS 38-88) sorghum under various aphid densities, and N fertilization levels. A complete factorial design consisting of 24 treatment combinations (2 cultivars x 4 infestation levels x 3 N fertilization levels) was used. Treatment combinations were randomized to 8-row (30-inch beds) x 50-ft plots (1000 ft^2 = 0.00667 ha/plot) with total of 48 plots (Figure 3.1). An early-maturing sorghum cultivar was planted in the buffer area to alleviate the impact of bird feeding on plots. The four aphid infestation levels were: (1) protected (bi-weekly insecticide applications), (2) low threshold level (spray at 50 aphids/leaf), (3) high threshold level (200 aphids/leaf), and (4) unprotected (no insecticides). Applications of flupyradifuron (Sivanto Prime, Bayer Crop Science, Research Triangle Park, NC) at 85 g A.I./ha were made using a CO_2 pressurized backpack sprayer calibrated to deliver 96 l/ha. Nitrogen was applied to each plot by hand in the form of urea to establish fertilization levels which corresponded to those evaluated in greenhouse assays (Chapter 2): no added N, low N (110 kg/ha), and high N (224 kg/ha). Sampling began approximately one week after the first aphids appeared and continued weekly for seven weeks. Samplings were done on July 12, July 19, July 26, August 2, August 16, and August 25 of 2017. All the leaves on ten plants/plot (4 center rows only) were examined, and the total numbers of aphids per plant were recorded.

Yield data were collected from the two center rows of each plot at harvest using a two-row combine harvester. Total grain was collected, weighed, and analyzed for moisture content with a grain moisture tester (Model GAC2500 AGRI, Dickey John Corp., Auburn, LA).

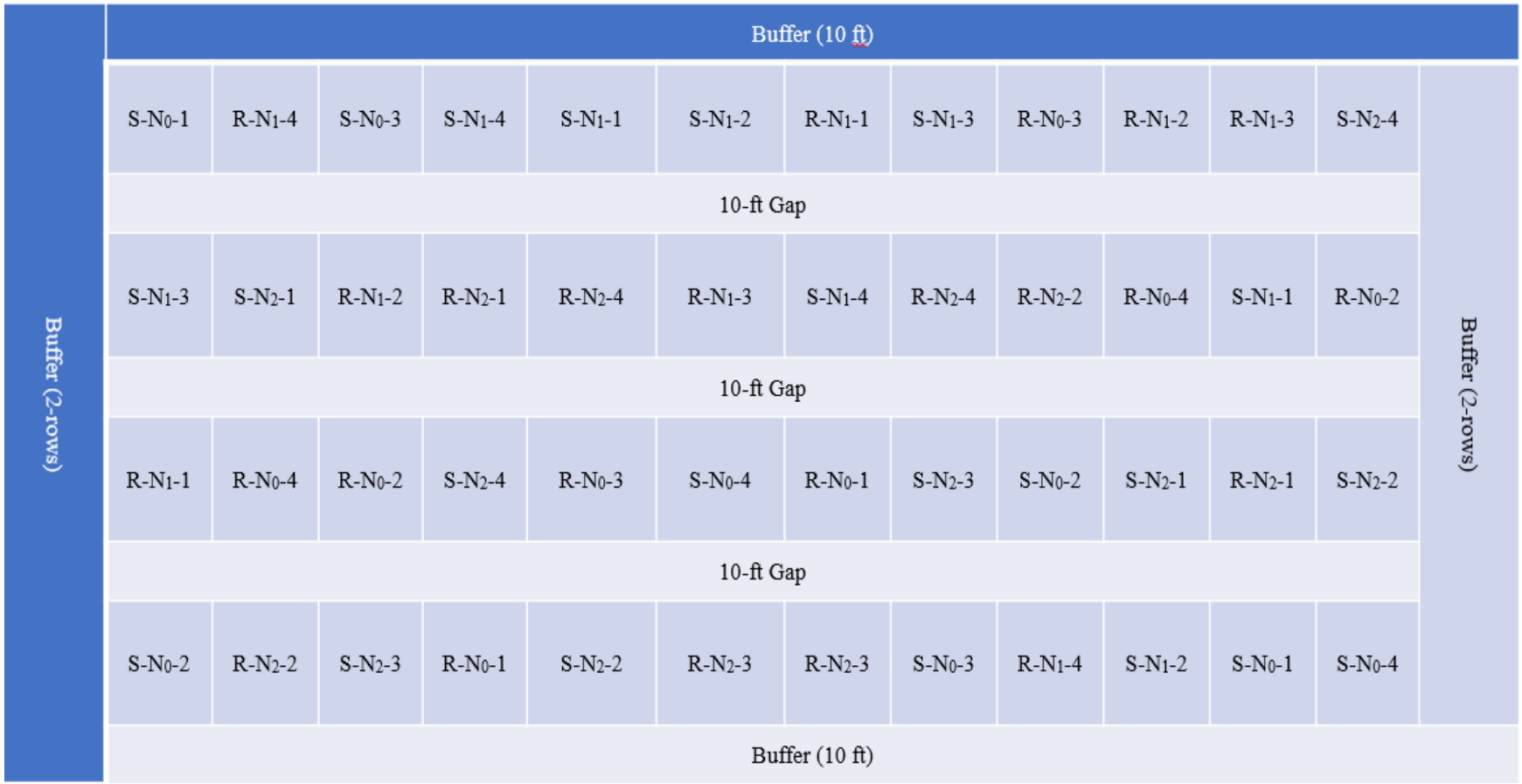

Figure 3.1. A plot map of Sorghum factorial field study. An experiment conducted at the LSU AgCenter Ben H Research Station (Baton Rouge, LA) in 2017 and 2018. Cultivars: S = DKS 38-88, R = DKS 37-07, N levels: N_0 = 0 kg/ha, N_1 = 110 kg/ha, N_3 = 224 kg/ha, Infestation levels: 1 = bi-weekly application of insecticide, 2 = application at 50aphids/leaf, and 3 = no application.

Plot yields were adjusted to a standard moisture content of grain as 14% (McNeill and Montross 2003).

Aphid data were analyzed with a mixed model analysis which included cultivar, infestation level, N fertilization, sampling week, and their interactions as fixed effects. Yield data were analyzed with the mixed model analysis with cultivar, N fertilization, infestation level, and their interactions as fixed effects. Multiple linear regression was conducted with cumulative aphid population, nitrogen rate as independent variables and yield as the dependent variable (PROC REG, SAS Institute 2002–2012). Cumulative aphid days was calculated using the formula:

$$\text{Cumulative aphid days} = \sum_{i=1}^{n} \left\{ \frac{x_i + x_{i-1}}{2} \right\} * t$$

Where n is the number of sample dates, x_i is the number of aphids per plant on sample date i, and t is the number of days since previous sample (Hanafi *et al.* 1989). Qualitative dummy variables, z1 and z2, were used to differentiate between yield differences associated with N fertilization rates (if N = 1 then z1 =1; if N = 2 then z2 =1).

3.3 Results

Significant aphid infestations were found in some plots at the first sampling date, and insecticides were applied to protected plots on Jul 12, Jul 26, and Aug 9. Insecticide applications were made to level 2 plots Jul 19 as infestations had reached >50 aphids/leaf in several plots but did not require additional applications. Infestations never reached the high threshold (>200 aphids/leaf) in any plots, so plots of both infestation levels three and four were left untreated. These two levels were considered the same during data analysis.

In leaf tissue analysis, Nitrogen fertilizer application had an effect on the leaf N content. Furthermore, both cultivar and infestation level had an effect on leaf nitrogen content; however, no significant effect of all the interactions were seen on Nitrogen content (Table 3.1).

Resistant cultivar DKS 37-07 had higher N leaf content in comparison to the susceptible cultivar DK 38-88 (Figure 3.4). Furthermore, as expected sorghum leaf with nitrogen fertilizer of highest rate (224 kg/ha) had highest nitrogen leaf content and other lower rate had lower leaf N content respectively (Figure 3.4). However, there was no significant difference among the leaf N content among the levels (Figure 3.4).

Table 3.1 Type 3 effect of fixed effects cultivar, N, and infestation level on leaf N content.

Effect	Num DF	Den DF	F Value	Pr > F
Cultivar	1	30	5.98	0.021
N	2	30	260.20	<.001
Cultivar*N	2	30	1.37	0.269
Level	2	30	4.83	0.015
Cultivar*Level	2	30	2.87	0.072
N*Level	4	30	1.00	0.422
Cultivar*N*Level	4	30	1.17	0.345

Populations of *M. sacchari* were influenced by cultivar, infestation level, and week as well as the cultivar × week, level × week, and N × level × week interactions (Table 3.2).

Differences were not detected among N rates or any of the other interactions for overall aphid populations (Figure 3.2 A). Cultivar influenced overall aphid population with a 3.7-fold lower number of aphids in resistant cultivar (37.85 aphids/plant) than susceptible cultivar

(139.43 aphids/plant) across all infestation levels, nitrogen rates, and weeks (Figure 3.2 B). Aphid populations in nontreated plots peaked 26-Jul and declined after that (Figure. 3.2 C). Insecticide applications effectively reduce aphid densities in protected plots and those sprayed at 50 aphids/leaf (Figure 3.2 C).

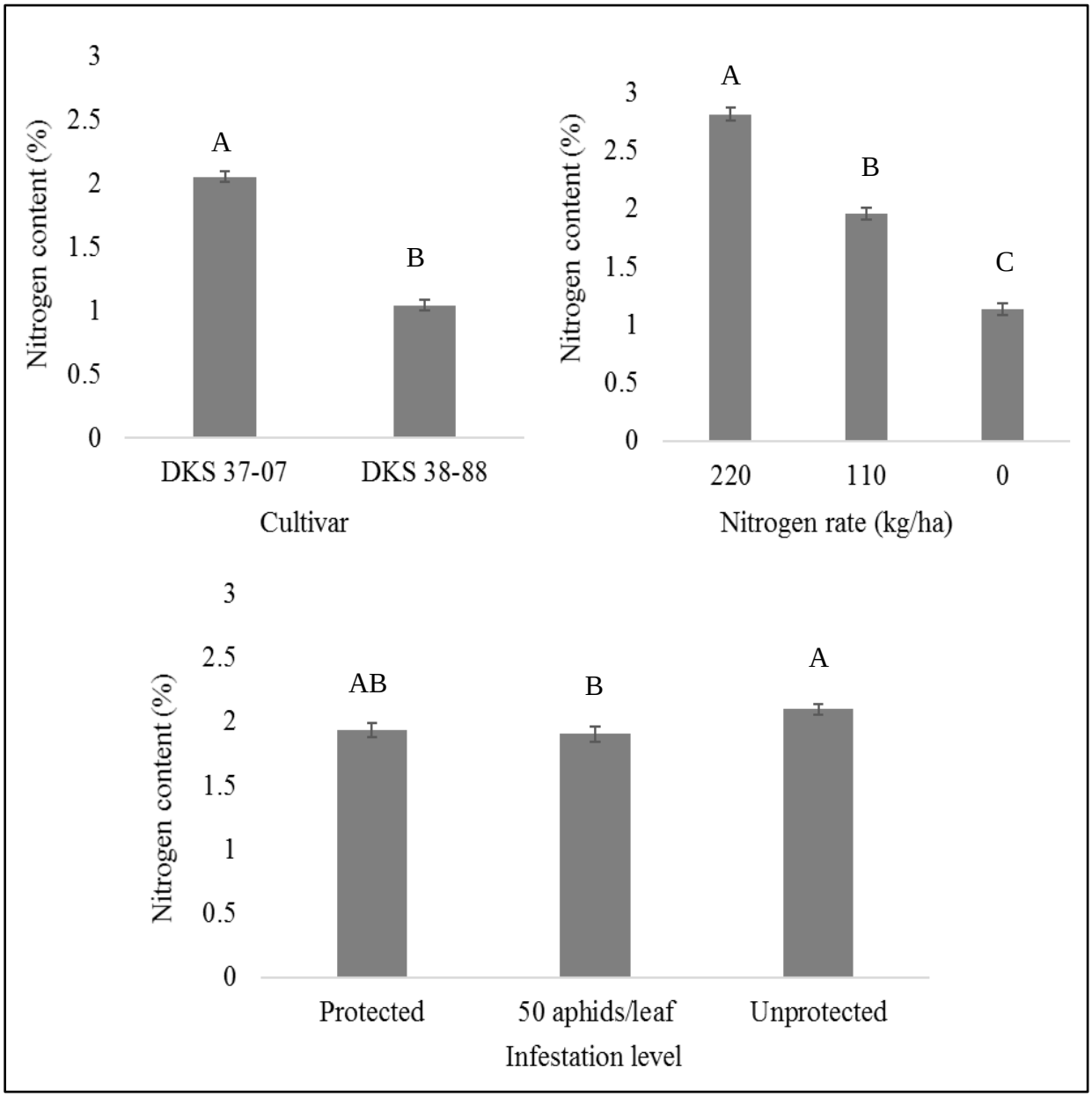

Figure 3.4: LS means (±SE) of Nitrogen content of sorghum as affected by cultivar, N rate and infestation level. Bars within each chart followed by the same letter are not significantly different ($P < 0.05$, Tukey's HSD).

Table 3.2: Type 3 tests of fixed effects such as cultivars, N= Nitrogen rates, Level = infestation level on *M. sacchari* population.

Effect	Num DF	Den DF	F Value	Pr > F
Cultivar	1	30	17.51	0.002
N	2	30	1.27	0.296
Cultivar*N	2	30	0.03	0.966
Level	2	30	11.71	0.002
Cultivar*Level	2	30	2.74	0.081
N*Level	4	30	0.75	0.567
Cultivar*N*Level	4	30	1.26	0.309
Week	6	180	10.32	<.001
Cultivar*Week	6	180	8.00	<.001
N*Week	12	180	0.42	0.955
Cultivar*N*Week	12	180	0.24	0.996
Level*Week	12	180	2.72	0.002
Cultivar*level*Week	12	180	0.54	0.883
N*Level*Week	24	180	1.91	0.009
Cultivar*N*Level*Week	24	180	1.33	0.149

Sorghum yields were affected by N rates, infestation levels, cultivar ($P< 0.1$), and the cultivar × N rate interaction, but not by any of the other interactions (Table 3.3). N had a positive effect on yield with a higher rate (224 kg/ha) of nitrogen giving highest sorghum yield followed by other two lower rates 110 kg/acre and no N respectively (Figure 3.3).

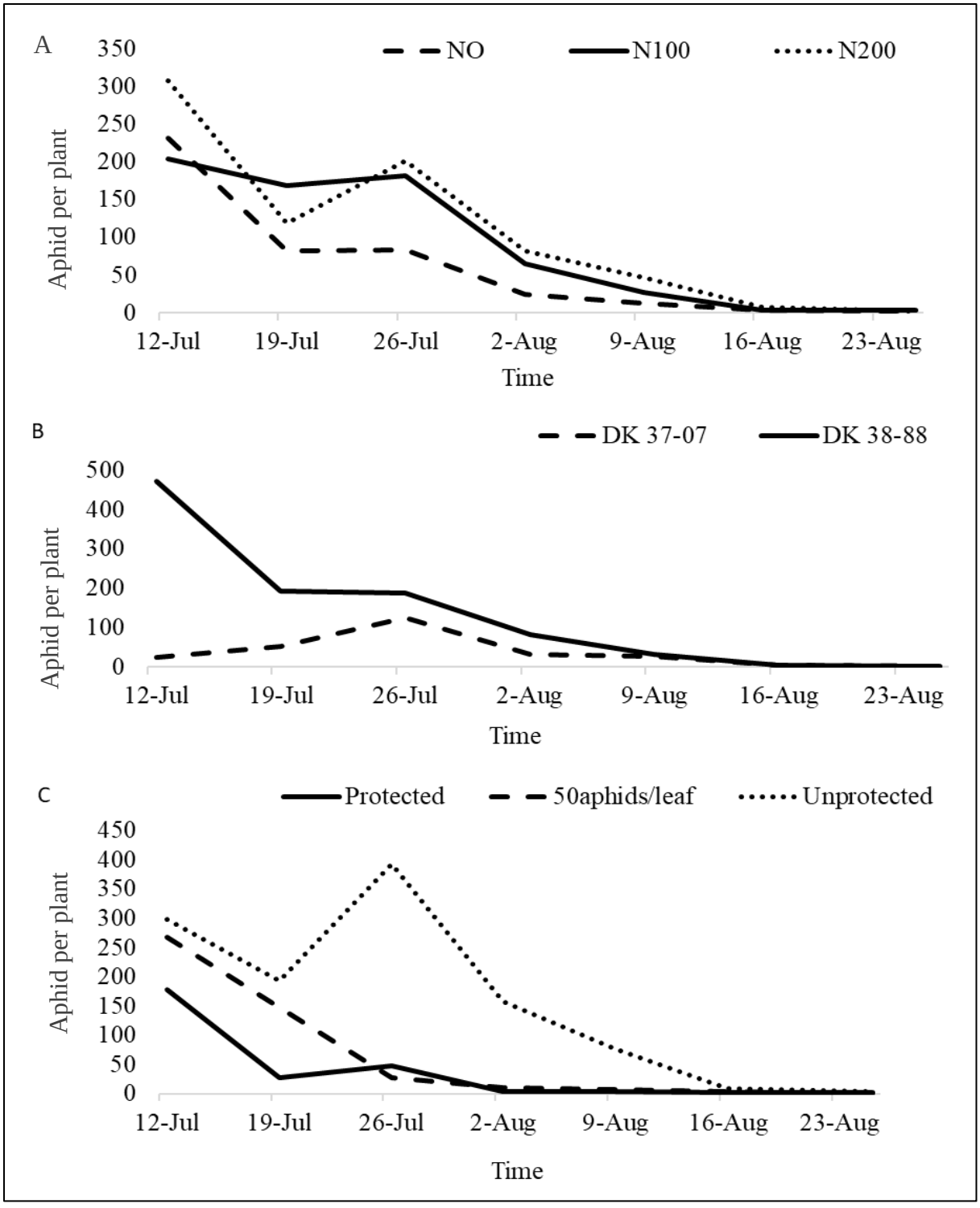

Figure 3.2. Effect of cultivars, nitrogen fertilizers (N), and infestation level (level) on aphid population throughout the time duration of seven weeks. N0 = 0kg/ha, N1 = 110 kg/ha, and N2 = 224 kg/ha.

Table 3.3: Type 3 Tests of Fixed Effects of Nitrogen, cultivar and infestation level on sorghum yield.

Effect	Num DF	Den DF	F Value	Pr > F
Cultivar	1	30	2.90	0.099
Nitrogen	2	30	180.81	<.0001
Cultivar* Nitrogen	2	30	3.80	0.034
Level	2	30	3.26	0.052
Cultivar * Level	2	30	1.22	0.310
Nitrogen * Level	4	30	0.96	0.442
Cultivar * Nitrogen *Level	4	30	0.98	0.433

Resistant cultivar (DKS 37-07) had higher yield compared to the susceptible cultivar (DKS 38-88). Furthermore, DKS 38-88 had a higher yield of 110 kg/ha; however, at 224kg/ha N rate DKS 37-07 had a higher yield than susceptible cultivar (DKS 38-88). Moreover, the effect of infestation level was detected on yield as treatment with insecticide spray at 50 aphids/leaf had the highest yield among the three levels (Figure 3.3).

A negative linear relationship occurred (F = 102.16; df = 3, 44; $P < 0.001$, $R^2 = 0.866$) between the mean number of aphids per plant across all weeks and the sorghum yield (Fig 3.4). The dummy variables, z1 and z2, improved the regression model (t = 11.2, $P < 0.001$ for z1; t = 16.3, $P < 0.001$ for z2) by increasing the intercept by 1,520.6 and 2,289.8 for N1 and N2 data, respectively (slope = -0.061; intercepts = N0: 844.2; N1: 2,364.8; N2: 3,134.0). It means that when there is an increase of one aphid/leaf, the average yield will reduce by 61 g/ha. Furthermore, in the absence of *M. sacchari*, average sorghum yield is 845kg/ha, 2365 kg/ha, and

3134 kg/ha at N rate of 0, 110, 220 kg/ha respectively. Moreover, 86% of the variation in the yield is explained by the nitrogen rates and cumulative aphid days as used in the model.

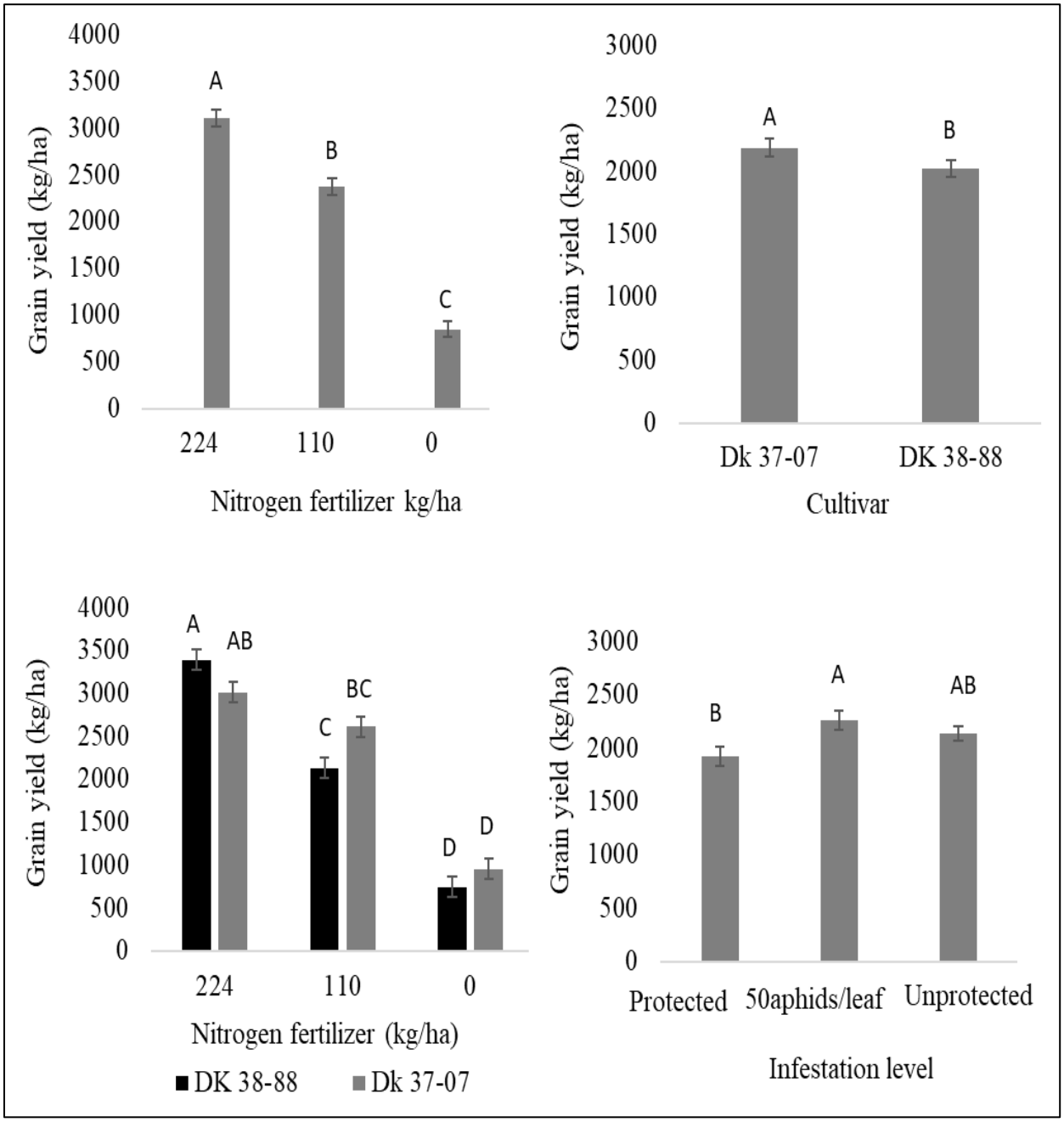

Fig 3.3: Grain yield as affected by Nitrogen fertilizer rates, Cultivars, Infestation level, and Nitrogen × cultivar. Bar graph with same letter are not significantly different (Tukey's HSD, $P < 0.05$). In cultivar and grain yield bar graph same letter are not significantly different (Tukey's HSD, $P < 0.1$)

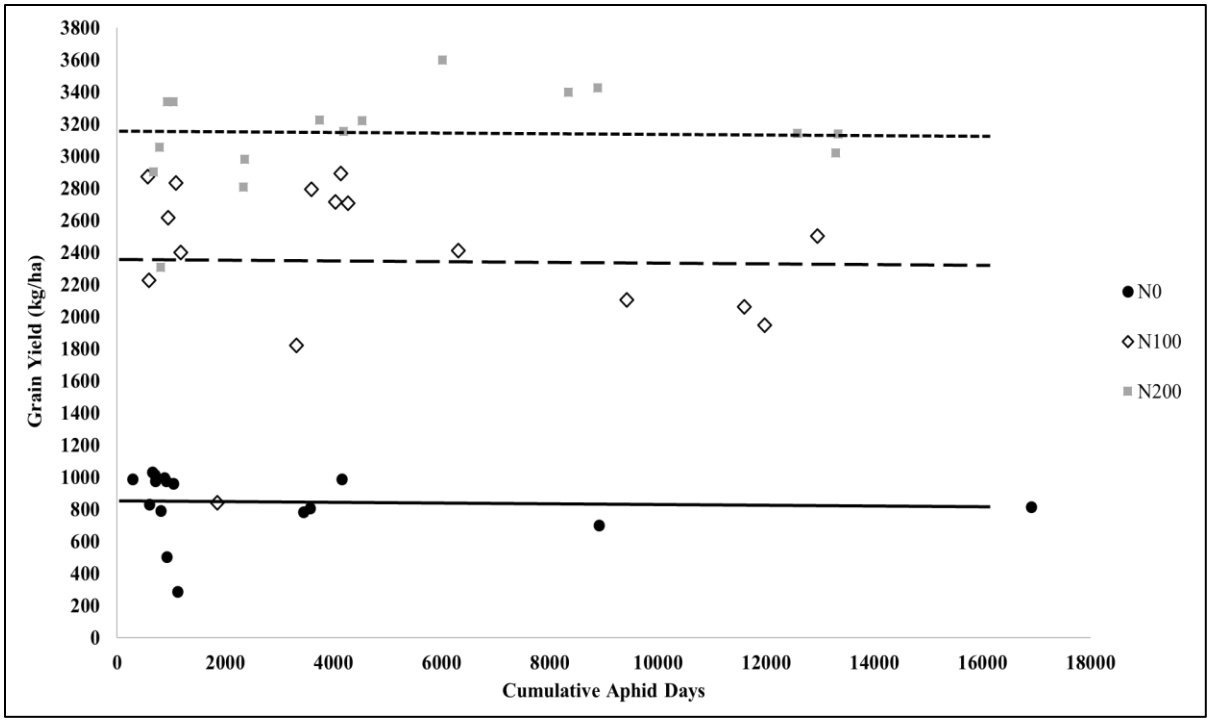

Figure 3.4: Relationship between grain yield and cumulative aphid days.

3.4 Discussion

This study provides the first assessment of the influence of both sorghum cultivar N fertilization on *M. sacchari* populations in the field. Further, our research demonstrates the potential for yield losses from *M. sacchari* under variable N levels and cultivars. The resistant cultivar DKS 37-07 reduced the aphid population compared to the susceptible cultivar DK 38-88 under field conditions; however, not to the extent of our greenhouse study, where none of the aphids survived to reproductive age on DKS 37-07. It might be because of greater aphid pressure in the field than in the greenhouse, as it is not possible under greenhouse conditions to replicate the number of possible inoculations which may occur in the field. The resistant cultivar DKS 37-07 is a greenbug resistance biotype E and has been reported as an intermediate resistant cultivar to *M. sacchari* in studies conducted by Trostle (2015) and Michaud and Zukoff (2017). Moreover, resistant cultivar had a higher yield than the susceptible cultivar at the N level of 224

kg/ha. Along with resistance, cultivar DKS 37-07 also gave substantial yield, which may not be common for other cultivars. Thus, these results suggest that use of resistant cultivar along with higher nitrogen rate (224kg/ha) in sorghum production can control sugarcane aphid without compromising the yield.

The comparable levels of aphid control between plots sprayed bi-weekly and those sprayed just once in our study suggest that application of Sivanto at the infestation level of 50 aphids/leaf may be sufficient to provide season-long control. These results are consistent with several other studies which have shown that Sivanto applications can control *M. sacchari* infestations effectively for up to 21 days (Buntin 2016, Bowling *et al.* 2016, and Steckel and Stewart 2016). However, greater pest pressure than what was observed in our study is not uncommon under commercial production conditions, and multiple insecticide applications are often required (Seiter *et al.* 2014). Furthermore, a study conducted by Gordy *et al.* (2016) reported economic threshold of *M. sacchari* to be 50–75 aphis/leaf. Therefore, insecticide application at an early stage of infestation with proper scouting can reduce the total cost for *M. sacchari* management.

There was no clear impact of insecticide applications on yields in our study, suggesting that factors other than *M. sacchari* infestations influence yields among plots. The fact that we did not control diseases or other pests of sorghum such as sorghum midge, and headworms, likely affected the sorghum yield results. Additionally, aphids were already present at treatable levels on the first sampling date, so earlier control may have improved results in our study. High yield variation among plots due to numerous treatment factors and limited replication likely limited ability of proc mixed procedure to detect impacts of aphids on yields. The slight negative relationship between aphid populations and yields observed with the multiple linear regression

which partially accounts for effects of N fertilization suggests *M. sacchari* infestations in our study did impact yields. Future studies should continue to examine the relationship between yield with the greater sampling frequency and improved management of other pests and diseases. Sorghum yields in our study greatly improved by high levels of N fertilization regardless of aphid infestations, which suggests that N fertilizer has a very important role in sorghum yield and its rate should not be reduced to manage aphids. This study adds to the growing body of work examining factors which influence *M. sacchari* population dynamics and management decisions in sorghum. Continued examination of aphid ecology and management is needed to develop IPM programs for this damaging pest.

CHAPTER 4. DISCUSSION AND CONCLUSION

This project examined *M. sacchari* population dynamics under greenhouse and field conditions. Two greenhouse studies with two cultivars of sorghum, three rates of N, two rates of Si fertilizer, and two phenological stages of sorghum were conducted in 2017. In both studies, N had a positive effect on *M. sacchari* growth and reproduction compared to unfertilized plants. However, this positive effect of N on aphid population growth was not observed under field conditions. Discrepancies between field and greenhouse studies are not uncommon, and cases have been reported where the effect of N on herbivore biology in the field does not correspond to greenhouse results with other aphid species (Alteiri and Nicholls 2003). Therefore, the effect of N on aphid populations might have been masked under other field conditions such as climate and natural enemies. However, N fertilization did affect sorghum yield, as the highest yield was associated with high rate of N (224 kg/ha). It suggests that even though N is can potentially increase the pest population in the field, it is not likely that manipulation of N rates will be adopted as a pest management strategy.

In case of Si, it did not affect any of the parameters such as aphid's lifespan, days to reproductive adult, and fecundity. It is the first study to see the effect of Si on *M. sacchari* biology, and our result indicates that there was no effect of Si despite increasing concentration of Si in the leaf tissue. However, effects of Si on other aphid species such as *S. graminum* and *A. gosypii* have been observed, where Si adversely affected aphid's biology. Although no effect of Si on *M. sacchari* was observed in this study, it may warrant further investigation with additional Si sources and application methods.

Resistant cultivar (DKS 37-07) showed a high level of resistance as aphids did not survive long enough to take data on their development for the life table parameters. Similarly, in

the field study, DKS 37-07 reduced aphid population when compared to the susceptible cultivar DKS 38-88. Therefore, this study shows that resistant cultivar DKS 37-07 has potential to reduce *M. sacchari* infestation and could be incorporated in future IPM strategies. In addition to this, the yield of DKS 37-07 cultivar was similar to the average yield of several sorghum hybrid cultivars grown in a yield trial in LSU AgCenter research stations (Fromme *et al.* 2018). Continued investigation into *M. sacchari* resistant cultivars has potential to improve management of this damaging pest greatly. Results from the field study manipulating aphid densities suggest the recommended economic threshold level for *M. sacchari* of 50–75 aphids per/leaf (Gordy *et al.* 2016) is likely effective in reducing yield losses from this pest. From the regression equation, we found a significant negative relation between aphids/leaf and sorghum yield, although this relationship needs further investigation.

This research highlights that resistant cultivars and insecticide applications at can provide effective *M. sacchcari* management. Although N fertilization is critical to achieving optimum grain yields, these fields may require additional pest management inputs as N content positively affects *M. sacchari* development and reproduction. Future studies regarding *M. sacchari* management programs should consider these components and how they may interact to influence aphid population dynamics.

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APPENDIX A: GREENHOUSE STUDY (PHENOLOGICAL STUDY)

DAYS TO REPRODUCTIVE ADULT OF *M. SACCHARI* AS AFFECTED BY SORGHUM PHENOLOGY, NITROGEN AND SILICON FERTILIZATION

```
dm 'log; clear; output; clear';
ods rtf;
data aphids;
input obs N $ Si $ Phen $ Trt $Rep dtr;
drop obs;
cards;
1     N0    S1    P2    N0S1P2      1     5
2     N0    S1    P2    N0S1P2      2     5
3     N0    S1    P2    N0S1P2      3     7
4     N0    S1    P2    N0S1P2      4     7
5     N0    S1    P2    N0S1P2      5     6
6     N0    S0    P2    N0S0P2      1     7
7     N0    S0    P2    N0S0P2      2     5
8     N0    S0    P2    N0S0P2      3     5
9     N0    S0    P2    N0S0P2      4     4
10    N0    S0    P2    N0S0P2      5     7
11    N1    S1    P2    N1S1P2      1     4
12    N1    S1    P2    N1S1P2      2     3
13    N1    S1    P2    N1S1P2      3     6
14    N1    S1    P2    N1S1P2      4     5
15    N1    S1    P2    N1S1P2      5     7
16    N1    S0    P2    N1S0P2      1     6
17    N1    S0    P2    N1S0P2      2     5
18    N1    S0    P2    N1S0P2      3     5
19    N1    S0    P2    N1S0P2      4     5
20    N1    S0    P2    N1S0P2      5     5
21    N2    S1    P2    N2S1P2      1     7
22    N2    S1    P2    N2S1P2      2     5
23    N2    S1    P2    N2S1P2      3     6
24    N2    S1    P2    N2S1P2      4     5
25    N2    S1    P2    N2S1P2      5     5
26    N2    S0    P2    N2S0P2      1     7
27    N2    S0    P2    N2S0P2      2     6
28    N2    S0    P2    N2S0P2      3     5
29    N2    S0    P2    N2S0P2      4     7
30    N2    S0    P2    N2S0P2      5     8
31    N0    S1    P1    N0S1P1      1     5
32    N0    S1    P1    N0S1P1      2     6
33    N0    S1    P1    N0S1P1      3     4
34    N0    S1    P1    N0S1P1      4     6
35    N0    S1    P1    N0S1P1      5     7
36    N0    S0    P1    N0S0P1      1     6
37    N0    S0    P1    N0S0P1      2     6
38    N0    S0    P1    N0S0P1      3     6
39    N0    S0    P1    N0S0P1      4     8
40    N0    S0    P1    N0S0P1      5     6
41    N1    S1    P1    N1S1P1      1     8
42    N1    S1    P1    N1S1P1      2     7
43    N1    S1    P1    N1S1P1      3     7
44    N1    S1    P1    N1S1P1      4     6
```

```
45    N1    S1    P1    N1S1P1    5    7
46    N1    S0    P1    N1S0P1    1    6
47    N1    S0    P1    N1S0P1    2    6
48    N1    S0    P1    N1S0P1    3    6
49    N1    S0    P1    N1S0P1    4    5
50    N1    S0    P1    N1S0P1    5    5
51    N2    S1    P1    N2S1P1    1    7
52    N2    S1    P1    N2S1P1    2    6
53    N2    S1    P1    N2S1P1    3    7
54    N2    S1    P1    N2S1P1    4    8
55    N2    S1    P1    N2S1P1    5    6
56    N2    S0    P1    N2S0P1    1    5
57    N2    S0    P1    N2S0P1    2    9
58    N2    S0    P1    N2S0P1    3    6
59    N2    S0    P1    N2S0P1    4    4
60    N2    S0    P1    N2S0P1    5    6
;
proc mixed data=aphids Cl METHOD=TYPE3;
CLASS N Si Phen;
TITLE3 'FACTORIAL DONE AS @ WAY ANOVA IN PROC MIXED';
MODEL dtr = N |Si|Phen / htype=3 ddfm = kr OUTP=ResidDATA ;
lsmeans N |Si|Phen / pdiff adjust=tukey cl ;
ods output diffs=ppp;
ods output lsmeans=mmm;
RUN;
%include 'C:/Users/llama1/Desktop/New folder/pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
run;quit;
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
run;
ods rtf close;
```

The mixed procedure

Model Information	
Data Set	WORK.APHIDS
Dependent Variable	dtr
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
N	3	N0 N1 N2
Si	2	S0 S1
Phen	2	P1 P2

Dimensions	
Covariance Parameters	1
Columns in X	36
Columns in Z	0
Subjects	1
Max Obs per Subject	60

Number of Observations	
Number of Observations Read	60
Number of Observations Used	60
Number of Observations Not Used	0

Fit Statistics	
-2 Res Log Likelihood	165.3
AIC (Smaller is Better)	167.3
AICC (Smaller is Better)	167.4
BIC (Smaller is Better)	169.1

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
Residual	1.2250	0.05	0.8519	1.9119

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
N	2	48	1.27	0.2914
Si	1	48	0.12	0.7279
N*Si	2	48	0.78	0.4662
Phen	1	48	3.93	0.0531

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
N*Phen	2	48	1.24	0.2990
Si*Phen	1	48	1.65	0.2056
N*Si*Phen	2	48	2.87	0.0664

Effect=N Method=Tukey(P<.05) Set=1

.	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1	N2			6.2500	0.2475	0.05	5.7524	6.7476	A
2	N0			5.9000	0.2475	0.05	5.4024	6.3976	A
3	N1			5.7000	0.2475	0.05	5.2024	6.1976	A

Effect=Si Method=Tukey(P<.05) Set=2

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
4		S1		6.0000	0.2021	0.05	5.5937	6.4063	A
5		S0		5.9000	0.2021	0.05	5.4937	6.3063	A

Effect=N*Si Method=Tukey(P<.05) Set=3

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
6	N2	S0		6.3000	0.3500	0.05	5.5963	7.0037	A
7	N2	S1		6.2000	0.3500	0.05	5.4963	6.9037	A
8	N0	S0		6.0000	0.3500	0.05	5.2963	6.7037	A
9	N1	S1		6.0000	0.3500	0.05	5.2963	6.7037	A
10	N0	S1		5.8000	0.3500	0.05	5.0963	6.5037	A
11	N1	S0		5.4000	0.3500	0.05	4.6963	6.1037	A

Effect=Phen Method=Tukey(P<.05) Set=4

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
12			P1	6.2333	0.2021	0.05	5.8270	6.6396	A
13			P2	5.6667	0.2021	0.05	5.2604	6.0730	A

Effect=N*Phen Method=Tukey(P<.05) Set=5

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
14	N2		P1	6.4000	0.3500	0.05	5.6963	7.1037	A
15	N1		P1	6.3000	0.3500	0.05	5.5963	7.0037	A
16	N2		P2	6.1000	0.3500	0.05	5.3963	6.8037	A
17	N0		P1	6.0000	0.3500	0.05	5.2963	6.7037	A
18	N0		P2	5.8000	0.3500	0.05	5.0963	6.5037	A
19	N1		P2	5.1000	0.3500	0.05	4.3963	5.8037	A

Effect=Si*Phen Method=Tukey(P<.05) Set=6

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
20		S1	P1	6.4667	0.2858	0.05	5.8921	7.0413	A
21		S0	P1	6.0000	0.2858	0.05	5.4254	6.5746	A
22		S0	P2	5.8000	0.2858	0.05	5.2254	6.3746	A
23		S1	P2	5.5333	0.2858	0.05	4.9587	6.1079	A

Effect=N*Si*Phen Method=Tukey(P<.05) Set=7

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
24	N1	S1	P1	7.0000	0.4950	0.05	6.0048	7.9952	A
25	N2	S1	P1	6.8000	0.4950	0.05	5.8048	7.7952	A
26	N2	S0	P2	6.6000	0.4950	0.05	5.6048	7.5952	A
27	N0	S0	P1	6.4000	0.4950	0.05	5.4048	7.3952	A
28	N0	S1	P2	6.0000	0.4950	0.05	5.0048	6.9952	A

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
29	N2	S0	P1	6.0000	0.4950	0.05	5.0048	6.9952	A
30	N2	S1	P2	5.6000	0.4950	0.05	4.6048	6.5952	A
31	N1	S0	P1	5.6000	0.4950	0.05	4.6048	6.5952	A
32	N0	S0	P2	5.6000	0.4950	0.05	4.6048	6.5952	A
33	N0	S1	P1	5.6000	0.4950	0.05	4.6048	6.5952	A
34	N1	S0	P2	5.2000	0.4950	0.05	4.2048	6.1952	A
35	N1	S1	P2	5.0000	0.4950	0.05	4.0048	5.9952	A

PROGENY OF *M. SACCHARI* AS AFFECTED BY SORGHUM PHENOLOGY, NITROGEN AND SILICON FERTILIZATION

```
dm 'log; clear; output; clear';
ods rtf;

data aphids;
input obs N $      Si     $ Phen $ Trt $     Rep Fecundity;
drop obs;
cards;
1       N0      S1      P2      N0S1P2          1       20
2       N0      S1      P2      N0S1P2          2       51
3       N0      S1      P2      N0S1P2          3       63
4       N0      S1      P2      N0S1P2          4       40
5       N0      S1      P2      N0S1P2          5       36
6       N0      S0      P2      N0S0P2          1       24
7       N0      S0      P2      N0S0P2          2       67
8       N0      S0      P2      N0S0P2          3       70
9       N0      S0      P2      N0S0P2          4       47
10      N0      S0      P2      N0S0P2          5       45
11      N1      S1      P2      N1S1P2          1       45
12      N1      S1      P2      N1S1P2          2       34
13      N1      S1      P2      N1S1P2          3       73
14      N1      S1      P2      N1S1P2          4       58
15      N1      S1      P2      N1S1P2          5       46
16      N1      S0      P2      N1S0P2          1       51
17      N1      S0      P2      N1S0P2          2       56
18      N1      S0      P2      N1S0P2          3       82
19      N1      S0      P2      N1S0P2          4       83
20      N1      S0      P2      N1S0P2          5       32
21      N2      S1      P2      N2S1P2          1       75
22      N2      S1      P2      N2S1P2          2       77
23      N2      S1      P2      N2S1P2          3       50
24      N2      S1      P2      N2S1P2          4       54
25      N2      S1      P2      N2S1P2          5       80
26      N2      S0      P2      N2S0P2          1       63
27      N2      S0      P2      N2S0P2          2       70
```

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28      N2      S0      P2      N2S0P2          3       74
29      N2      S0      P2      N2S0P2          4       70
30      N2      S0      P2      N2S0P2          5       35
31      N0      S1      P1      N0S1P1          1       70
32      N0      S1      P1      N0S1P1          2       28
33      N0      S1      P1      N0S1P1          3       72
34      N0      S1      P1      N0S1P1          4       71
35      N0      S1      P1      N0S1P1          5       31
36      N0      S0      P1      N0S0P1          1       24
37      N0      S0      P1      N0S0P1          2       52
38      N0      S0      P1      N0S0P1          3       38
39      N0      S0      P1      N0S0P1          4       45
40      N0      S0      P1      N0S0P1          5       15
41      N1      S1      P1      N1S1P1          1       65
42      N1      S1      P1      N1S1P1          2       73
43      N1      S1      P1      N1S1P1          3       55
44      N1      S1      P1      N1S1P1          4       55
45      N1      S1      P1      N1S1P1          5       63
46      N1      S0      P1      N1S0P1          1       90
47      N1      S0      P1      N1S0P1          2       65
48      N1      S0      P1      N1S0P1          3       71
49      N1      S0      P1      N1S0P1          4       63
50      N1      S0      P1      N1S0P1          5       71
51      N2      S1      P1      N2S1P1          1       56
52      N2      S1      P1      N2S1P1          2       60
53      N2      S1      P1      N2S1P1          3       60
54      N2      S1      P1      N2S1P1          4       60
55      N2      S1      P1      N2S1P1          5       86
56      N2      S0      P1      N2S0P1          1       43
57      N2      S0      P1      N2S0P1          2       68
58      N2      S0      P1      N2S0P1          3       66
59      N2      S0      P1      N2S0P1          4       44
60      N2      S0      P1      N2S0P1          5       69
;

proc mixed data=aphids Cl METHOD=TYPE3;
CLASS N Si Phen;
TITLE3 'FACTORIAL DONE AS @ WAY ANOVA IN PROC MIXED';
MODEL Fecundity = N |Si|Phen / htype=3 ddfm = kr OUTP=ResidDATA;
lsmeans N |Si|Phen / pdiff adjust=tukey cl ;
ods output diffs=ppp;
ods output lsmeans=mmm;
*ods listing exclude diffs;
*ods listing exclude lsmeans;
RUN;
%include 'C:/Users/llama1/Desktop/New folder/pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
run;quit;
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
run;
ods rtf close;
```

The mixed procedure

Model Information	
Data Set	WORK.APHIDS
Dependent Variable	Fecundity
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
N	3	N0 N1 N2
Si	2	S0 S1
Phen	2	P1 P2

Dimensions	
Covariance Parameters	1
Columns in X	36
Columns in Z	0
Subjects	1
Max Obs per Subject	60

Number of Observations	
Number of Observations Read	60
Number of Observations Used	60
Number of Observations Not Used	0

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
Residual	249.73	0.05	173.66	389.76

Fit Statistics	
-2 Res Log Likelihood	420.5
AIC (Smaller is Better)	422.5
AICC (Smaller is Better)	422.6
BIC (Smaller is Better)	424.4

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
N	2	48	7.60	0.0014
Si	1	48	0.01	0.9094
N*Si	2	48	1.55	0.2222
Phen	1	48	0.22	0.6378
N*Phen	2	48	1.28	0.2874
Si*Phen	1	48	1.46	0.2326
N*Si*Phen	2	48	1.27	0.2912

Effect=N Method=Tukey(P<.05) Set=1

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1	N2			63.0000	3.5336	0.05	55.8952	70.1048	A
2	N1			61.5500	3.5336	0.05	54.4452	68.6548	A
3	N0			45.4500	3.5336	0.05	38.3452	52.5548	B

Effect=Si Method=Tukey(P<.05) Set=2

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
4		S1		56.9000	2.8852	0.05	51.0990	62.7010	A
5		S0		56.4333	2.8852	0.05	50.6323	62.2343	A

Effect=N*Si Method=Tukey(P<.05) Set=3

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
6	N1	S0		66.4000	4.9972	0.05	56.3524	76.4476	A
7	N2	S1		65.8000	4.9972	0.05	55.7524	75.8476	A
8	N2	S0		60.2000	4.9972	0.05	50.1524	70.2476	AB
9	N1	S1		56.7000	4.9972	0.05	46.6524	66.7476	AB
10	N0	S1		48.2000	4.9972	0.05	38.1524	58.2476	AB
11	N0	S0		42.7000	4.9972	0.05	32.6524	52.7476	B

Effect=Phen Method=Tukey(P<.05) Set=4

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
12			P1	57.6333	2.8852	0.05	51.8323	63.4343	A
13			P2	55.7000	2.8852	0.05	49.8990	61.5010	A

Effect=N*Phen Method=Tukey(P<.05) Set=5

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
14	N1		P1	67.1000	4.9972	0.05	57.0524	77.1476	A
15	N2		P2	64.8000	4.9972	0.05	54.7524	74.8476	AB
16	N2		P1	61.2000	4.9972	0.05	51.1524	71.2476	AB
17	N1		P2	56.0000	4.9972	0.05	45.9524	66.0476	AB
18	N0		P2	46.3000	4.9972	0.05	36.2524	56.3476	AB
19	N0		P1	44.6000	4.9972	0.05	34.5524	54.6476	B

Effect=Si*Phen Method=Tukey(P<.05) Set=6

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
20		S1	P1	60.3333	4.0802	0.05	52.1295	68.5372	A
21		S0	P2	57.9333	4.0802	0.05	49.7295	66.1372	A
22		S0	P1	54.9333	4.0802	0.05	46.7295	63.1372	A
23		S1	P2	53.4667	4.0802	0.05	45.2628	61.6705	A

Effect=N*Si*Phen Method=Tukey(P<.05) Set=7

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
24	N1	S0	P1	72.0000	7.0672	0.05	57.7905	86.2095	A
25	N2	S1	P2	67.2000	7.0672	0.05	52.9905	81.4095	AB
26	N2	S1	P1	64.4000	7.0672	0.05	50.1905	78.6095	AB
27	N2	S0	P2	62.4000	7.0672	0.05	48.1905	76.6095	AB
28	N1	S1	P1	62.2000	7.0672	0.05	47.9905	76.4095	AB
29	N1	S0	P2	60.8000	7.0672	0.05	46.5905	75.0095	AB
30	N2	S0	P1	58.0000	7.0672	0.05	43.7905	72.2095	AB
31	N0	S1	P1	54.4000	7.0672	0.05	40.1905	68.6095	AB
32	N1	S1	P2	51.2000	7.0672	0.05	36.9905	65.4095	AB
33	N0	S0	P2	50.6000	7.0672	0.05	36.3905	64.8095	AB
34	N0	S1	P2	42.0000	7.0672	0.05	27.7905	56.2095	AB
35	N0	S0	P1	34.8000	7.0672	0.05	20.5905	49.0095	B

PROGENY OF *M. SACCHARI* AS AFFECTED BY SORGHUM PHENOLOGY, NITROGEN AND SILICON FERTILIZATION

```
dm 'log; clear; output; clear';
title1 'Life span of Sugarcane aphid';
ods rtf;

data aphids;
input obs N $      Si     $ Phen $ Trt $     Rep lifespan;
drop obs;
cards;
1      N0     S1     P2     N0S1P2       1      23
```

2	N0	S1	P2	N0S1P2	2	23
3	N0	S1	P2	N0S1P2	3	26
4	N0	S1	P2	N0S1P2	4	26
5	N0	S1	P2	N0S1P2	5	22
6	N0	S0	P2	N0S0P2	1	18
7	N0	S0	P2	N0S0P2	2	29
8	N0	S0	P2	N0S0P2	3	32
9	N0	S0	P2	N0S0P2	4	15
10	N0	S0	P2	N0S0P2	5	28
11	N1	S1	P2	N1S1P2	1	30
12	N1	S1	P2	N1S1P2	2	27
13	N1	S1	P2	N1S1P2	3	33
14	N1	S1	P2	N1S1P2	4	25
15	N1	S1	P2	N1S1P2	5	27
16	N1	S0	P2	N1S0P2	1	21
17	N1	S0	P2	N1S0P2	2	31
18	N1	S0	P2	N1S0P2	3	41
19	N1	S0	P2	N1S0P2	4	33
20	N1	S0	P2	N1S0P2	5	13
21	N2	S1	P2	N2S1P2	1	37
22	N2	S1	P2	N2S1P2	2	40
23	N2	S1	P2	N2S1P2	3	38
24	N2	S1	P2	N2S1P2	4	31
25	N2	S1	P2	N2S1P2	5	28
26	N2	S0	P2	N2S0P2	1	36
27	N2	S0	P2	N2S0P2	2	37
28	N2	S0	P2	N2S0P2	3	36
29	N2	S0	P2	N2S0P2	4	27
30	N2	S0	P2	N2S0P2	5	28
31	N0	S1	P1	N0S1P1	1	28
32	N0	S1	P1	N0S1P1	2	14
33	N0	S1	P1	N0S1P1	3	19
34	N0	S1	P1	N0S1P1	4	22
35	N0	S1	P1	N0S1P1	5	17
36	N0	S0	P1	N0S0P1	1	14
37	N0	S0	P1	N0S0P1	2	23
38	N0	S0	P1	N0S0P1	3	24
39	N0	S0	P1	N0S0P1	4	29
40	N0	S0	P1	N0S0P1	5	11
41	N1	S1	P1	N1S1P1	1	35
42	N1	S1	P1	N1S1P1	2	34
43	N1	S1	P1	N1S1P1	3	27
44	N1	S1	P1	N1S1P1	4	25
45	N1	S1	P1	N1S1P1	5	22
46	N1	S0	P1	N1S0P1	1	31
47	N1	S0	P1	N1S0P1	2	25
48	N1	S0	P1	N1S0P1	3	26
49	N1	S0	P1	N1S0P1	4	31
50	N1	S0	P1	N1S0P1	5	29
51	N2	S1	P1	N2S1P1	1	23
52	N2	S1	P1	N2S1P1	2	24
53	N2	S1	P1	N2S1P1	3	36
54	N2	S1	P1	N2S1P1	4	34
55	N2	S1	P1	N2S1P1	5	31
56	N2	S0	P1	N2S0P1	1	16
57	N2	S0	P1	N2S0P1	2	32
58	N2	S0	P1	N2S0P1	3	32

```
59     N2     S0     P1     N2S0P1       4      28
60     N2     S0     P1     N2S0P1       5      32
;

proc mixed data=aphids Cl METHOD=TYPE3;
CLASS N Si Phen;
TITLE3 'FACTORIAL DONE AS @ WAY ANOVA IN PROC MIXED';
MODEL lifespan = N |Si|Phen / htype=3 ddfm = kr OUTP=ResidDATA;
lsmeans N |Si|Phen / pdiff adjust=tukey cl ;
ods output diffs=ppp;
ods output lsmeans=mmm;
*ods listing exclude diffs;
*ods listing exclude lsmeans;
RUN;
%include 'C:/Users/llama1/Desktop/New folder/pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
run;quit;
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
run;
ods rtf close;
```

The mixed procedure

Model Information	
Data Set	WORK.APHIDS
Dependent Variable	lifespan
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
N	3	N0 N1 N2
Si	2	S0 S1
Phen	2	P1 P2

Dimensions	
Covariance Parameters	1
Columns in X	36
Columns in Z	0
Subjects	1
Max Obs per Subject	60

Number of Observations	
Number of Observations Read	60
Number of Observations Used	60
Number of Observations Not Used	0

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
Residual	36.9833	0.05	25.7191	57.7216

Fit Statistics	
-2 Res Log Likelihood	328.8
AIC (Smaller is Better)	330.8
AICC (Smaller is Better)	330.9
BIC (Smaller is Better)	332.7

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
N	2	48	11.77	<.0001
Si	1	48	0.16	0.6885
N*Si	2	48	0.15	0.8572
Phen	1	48	3.41	0.0709
N*Phen	2	48	1.13	0.3310

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Si*Phen	1	48	0.00	0.9495
N*Si*Phen	2	48	0.00	0.9960

Effect=N Method=Tukey(P<.05) Set=1

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1	N2			31.3000	1.3598	0.05	28.5659	34.0341	A
2	N1			28.3000	1.3598	0.05	25.5659	31.0341	A
3	N0			22.1500	1.3598	0.05	19.4159	24.8841	B

Effect=Si Method=Tukey(P<.05) Set=2

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
4		S1		27.5667	1.1103	0.05	25.3342	29.7991	A
5		S0		26.9333	1.1103	0.05	24.7009	29.1658	A

Effect=N*Si Method=Tukey(P<.05) Set=3

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
6	N2	S1		32.2000	1.9231	0.05	28.3333	36.0667	A
7	N2	S0		30.4000	1.9231	0.05	26.5333	34.2667	A
8	N1	S1		28.5000	1.9231	0.05	24.6333	32.3667	AB
9	N1	S0		28.1000	1.9231	0.05	24.2333	31.9667	AB
10	N0	S0		22.3000	1.9231	0.05	18.4333	26.1667	B
11	N0	S1		22.0000	1.9231	0.05	18.1333	25.8667	B

Effect=Phen Method=Tukey(P<.05) Set=4

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
12			P2	28.7000	1.1103	0.05	26.4676	30.9324	A
13			P1	25.8000	1.1103	0.05	23.5676	28.0324	A

Effect=N*Phen Method=Tukey(P<.05) Set=5

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
14	N2		P2	33.8000	1.9231	0.05	29.9333	37.6667	A
15	N2		P1	28.8000	1.9231	0.05	24.9333	32.6667	AB
16	N1		P1	28.5000	1.9231	0.05	24.6333	32.3667	AB
17	N1		P2	28.1000	1.9231	0.05	24.2333	31.9667	ABC
18	N0		P2	24.2000	1.9231	0.05	20.3333	28.0667	BC
19	N0		P1	20.1000	1.9231	0.05	16.2333	23.9667	C

Effect=Si*Phen Method=Tukey(P<.05) Set=6

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
20		S1	P2	29.0667	1.5702	0.05	25.9096	32.2238	A
21		S0	P2	28.3333	1.5702	0.05	25.1762	31.4904	A
22		S1	P1	26.0667	1.5702	0.05	22.9096	29.2238	A
23		S0	P1	25.5333	1.5702	0.05	22.3762	28.6904	A

Effect=N*Si*Phen Method=Tukey(P<.05) Set=7

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
24	N2	S1	P2	34.8000	2.7197	0.05	29.3317	40.2683	A
25	N2	S0	P2	32.8000	2.7197	0.05	27.3317	38.2683	AB
26	N2	S1	P1	29.6000	2.7197	0.05	24.1317	35.0683	AB
27	N1	S1	P1	28.6000	2.7197	0.05	23.1317	34.0683	AB
28	N1	S0	P1	28.4000	2.7197	0.05	22.9317	33.8683	AB

Obs	N	Si	Phen	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
29	N1	S1	P2	28.4000	2.7197	0.05	22.9317	33.8683	AB
30	N2	S0	P1	28.0000	2.7197	0.05	22.5317	33.4683	AB
31	N1	S0	P2	27.8000	2.7197	0.05	22.3317	33.2683	AB
32	N0	S0	P2	24.4000	2.7197	0.05	18.9317	29.8683	AB
33	N0	S1	P2	24.0000	2.7197	0.05	18.5317	29.4683	AB
34	N0	S0	P1	20.2000	2.7197	0.05	14.7317	25.6683	B
35	N0	S1	P1	20.0000	2.7197	0.05	14.5317	25.4683	B

APPENDIX B: GREENHOUSE STUDY (CULTIVAR STUDY)

DAYS TO REPRODUCTIVE ADULT OF *M. SACCHARI* AS AFFECTED BY SORGHUM CULTIVAR, NITROGEN AND SILICON FERTILIZATION

```
dm 'log; clear; output; clear';
ods rtf;

data aphids;
input obs  N $ Si $Trt$ Rep   dtr;
drop obs;
cards;
1      N0     S0     N0S0   1      7
2      N0     S0     N0S0   2      7
3      N0     S0     N0S0   3      8
4      N0     S0     N0S0   4      7
5      N0     S0     N0S0   5      9
6      N0     S1     N0S1   1      6
7      N0     S1     N0S1   2      9
8      N0     S1     N0S1   3      7
9      N0     S1     N0S1   4      9
10     N0     S1     N0S1   5      6
11     N1     S0     N1S0   1      7
12     N1     S0     N1S0   2      5
13     N1     S0     N1S0   3      6
14     N1     S0     N1S0   4      6
15     N1     S0     N1S0   5      6
16     N1     S1     N1S1   1      6
17     N1     S1     N1S1   2      6
18     N1     S1     N1S1   3      6
19     N1     S1     N1S1   4      7
20     N1     S1     N1S1   5      6
21     N2     S0     N2S0   1      7
22     N2     S0     N2S0   2      5
23     N2     S0     N2S0   3      3
24     N2     S0     N2S0   4      6
25     N2     S0     N2S0   5      7
26     N2     S1     N2S1   1      10
27     N2     S1     N2S1   2      7
28     N2     S1     N2S1   3      6
29     N2     S1     N2S1   4      5
30     N2     S1     N2S1   5      5
;
proc mixed data=aphids Cl METHOD=TYPE3; CLASS N Si;
TITLE3 'FACTORIAL DONE AS @ WAY ANOVA IN PROC MIXED';
MODEL dtr= N Si N*Si/ htype=3 ddfm = kr OUTP=ResidDATA;
lsmeans N N N*N/pdiff adjust=tukey cl;
ods output diffs=ppp;
ods output lsmeans=mmm;
RUN;
%include 'C:\Users\llama1\Desktop\New folder\pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
run;quit;
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
run;
```

```
ods rtf close;
```

The mixed procedure

Model Information	
Data Set	WORK.APHIDS
Dependent Variable	dtr
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
N	3	N0 N1 N2
Si	2	S0 S1

Dimensions	
Covariance Parameters	1
Columns in X	12
Columns in Z	0
Subjects	1
Max Obs per Subject	30

Number of Observations	
Number of Observations Read	30
Number of Observations Used	30
Number of Observations Not Used	0

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
Residual	1.8167	0.05	1.1076	3.5158

Fit Statistics	
-2 Res Log Likelihood	92.1
AIC (Smaller is Better)	94.1
AICC (Smaller is Better)	94.3
BIC (Smaller is Better)	95.3

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
N	2	24	3.60	0.0430
Si	1	24	0.46	0.5047
N*Si	2	24	0.51	0.6047

Effect=N Method=Tukey(P<.05) Set=1

Obs	N	Si	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1	N0		7.5000	0.4262	0.05	6.6203	8.3797	A
2	N1		6.1000	0.4262	0.05	5.2203	6.9797	A
3	N2		6.1000	0.4262	0.05	5.2203	6.9797	A

Effect=Si Method=Tukey(P<.05) Set=2

Obs	N	Si	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
4		S1	6.7333	0.3480	0.05	6.0151	7.4516	A
5		S0	6.4000	0.3480	0.05	5.6817	7.1183	A

Effect=N*Si Method=Tukey(P<.05) Set=3

Obs	N	Si	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
6	N0	S0	7.6000	0.6028	0.05	6.3559	8.8441	A
7	N0	S1	7.4000	0.6028	0.05	6.1559	8.6441	A
8	N2	S1	6.6000	0.6028	0.05	5.3559	7.8441	A
9	N1	S1	6.2000	0.6028	0.05	4.9559	7.4441	A
10	N1	S0	6.0000	0.6028	0.05	4.7559	7.2441	A
11	N2	S0	5.6000	0.6028	0.05	4.3559	6.8441	A

PROGENY OF *M. SACCHARI* AS AFFECTED BY SORGHUM CULTIVAR, NITROGEN AND SILICON FERTILIZATION

```
dm 'log; clear; output; clear';
ods rtf;

data aphids;
input obs N $Si   $Trt$ Rep   progeny;
drop obs;
cards;
1      N0     S0     NoSo   1      50
2      N0     S0     NoSo   2      20
3      N0     S0     NoSo   3      16
4      N0     S0     NoSo   4      34
5      N0     S0     NoSo   5      27
6      N0     S1     N0S1   1      32
7      N0     S1     N0S1   2      19
8      N0     S1     N0S1   3      36
9      N0     S1     N0S1   4      23
10     N0     S1     N0S1   5      59
11     N1     S0     N1S0   1      74
12     N1     S0     N1S0   2      25
13     N1     S0     N1S0   3      80
14     N1     S0     N1S0   4      91
15     N1     S0     N1S0   5      88
16     N1     S1     N1S1   1      79
17     N1     S1     N1S1   2      77
18     N1     S1     N1S1   3      88
19     N1     S1     N1S1   4      60
20     N1     S1     N1S1   5      60
21     N2     S0     N2S0   1      95
22     N2     S0     N2S0   2      62
23     N2     S0     N2S0   3      45
24     N2     S0     N2S0   4      78
25     N2     S0     N2S0   5      65
26     N2     S1     N2S1   1      61
27     N2     S1     N2S1   2      85
28     N2     S1     N2S1   3      91
```

```
29     N2     S1     N2S1   4      88
30     N2     S1     N2S1   5      81
;
proc mixed data=aphids Cl METHOD=TYPE3; CLASS N Si;
TITLE3 'FACTORIAL DONE AS @ WAY ANOVA IN PROC MIXED';
MODEL progeny= N Si N*Si/ htype=3 ddfm = kr OUTP=ResidDATA;
lsmeans N Si N*Si/ pdiff adjust=tukey cl;
ods output diffs=ppp;
ods output lsmeans=mmm;
RUN;
%include 'C:\Users\llama1\Desktop\New folder\pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
run; quit;
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
run;
ods rtf close;
```

The mixed procedure

Model Information	
Data Set	WORK.APHIDS
Dependent Variable	progeny
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
N	3	N0 N1 N2
Si	2	S0 S1

Dimensions	
Covariance Parameters	1
Columns in X	12
Columns in Z	0
Subjects	1
Max Obs per Subject	30

Number of Observations	
Number of Observations Read	30
Number of Observations Used	30
Number of Observations Not Used	0

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
Residual	298.70	0.05	182.12	578.08

Fit Statistics	
-2 Res Log Likelihood	214.6
AIC (Smaller is Better)	216.6
AICC (Smaller is Better)	216.7
BIC (Smaller is Better)	217.7

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
N	2	24	19.80	<.0001
Si	1	24	0.88	0.3565
N*Si	2	24	0.27	0.7672

Effect=N Method=Tukey(P<.05) Set=1

Obs	N	Si	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1	N2		75.1000	5.4653	0.05	63.8201	86.3799	A
2	N1		72.2000	5.4653	0.05	60.9201	83.4799	A
3	N0		31.6000	5.4653	0.05	20.3201	42.8799	B

Effect=Si Method=Tukey(P<.05) Set=2

Obs	N	Si	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
4		S1	62.6000	4.4624	0.05	53.3900	71.8100	A
5		S0	56.6667	4.4624	0.05	47.4567	65.8767	A

Effect=N*Si Method=Tukey(P<.05) Set=3

Obs	N	Si	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
6	N2	S1	81.2000	7.7292	0.05	65.2478	97.1522	A
7	N1	S1	72.8000	7.7292	0.05	56.8478	88.7522	A
8	N1	S0	71.6000	7.7292	0.05	55.6478	87.5522	A
9	N2	S0	69.0000	7.7292	0.05	53.0478	84.9522	A
10	N0	S1	33.8000	7.7292	0.05	17.8478	49.7522	B
11	N0	S0	29.4000	7.7292	0.05	13.4478	45.3522	B

LIFESPAN OF *M. SACCHARI* AS AFFECTED BY SORGHUM CULTIVAR, NITROGEN AND SILICON FERTILIZATION

```
dm 'log; clear; output; clear';
options nodate nocenter pageno = 1 ls=78 ps=53;
title1 'EXST7015 lab 9, Name, Section#';
ods rtf;

data aphids;
input obs N $ Si  $Trt$ Rep   lifespan;
drop obs;
cards;
1      N0     S0     NoSo   1      31
2      N0     S0     NoSo   2      16
3      N0     S0     NoSo   3      30
4      N0     S0     NoSo   4      25
5      N0     S0     NoSo   5      21
6      N0     S1     N0S1   1      19
7      N0     S1     N0S1   2      25
8      N0     S1     N0S1   3      25
9      N0     S1     N0S1   4      22
10     N0     S1     N0S1   5      24
11     N1     S0     N1S0   1      29
12     N1     S0     N1S0   2      08
13     N1     S0     N1S0   3      29
14     N1     S0     N1S0   4      32
15     N1     S0     N1S0   5      31
16     N1     S1     N1S1   1      29
17     N1     S1     N1S1   2      25
```

```
18      N1      S1      N1S1    3       25
19      N1      S1      N1S1    4       20
20      N1      S1      N1S1    5       29
21      N2      S0      N2S0    1       20
22      N2      S0      N2S0    2       16
23      N2      S0      N2S0    3       24
24      N2      S0      N2S0    4       23
25      N2      S0      N2S0    5       29
26      N2      S1      N2S1    1       36
27      N2      S1      N2S1    2       29
28      N2      S1      N2S1    3       32
29      N2      S1      N2S1    4       17
30      N2      S1      N2S1    5       23
;
proc mixed data=aphids Cl METHOD=TYPE3; CLASS cultivar factor2;
TITLE3 'FACTORIAL DONE AS @ WAY ANOVA IN PROC MIXED';
MODEL lifespan = N Si N*Si/ htype=3 ddfm = kr OUTP=ResidDATA;
lsmeans N Si N*Si/pdiff adjust=tukey cl;
ods output diffs=ppp;
ods output lsmeans=mmm;
RUN;
%include 'C:\Users\llama1\Desktop\New folder\pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
run; quit;
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
run;
ods rtf close;
```

The mixed procedure

Model Information	
Data Set	WORK.APHIDS
Dependent Variable	lifespan
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
cultivar	3	N0 N1 N2
Si	2	S0 S1

Dimensions	
Covariance Parameters	1
Columns in X	12
Columns in Z	0
Subjects	1
Max Obs per Subject	30

Number of Observations	
Number of Observations Read	30
Number of Observations Used	30
Number of Observations Not Used	0

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
Residual	39.9833	0.05	24.3776	77.3799

Fit Statistics	
-2 Res Log Likelihood	166.3
AIC (Smaller is Better)	168.3
AICC (Smaller is Better)	168.5
BIC (Smaller is Better)	169.5

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
cultivar	2	24	0.23	0.7981
Si	1	24	0.21	0.6483
cultivar*Si	2	24	0.76	0.4803

Effect=cultivar Method=Tukey(P<.05) Set=1

Obs	N	Si	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1	N1		25.7000	1.9996	0.05	21.5731	29.8269	A
2	N2		24.9000	1.9996	0.05	20.7731	29.0269	A
3	N0		23.8000	1.9996	0.05	19.6731	27.9269	A

Effect=Si Method=Tukey(P<.05) Set=2

Obs	N	Si	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
4		S1	25.3333	1.6327	0.05	21.9637	28.7030	A
5		S0	24.2667	1.6327	0.05	20.8970	27.6363	A

Effect=N*Si Method=Tukey(P<.05) Set=3

Obs	N	Si	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
6	N2	S1	27.4000	2.8278	0.05	21.5636	33.2364	A
7	N1	S0	25.8000	2.8278	0.05	19.9636	31.6364	A
8	N1	S1	25.6000	2.8278	0.05	19.7636	31.4364	A
9	N0	S0	24.6000	2.8278	0.05	18.7636	30.4364	A
10	N0	S1	23.0000	2.8278	0.05	17.1636	28.8364	A
11	N2	S0	22.4000	2.8278	0.05	16.5636	28.2364	A

NITROGEN CONTENT ANALYSIS

```
dm 'log; clear; output; clear';
options nodate nocenter pageno = 1 ls=78 ps=53;
title1 'EXST7015 lab 9, Name, Section#';
ods rtf;
```

```
data aphids;
input id  cultivar $ Si $ N $ Trt$  Rep   N;
drop id;
cards;
1      R0     S0     N0     R0S0N0       1      1.196
2      R0     S0     N0     R0S0N0       2      0.927
3      R0     S0     N0     R0S0N0       3      0.635
4      R0     S0     N0     R0S0N0       4      0.073
5      R0     S0     N0     R0S0N0       5      0.713
6      R0     S0     N1     R0S0N1       1      1.147
7      R0     S0     N1     R0S0N1       2      0.997
8      R0     S0     N1     R0S0N1       3      1.906
9      R0     S0     N1     R0S0N1       4      0.898
10     R0     S0     N1     R0S0N1       5      0.971
11     R0     S0     N2     R0S0N2       1      .
12     R0     S0     N2     R0S0N2       2      1.381
13     R0     S0     N2     R0S0N2       3      .
14     R0     S0     N2     R0S0N2       4      1.585
15     R0     S0     N2     R0S0N2       5      1.304
16     R0     S1     N0     R0S1N0       1      0.718
17     R0     S1     N0     R0S1N0       2      0.603
18     R0     S1     N0     R0S1N0       3      0.722
19     R0     S1     N0     R0S1N0       4      0.802
20     R0     S1     N0     R0S1N0       5      .
21     R0     S1     N1     R0S1N1       1      0.730
22     R0     S1     N1     R0S1N1       2      0.923
23     R0     S1     N1     R0S1N1       3      0.920
24     R0     S1     N1     R0S1N1       4      0.869
25     R0     S1     N1     R0S1N1       5      0.907
26     R0     S1     N2     R0S1N2       1      0.809
27     R0     S1     N2     R0S1N2       2      0.879
28     R0     S1     N2     R0S1N2       3      0.963
29     R0     S1     N2     R0S1N2       4      0.603
30     R0     S1     N2     R0S1N2       5      .
31     R1     S0     N0     R1S0N0       1      0.926
32     R1     S0     N0     R1S0N0       2      0.624
33     R1     S0     N0     R1S0N0       3      0.946
34     R1     S0     N0     R1S0N0       4      0.915
35     R1     S0     N0     R1S0N0       5      0.753
36     R1     S0     N1     R1S0N1       1      1.134
37     R1     S0     N1     R1S0N1       2      0.776
38     R1     S0     N1     R1S0N1       3      0.798
39     R1     S0     N1     R1S0N1       4      0.999
40     R1     S0     N1     R1S0N1       5      1.157
41     R1     S0     N2     R1S0N2       1      1.217
42     R1     S0     N2     R1S0N2       2      0.611
43     R1     S0     N2     R1S0N2       3      0.729
44     R1     S0     N2     R1S0N2       4      1.221
45     R1     S0     N2     R1S0N2       5      0.758
46     R1     S1     N0     R1S1N0       1      0.546
47     R1     S1     N0     R1S1N0       2      0.548
48     R1     S1     N0     R1S1N0       3      0.682
49     R1     S1     N0     R1S1N0       4      0.591
50     R1     S1     N0     R1S1N0       5      0.653
51     R1     S1     N1     R1S1N1       1      0.859
52     R1     S1     N1     R1S1N1       2      0.705
```

```
53     R1     S1     N1     R1S1N1        3      0.798
54     R1     S1     N1     R1S1N1        4      0.863
55     R1     S1     N1     R1S1N1        5      0.814
56     R1     S1     N2     R1S1N2        1      1.028
57     R1     S1     N2     R1S1N2        2      0.870
58     R1     S1     N2     R1S1N2        3      0.786
59     R1     S1     N2     R1S1N2        4      0.703
60     R1     S1     N2     R1S1N2        5      0.614
;
proc mixed data=aphids Cl METHOD=TYPE3; CLASS cultivar Si N;
TITLE3 'FACTORIAL DONE AS @ WAY ANOVA IN PROC MIXED';
MODEL N = cultivar|Si| N / htype=3 ddfm = kr OUTP=ResidDATA;
lsmeans cultivar|Si|N/pdiff adjust=tukey cl;
ods output diffs=ppp;
ods output lsmeans=mmm;
RUN;
%include 'C:\Users\llama1\Desktop\New folder\pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
run; quit;
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
run;
ods rtf close;
```

The mixed procedure

Model Information	
Data Set	WORK.APHIDS
Dependent Variable	N
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
cultivar	2	R0 R1
Si	2	S0 S1
factor3	3	N0 N1 N2

Dimensions	
Covariance Parameters	1
Columns in X	36
Columns in Z	0
Subjects	1
Max Obs per Subject	56

Number of Observations	
Number of Observations Read	60
Number of Observations Used	56
Number of Observations Not Used	4

Type 3 Analysis of Variance		
Source	**F Value**	**Pr > F**
cultivar	4.65	0.0365
Si	15.25	0.0003
factor3	8.17	0.0010
cultivar*Si	1.33	0.2544
cultivar*factor3	1.63	0.2083
Si*factor3	1.31	0.2807
factor*factor*factor	2.93	0.0638
Residual	.	.

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
Residual	0.05036	0.05	0.03452	0.08036

Fit Statistics	
-2 Res Log Likelihood	11.7
AIC (Smaller is Better)	13.7
AICC (Smaller is Better)	13.8
BIC (Smaller is Better)	15.5

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
cultivar	1	44	4.65	0.0365
Si	1	44	15.25	0.0003
N	2	44	8.17	0.0010
cultivar*Si	1	44	1.33	0.2544
cultivar*N	2	44	1.63	0.2083
Si*N	2	44	1.31	0.2807
factor*factor*factor	2	44	2.93	0.0638

Effect=cultivar Method=Tukey-Kramer(P<.05) Set=1

Obs	cultivar	Si	N	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1	R0			0.9517	0.04478	0.05	0.8615	1.0420	A
2	R1			0.8208	0.04097	0.05	0.7382	0.9034	B

Effect=Si Method=Tukey-Kramer(P<.05) Set=2

Obs	cultivar	Si	N	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
3		S0		1.0048	0.04319	0.05	0.9177	1.0918	A
4		S1		0.7678	0.04265	0.05	0.6818	0.8537	B

Effect=N Method=Tukey-Kramer(P<.05) Set=3

Obs	cultivar	Si	N	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
5			N2	0.9861	0.05563	0.05	0.8739	1.0982	A
6			N1	0.9586	0.05018	0.05	0.8574	1.0597	A
7			N0	0.7142	0.05172	0.05	0.6100	0.8185	B

Effect=cultivar*Si Method=Tukey-Kramer(P<.05) Set=4

Obs	cultivar	Si	N	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
8	R0	S0		1.1053	0.06406	0.05	0.9762	1.2344	A
9	R1	S0		0.9043	0.05794	0.05	0.7875	1.0210	AB
10	R0	S1		0.7982	0.06259	0.05	0.6720	0.9243	B
11	R1	S1		0.7373	0.05794	0.05	0.6206	0.8541	B

Effect=cultivar*N Method=Tukey-Kramer(P<.05) Set=5

Obs	cultivar	Si	N	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
12	R0		N2	1.1184	0.08570	0.05	0.9457	1.2911	A
13	R0		N1	1.0268	0.07097	0.05	0.8838	1.1698	A
14	R1		N1	0.8903	0.07097	0.05	0.7473	1.0333	AB
15	R1		N2	0.8537	0.07097	0.05	0.7107	0.9967	AB
16	R1		N0	0.7184	0.07097	0.05	0.5754	0.8614	B
17	R0		N0	0.7100	0.07527	0.05	0.5583	0.8617	B

Effect=Si*N Method=Tukey-Kramer(P<.05) Set=6

Obs	cultivar	Si	N	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
18		S0	N2	1.1653	0.08194	0.05	1.0001	1.3304	A
19		S0	N1	1.0783	0.07097	0.05	0.9353	1.2213	AB
20		S1	N1	0.8388	0.07097	0.05	0.6958	0.9818	BC
21		S1	N2	0.8069	0.07527	0.05	0.6552	0.9585	BC

Obs	cultivar	Si	N	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
22		S0	N0	0.7708	0.07097	0.05	0.6278	0.9138	C
23		S1	N0	0.6576	0.07527	0.05	0.5059	0.8093	C

Effect=factor*factor*factor Method=Tukey-Kramer(P<.05) Set=7

Obs	cultivar	Si	N	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
24	R0	S0	N2	1.4233	0.1296	0.05	1.1622	1.6845	A
25	R0	S0	N1	1.1838	0.1004	0.05	0.9815	1.3861	AB
26	R1	S0	N1	0.9728	0.1004	0.05	0.7705	1.1751	ABC
27	R1	S0	N2	0.9072	0.1004	0.05	0.7049	1.1095	ABC
28	R0	S1	N1	0.8698	0.1004	0.05	0.6675	1.0721	ABC
29	R1	S0	N0	0.8328	0.1004	0.05	0.6305	1.0351	BC
30	R0	S1	N2	0.8135	0.1122	0.05	0.5874	1.0396	BC
31	R1	S1	N1	0.8078	0.1004	0.05	0.6055	1.0101	BC
32	R1	S1	N2	0.8002	0.1004	0.05	0.5979	1.0025	BC
33	R0	S1	N0	0.7112	0.1122	0.05	0.4851	0.9374	BC
34	R0	S0	N0	0.7088	0.1004	0.05	0.5065	0.9111	BC
35	R1	S1	N0	0.6040	0.1004	0.05	0.4017	0.8063	C

SILICON CONTENT ANALYSIS

```
dm 'log; clear; output; clear';
options nodate nocenter pageno = 1 ls=78 ps=53;
title1 'EXST7015 lab 9, Name, Section#';
ods rtf;

data aphids;
input id  cultivar $ N  $ Si $ Trt  $ Rep SI;
drop id;
cards;
1      R0     N0     S0     R0N0S0       1      1.99
2      R0     N0     S0     R0N0S0       2      2.03
3      R0     N0     S0     R0N0S0       3      1.81
4      R0     N0     S0     R0N0S0       4      1.83
5      R0     N0     S0     R0N0S0       5      1.83
6      R0     N0     S1     R0N0S1       1      3.61
7      R0     N0     S1     R0N0S1       2      5.12
8      R0     N0     S1     R0N0S1       3      5.01
9      R0     N0     S1     R0N0S1       4      3.35
10     R0     N0     S1     R0N0S1       5      2.96
```

```
11    R0    N1    S0    R0N1S0    1    0.98
12    R0    N1    S0    R0N1S0    2    0.92
13    R0    N1    S0    R0N1S0    3    0.95
14    R0    N1    S0    R0N1S0    4    1.03
15    R0    N1    S0    R0N1S0    5    0.91
16    R0    N1    S1    R0N1S1    1    3.08
17    R0    N1    S1    R0N1S1    2    3.42
18    R0    N1    S1    R0N1S1    3    2.76
19    R0    N1    S1    R0N1S1    4    3.21
20    R0    N1    S1    R0N1S1    5    3.18
21    R0    N2    S0    R0N2S0    1    0.63
22    R0    N2    S0    R0N2S0    2    1.06
23    R0    N2    S0    R0N2S0    3    0.74
24    R0    N2    S0    R0N2S0    4    0.88
25    R0    N2    S0    R0N2S0    5    0.98
26    R0    N2    S1    R0N2S1    1    3.55
27    R0    N2    S1    R0N2S1    2    2.31
28    R0    N2    S1    R0N2S1    3    2.72
29    R0    N2    S1    R0N2S1    4    3.22
30    R0    N2    S1    R0N2S1    5    2.84
31    R1    N0    S0    R1N0S0    1    .
32    R1    N0    S0    R1N0S0    2    1.92
33    R1    N0    S0    R1N0S0    3    2.06
34    R1    N0    S0    R1N0S0    4    1.65
35    R1    N0    S0    R1N0S0    5    1.33
36    R1    N0    S1    R1N0S1    1    2.79
37    R1    N0    S1    R1N0S1    2    6.34
38    R1    N0    S1    R1N0S1    3    3.2
39    R1    N0    S1    R1N0S1    4    3.85
40    R1    N0    S1    R1N0S1    5    3.77
41    R1    N1    S0    R1N1S0    1    0.93
42    R1    N1    S0    R1N1S0    2    1.01
43    R1    N1    S0    R1N1S0    3    0.85
44    R1    N1    S0    R1N1S0    4    0.75
45    R1    N1    S0    R1N1S0    5    0.71
46    R1    N1    S1    R1N1S1    1    2.18
47    R1    N1    S1    R1N1S1    2    2.92
48    R1    N1    S1    R1N1S1    3    3.57
49    R1    N1    S1    R1N1S1    4    2.6
50    R1    N1    S1    R1N1S1    5    3.45
51    R1    N2    S0    R1N2S0    1    0.63
52    R1    N2    S0    R1N2S0    2    0.68
53    R1    N2    S0    R1N2S0    3    0.66
54    R1    N2    S0    R1N2S0    4    0.67
55    R1    N2    S0    R1N2S0    5    1.41
56    R1    N2    S1    R1N2S1    1    2.57
57    R1    N2    S1    R1N2S1    2    2.95
58    R1    N2    S1    R1N2S1    3    2.95
59    R1    N2    S1    R1N2S1    4    6.2
60    R1    N2    S1    R1N2S1    5    3.84
;
proc mixed data=aphids Cl METHOD=TYPE3; CLASS cultivar cN factor3;
TITLE3 'FACTORIAL DONE AS @ WAY ANOVA IN PROC MIXED';
MODEL SI = cultivar | N | Si/ htype=3 ddfm = kr OUTP=ResidDATA;
lsmeans cultivar | N| Si/pdiff adjust=tukey;
ods output diffs=ppp;
ods output lsmeans=mmm;
```

```
RUN;
%include 'C:/Users/llama1/Desktop/New folder/pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
run;quit;
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
RUN;
ods rtf close;
```

The mixed procedure

Model Information	
Data Set	WORK.APHIDS
Dependent Variable	SI
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
factor1	2	R0 R1
factor2	3	N0 N1 N2
factor3	2	S0 S1

Dimensions	
Covariance Parameters	1
Columns in X	36
Columns in Z	0
Subjects	1
Max Obs per Subject	59

Number of Observations	
Number of Observations Read	60
Number of Observations Used	59
Number of Observations Not Used	1

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
Residual	0.5052	0.05	0.3501	0.7926

Fit Statistics	
-2 Res Log Likelihood	120.4
AIC (Smaller is Better)	122.4
AICC (Smaller is Better)	122.5
BIC (Smaller is Better)	124.2

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
factor1	1	47	0.05	0.8204
factor2	2	47	10.07	0.0002
factor3	1	47	149.22	<.0001
factor1*factor2	2	47	0.77	0.4703
factor1*factor3	1	47	0.63	0.4319
factor2*factor3	2	47	0.35	0.7065
factor*factor*factor	2	47	0.54	0.5838

Effect=factor1 Method=Tukey-Kramer(P<.05) Set=1

Obs	factor1	factor2	factor3	Estimate	Standard Error	Letter Group
1	R1			2.3393	0.1324	A
2	R0			2.2970	0.1298	A

Effect=factor2 Method=Tukey-Kramer(P<.05) Set=2

Obs	factor1	factor2	factor3	Estimate	Standard Error	Letter Group
3		N0		2.9095	0.1638	A
4		N2		2.0745	0.1589	B
5		N1		1.9705	0.1589	B

Effect=factor3 Method=Tukey-Kramer(P<.05) Set=3

Obs	factor1	factor2	factor3	Estimate	Standard Error	Letter Group
6			S1	3.4507	0.1298	A
7			S0	1.1857	0.1324	B

Effect=factor1*factor2 Method=Tukey-Kramer(P<.05) Set=4

Obs	factor1	factor2	factor3	Estimate	Standard Error	Letter Group
8	R0	N0		2.9540	0.2248	A
9	R1	N0		2.8650	0.2384	AB
10	R1	N2		2.2560	0.2248	AB
11	R0	N1		2.0440	0.2248	AB
12	R1	N1		1.8970	0.2248	B
13	R0	N2		1.8930	0.2248	B

Effect=factor1*factor3 Method=Tukey-Kramer(P<.05) Set=5

Obs	factor1	factor2	factor3	Estimate	Standard Error	Letter Group
14	R1		S1	3.5453	0.1835	A
15	R0		S1	3.3560	0.1835	A
16	R0		S0	1.2380	0.1835	B
17	R1		S0	1.1333	0.1910	B

Effect=factor2*factor3 Method=Tukey-Kramer(P<.05) Set=6

Obs	factor1	factor2	factor3	Estimate	Standard Error	Letter Group
18		N0	S1	4.0000	0.2248	A
19		N2	S1	3.3150	0.2248	AB
20		N1	S1	3.0370	0.2248	B
21		N0	S0	1.8190	0.2384	C
22		N1	S0	0.9040	0.2248	CD
23		N2	S0	0.8340	0.2248	D

Effect=factor*factor*factor Method=Tukey-Kramer(P<.05) Set=7

Obs	factor1	factor2	factor3	Estimate	Standard Error	Letter Group
24	R0	N0	S1	4.0100	0.3179	A
25	R1	N0	S1	3.9900	0.3179	A
26	R1	N2	S1	3.7020	0.3179	A
27	R0	N1	S1	3.1300	0.3179	AB
28	R1	N1	S1	2.9440	0.3179	AB
29	R0	N2	S1	2.9280	0.3179	AB
30	R0	N0	S0	1.8980	0.3179	BC
31	R1	N0	S0	1.7400	0.3554	BC
32	R0	N1	S0	0.9580	0.3179	C
33	R0	N2	S0	0.8580	0.3179	C

Obs	factor1	factor2	factor3	Estimate	Standard Error	Letter Group
34	R1	N1	S0	0.8500	0.3179	C
35	R1	N2	S0	0.8100	0.3179	C

APPENDIX C: FIELD STUDY

LEAF N CONTENT ANALYSIS

```
dm 'log; clear; output; clear';
options nodate nocenter pageno = 1 ls=78 ps=53;
title1 'N content analysis#';
ods rtf;

data aphids;
input id  cultivar $ N  $ level Rep Ncontent;

cards;
1     S     N0    1     1     1.1554
2     S     N0    1     2     0.9688
3     S     N0    2     1     1.1377
4     S     N0    2     2     0.9368
5     S     N0    3     1     1.2836
6     S     N0    3     2     1.5672
7     S     N0    3     3     1.0239
8     S     N0    3     4     1.2755
9     S     N100  1     1     1.2993
10    S     N100  1     2     1.9403
11    S     N100  2     1     1.7525
12    S     N100  2     2     2.1209
13    S     N100  3     1     2.1691
14    S     N100  3     2     1.8693
15    S     N100  3     3     2.1758
16    S     N100  3     4     2.1332
17    S     N200  1     1     2.5635
18    S     N200  1     2     2.6639
19    S     N200  2     1     2.5266
20    S     N200  2     2     2.669
21    S     N200  3     1     2.6916
22    S     N200  3     2     3.0008
23    S     N200  3     3     2.9651
24    S     N200  3     4     2.9361
25    R     N0    1     1     1.3491
26    R     N0    1     2     1.2844
27    R     N0    2     1     0.6555
28    R     N0    2     2     1.0202
29    R     N0    3     1     1.2866
30    R     N0    3     2     1.3661
31    R     N0    3     3     1.1777
32    R     N0    3     4     1.1729
33    R     N100  1     1     2.1135
34    R     N100  1     2     2.2749
35    R     N100  2     1     2.0511
36    R     N100  2     2     2.0147
37    R     N100  3     1     2.3709
38    R     N100  3     2     2.215
39    R     N100  3     3     1.7465
40    R     N100  3     4     1.9111
41    R     N200  1     1     2.9168
42    R     N200  1     2     2.7056
43    R     N200  2     1     2.8327
44    R     N200  2     2     3.113
```

```
45     R      N200   3      1      2.7321
46     R      N200   3      2      3.1973
47     R      N200   3      3      2.8431
48     R      N200   3      4      3.1754
;
proc mixed data=aphids Cl METHOD=TYPE3; CLASS cultivar N level;
TITLE3 'FACTORIAL DONE AS @ WAY ANOVA IN PROC MIXED';
MODEL Ncontent = cultivar | N |level/ htype=3 OUTP=ResidDATA;
lsmeans cultivar | N |level /pdiff adjust=tukey cl;
ods output diffs=ppp;
ods output lsmeans=mmm;
*ods listing exclude diffs;
*ods listing exclude lsmeans;
RUN;
%include 'C:\Users\llama1\Desktop\New folder\pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
ods rtf close;
```

The mixed procedure

Model Information	
Data Set	WORK.APHIDS
Dependent Variable	Ncontent
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
cultivar	2	R S
N	3	N0 N100 N200
level	3	1 2 3

Dimensions	
Covariance Parameters	1
Columns in X	48
Columns in Z	0
Subjects	1
Max Obs per Subject	48

Number of Observations	
Number of Observations Read	48
Number of Observations Used	48
Number of Observations Not Used	0

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
Residual	0.03912	0.05	0.02498	0.06990

Fit Statistics	
-2 Res Log Likelihood	4.5
AIC (Smaller is Better)	6.5
AICC (Smaller is Better)	6.7
BIC (Smaller is Better)	7.9

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
cultivar	1	30	5.98	0.0206
N	2	30	260.20	<.0001
cultivar*N	2	30	1.37	0.2690
level	2	30	4.83	0.0152
cultivar*level	2	30	2.87	0.0724
N*level	4	30	1.00	0.4219
cultivar*N*level	4	30	1.17	0.3454

Effect=cultivar Method=Tukey(P<.05) Set=1

Obs	cultivar	N	level	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1	R		_	2.0516	0.04256	0.05	1.9647	2.1385	A
2	S		_	1.9045	0.04256	0.05	1.8175	1.9914	B

Effect=N Method=Tukey(P<.05) Set=2

Obs	cultivar	N	level	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
3		N200	_	2.8135	0.05212	0.05	2.7070	2.9199	A
4		N100	_	1.9886	0.05212	0.05	1.8821	2.0950	B
5		N0	_	1.1321	0.05212	0.05	1.0256	1.2385	C

Effect=cultivar*N Method=Tukey(P<.05) Set=3

Obs	cultivar	N	level	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
6	R	N200	_	2.9237	0.07371	0.05	2.7731	3.0742	A
7	S	N200	_	2.7033	0.07371	0.05	2.5528	2.8538	A
8	R	N100	_	2.0960	0.07371	0.05	1.9455	2.2465	B
9	S	N100	_	1.8811	0.07371	0.05	1.7306	2.0317	B
10	R	N0	_	1.1351	0.07371	0.05	0.9846	1.2857	C
11	S	N0	_	1.1290	0.07371	0.05	0.9784	1.2795	C

Effect=level Method=Tukey-Kramer(P<.05) Set=4

Obs	cultivar	N	level	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
12			3	2.0952	0.04037	0.05	2.0128	2.1777	A
13			1	1.9363	0.05710	0.05	1.8197	2.0529	AB
14			2	1.9026	0.05710	0.05	1.7860	2.0192	B

Effect=cultivar*level Method=Tukey-Kramer(P<.05) Set=5

Obs	cultivar	N	level	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
15	R		1	2.1074	0.08075	0.05	1.9425	2.2723	AB
16	R		3	2.0996	0.05710	0.05	1.9830	2.2162	A
17	S		3	2.0909	0.05710	0.05	1.9743	2.2075	A
18	R		2	1.9479	0.08075	0.05	1.7830	2.1128	AB
19	S		2	1.8572	0.08075	0.05	1.6923	2.0222	AB
20	S		1	1.7652	0.08075	0.05	1.6003	1.9301	B

Effect=N*level Method=Tukey-Kramer(P<.05) Set=6

Obs	cultivar	N	level	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
21		N200	3	2.9427	0.06993	0.05	2.7999	3.0855	A
22		N200	2	2.7853	0.09889	0.05	2.5834	2.9873	A
23		N200	1	2.7124	0.09889	0.05	2.5105	2.9144	A
24		N100	3	2.0739	0.06993	0.05	1.9310	2.2167	B
25		N100	2	1.9848	0.09889	0.05	1.7828	2.1868	B
26		N100	1	1.9070	0.09889	0.05	1.7050	2.1090	B
27		N0	3	1.2692	0.06993	0.05	1.1264	1.4120	C
28		N0	1	1.1894	0.09889	0.05	0.9875	1.3914	C
29		N0	2	0.9375	0.09889	0.05	0.7356	1.1395	C

Effect=cultivar*N*level Method=Tukey-Kramer(P<.05) Set=7

Obs	cultivar	N	level	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
30	R	N200	3	2.9870	0.09889	0.05	2.7850	3.1889	A
31	R	N200	2	2.9729	0.1399	0.05	2.6872	3.2585	A
32	S	N200	3	2.8984	0.09889	0.05	2.6964	3.1004	A
33	R	N200	1	2.8112	0.1399	0.05	2.5256	3.0968	AB
34	S	N200	1	2.6137	0.1399	0.05	2.3281	2.8993	ABC
35	S	N200	2	2.5978	0.1399	0.05	2.3122	2.8834	ABC
36	R	N100	1	2.1942	0.1399	0.05	1.9086	2.4798	BCD
37	S	N100	3	2.0869	0.09889	0.05	1.8849	2.2888	CD
38	R	N100	3	2.0609	0.09889	0.05	1.8589	2.2628	CD
39	R	N100	2	2.0329	0.1399	0.05	1.7473	2.3185	CDE
40	S	N100	2	1.9367	0.1399	0.05	1.6511	2.2223	CDEF
41	S	N100	1	1.6198	0.1399	0.05	1.3342	1.9054	DEFG
42	R	N0	1	1.3167	0.1399	0.05	1.0311	1.6024	EFGH
43	S	N0	3	1.2876	0.09889	0.05	1.0856	1.4895	FGH
44	R	N0	3	1.2508	0.09889	0.05	1.0489	1.4528	GH
45	S	N0	1	1.0621	0.1399	0.05	0.7765	1.3477	GH
46	S	N0	2	1.0372	0.1399	0.05	0.7516	1.3229	GH
47	R	N0	2	0.8378	0.1399	0.05	0.5522	1.1235	H

APHID POPULATION AS AFFECTED BY SORGHUM CULTIVAR, N RATE, AND INFESTATION LEVEL

```
dm 'log;clear;output;clear';
ods html close; ods html;
ods graphics on;

options nodate nocenter pageno=1 ls=90 ps=56;
ODS listing;
ods rtf;
data aphid;
input var $ N     level       plot  week  no_of_aphid;
datalines;

R      0      1      1      1      19
R      0      1      1      2      11.8
R      0      1      1      3      57.7
R      0      1      1      4      0.8
R      0      1      1      5      0.9
R      0      1      1      6      3.7
R      0      1      1      7      0.7
R      0      1      2      1      22.5
R      0      1      2      2      12.2
R      0      1      2      3      44.4
R      0      1      2      4      10
R      0      1      2      5      11.2
R      0      1      2      6      7.6
R      0      1      2      7      4.3
R      0      2      3      1      9.2
R      0      2      3      2      74.7
R      0      2      3      3      4.3
R      0      2      3      4      11.6
R      0      2      3      5      2.9
R      0      2      3      6      1.9
R      0      2      3      7      2.6
R      0      2      4      1      24.6
R      0      2      4      2      11.5
R      0      2      4      3      4.9
R      0      2      4      4      1.7
R      0      2      4      5      5.4
R      0      2      4      6      4.2
R      0      2      4      7      2
R      0      3      5      1      10.6
R      0      3      5      2      15
R      0      3      5      3      51.2
R      0      3      5      4      27.4
R      0      3      5      5      9.2
R      0      3      5      6      4.6
R      0      3      5      7      3.3
R      0      3      6      1      15.9
R      0      3      6      2      23
R      0      3      6      3      60.5
R      0      3      6      4      14
```

R	0	3	6	5	15.9
R	0	3	6	6	5.1
R	0	3	6	7	4.3
R	0	3	7	1	28.2
R	0	3	7	2	14.5
R	0	3	7	3	56.7
R	0	3	7	4	33.4
R	0	3	7	5	5.2
R	0	3	7	6	0.2
R	0	3	7	7	2.1
R	0	3	8	1	2.5
R	0	3	8	2	17.3
R	0	3	8	3	52.2
R	0	3	8	4	9.7
R	0	3	8	5	6.9
R	0	3	8	6	4.8
R	0	3	8	7	0.9
R	100	1	9	1	29.9
R	100	1	9	2	29.1
R	100	1	9	3	28.7
R	100	1	9	4	3.7
R	100	1	9	5	1.6
R	100	1	9	6	1.7
R	100	1	9	7	2.4
R	100	1	10	1	13.7
R	100	1	10	2	48.3
R	100	1	10	3	106.6
R	100	1	10	4	4.3
R	100	1	10	5	1.3
R	100	1	10	6	0.3
R	100	1	10	7	0.5
R	100	2	11	1	18.8
R	100	2	11	2	88
R	100	2	11	3	21.5
R	100	2	11	4	2.2
R	100	2	11	5	11.6
R	100	2	11	6	1
R	100	2	11	7	1.3
R	100	2	12	1	28.9
R	100	2	12	2	100.1
R	100	2	12	3	32.2
R	100	2	12	4	3.5
R	100	2	12	5	2.4
R	100	2	12	6	2.9
R	100	2	12	7	0.6
R	100	3	13	1	27.5
R	100	3	13	2	60
R	100	3	13	3	146.7
R	100	3	13	4	64
R	100	3	13	5	224.8
R	100	3	13	6	3
R	100	3	13	7	2.4
R	100	3	14	1	21.4
R	100	3	14	2	58.7
R	100	3	14	3	727.3
R	100	3	14	4	78.1
R	100	3	14	5	20.3

R	100	3	14	6	4.7
R	100	3	14	7	3.5
R	100	3	15	1	50.6
R	100	3	15	2	123.3
R	100	3	15	3	228.9
R	100	3	15	4	87.2
R	100	3	15	5	3.8
R	100	3	15	6	4.5
R	100	3	15	7	2.7
R	100	3	16	1	66.3
R	100	3	16	2	216.2
R	100	3	16	3	275.7
R	100	3	16	4	25.4
R	100	3	16	5	37.5
R	100	3	16	6	3.1
R	100	3	16	7	0.8
R	200	1	17	1	35.4
R	200	1	17	2	15.6
R	200	1	17	3	69.8
R	200	1	17	4	3.9
R	200	1	17	5	3.9
R	200	1	17	6	3.4
R	200	1	17	7	0.8
R	200	1	18	1	22.6
R	200	1	18	2	61
R	200	1	18	3	52.9
R	200	1	18	4	4.1
R	200	1	18	5	2.7
R	200	1	18	6	0.4
R	200	1	18	7	0.6
R	200	2	19	1	49.2
R	200	2	19	2	38.4
R	200	2	19	3	76.6
R	200	2	19	4	2.2
R	200	2	19	5	3.5
R	200	2	19	6	3.6
R	200	2	19	7	0.2
R	200	2	20	1	14.2
R	200	2	20	2	44.4
R	200	2	20	3	38.1
R	200	2	20	4	2.2
R	200	2	20	5	2.7
R	200	2	20	6	1.1
R	200	2	20	7	0.6
R	200	3	21	1	13.7
R	200	3	21	2	50.6
R	200	3	21	3	449
R	200	3	21	4	60.4
R	200	3	21	5	76.2
R	200	3	21	6	2.4
R	200	3	21	7	1.8
R	200	3	22	1	33.3
R	200	3	22	2	95.1
R	200	3	22	3	896
R	200	3	22	4	508.7
R	200	3	22	5	381.6
R	200	3	22	6	6.2

R	200	3	22	7	1
R	200	3	23	1	8.8
R	200	3	23	2	30.4
R	200	3	23	3	179.5
R	200	3	23	4	42.7
R	200	3	23	5	17.8
R	200	3	23	6	58.8
R	200	3	23	7	1.3
R	200	3	24	1	13.7
R	200	3	24	2	78.5
R	200	3	24	3	275.2
R	200	3	24	4	84.1
R	200	3	24	5	75
R	200	3	24	6	13.5
R	200	3	24	7	2
S	0	1	25	1	160.8
S	0	1	25	2	21.3
S	0	1	25	3	17.3
S	0	1	25	4	1.2
S	0	1	25	5	6.6
S	0	1	25	6	4.2
S	0	1	25	7	0.9
S	0	1	26	1	783.5
S	0	1	26	2	20.9
S	0	1	26	3	73.8
S	0	1	26	4	1.6
S	0	1	26	5	1.6
S	0	1	26	6	2.2
S	0	1	26	7	1.2
S	0	2	27	1	52
S	0	2	27	2	90.8
S	0	2	27	3	39.1
S	0	2	27	4	1.5
S	0	2	27	5	0.4
S	0	2	27	6	1.4
S	0	2	27	7	1.6
S	0	2	28	1	97.3
S	0	2	28	2	39.4
S	0	2	28	3	50
S	0	2	28	4	0.9
S	0	2	28	5	3.7
S	0	2	28	6	5.6
S	0	2	28	7	1.1
S	0	3	29	1	556.6
S	0	3	29	2	145.4
S	0	3	29	3	467.8
S	0	3	29	4	227.6
S	0	3	29	5	143.1
S	0	3	29	6	10
S	0	3	29	7	0.9
S	0	3	30	1	2067
S	0	3	30	2	938
S	0	3	30	3	397
S	0	3	30	4	25.6
S	0	3	30	5	11.3
S	0	3	30	6	7.9
S	0	3	30	7	2

S	0	3	31	1	408.6
S	0	3	31	2	40.2
S	0	3	31	3	42
S	0	3	31	4	171.1
S	0	3	31	5	47.3
S	0	3	31	6	3.2
S	0	3	31	7	2.5
S	0	3	32	1	144.3
S	0	3	32	2	199.8
S	0	3	32	3	300.4
S	0	3	32	4	11.2
S	0	3	32	5	5.1
S	0	3	32	6	1.2
S	0	3	32	7	6.1
S	100	1	33	1	58.2
S	100	1	33	2	7.2
S	100	1	33	3	39.6
S	100	1	33	4	0.8
S	100	1	33	5	5
S	100	1	33	6	1.1
S	100	1	33	7	2.5
S	100	1	34	1	399.3
S	100	1	34	2	25.9
S	100	1	34	3	31
S	100	1	34	4	3.8
S	100	1	34	5	1.9
S	100	1	34	6	1.6
S	100	1	34	7	0.6
S	100	2	35	1	48.6
S	100	2	35	2	552.7
S	100	2	35	3	10
S	100	2	35	4	3.4
S	100	2	35	5	13.3
S	100	2	35	6	5.3
S	100	2	35	7	1.6
S	100	2	36	1	335
S	100	2	36	2	303.3
S	100	2	36	3	22.5
S	100	2	36	4	67.7
S	100	2	36	5	12.6
S	100	2	36	6	2.1
S	100	2	36	7	0.3
S	100	3	37	1	644.5
S	100	3	37	2	173.2
S	100	3	37	3	647.9
S	100	3	37	4	450.2
S	100	3	37	5	108.6
S	100	3	37	6	1.6
S	100	3	37	7	13.7
S	100	3	38	1	159.7
S	100	3	38	2	637.1
S	100	3	38	3	385.2
S	100	3	38	4	220.2
S	100	3	38	5	9.9
S	100	3	38	6	12.8
S	100	3	38	7	2.3
S	100	3	39	1	1271.1

S	100	3	39	2	151.7
S	100	3	39	3	850
S	100	3	39	4	107.1
S	100	3	39	5	87.7
S	100	3	39	6	5.9
S	100	3	39	7	23.4
S	100	3	40	1	789.3
S	100	3	40	2	313.5
S	100	3	40	3	532.9
S	100	3	40	4	354.5
S	100	3	40	5	45.5
S	100	3	40	6	12.8
S	100	3	40	7	6.7
S	200	1	41	1	146.5
S	200	1	41	2	20.8
S	200	1	41	3	2.8
S	200	1	41	4	4.2
S	200	1	41	5	9.6
S	200	1	41	6	1.4
S	200	1	41	7	1.1
S	200	1	42	1	444.7
S	200	1	42	2	48.8
S	200	1	42	3	54.8
S	200	1	42	4	6.3
S	200	1	42	5	1.6
S	200	1	42	6	1.9
S	200	1	42	7	0.7
S	200	2	43	1	374.4
S	200	2	43	2	372.8
S	200	2	43	3	17.8
S	200	2	43	4	9.7
S	200	2	43	5	6.9
S	200	2	43	6	3
S	200	2	43	7	0.3
S	200	2	44	1	2164.3
S	200	2	44	2	62.9
S	200	2	44	3	13
S	200	2	44	4	14.8
S	200	2	44	5	12.4
S	200	2	44	6	6.8
S	200	2	44	7	0.6
S	200	3	45	1	141
S	200	3	45	2	263.2
S	200	3	45	3	1142
S	200	3	45	4	135.8
S	200	3	45	5	270.1
S	200	3	45	6	15.7
S	200	3	45	7	2.1
S	200	3	46	1	347.5
S	200	3	46	2	581
S	200	3	46	3	435.1
S	200	3	46	4	28.6
S	200	3	46	5	20.8
S	200	3	46	6	25.5
S	200	3	46	7	8.7
S	200	3	47	1	265.7
S	200	3	47	2	68

```
S       200     3       47      3       291.8
S       200     3       47      4       296.8
S       200     3       47      5       60.3
S       200     3       47      6       6.4
S       200     3       47      7       6.3
S       200     3       48      1       65.6
S       200     3       48      2       345.9
S       200     3       48      3       526.1
S       200     3       48      4       734
S       200     3       48      5       150.1
S       200     3       48      6       4.9
S       200     3       48      7       6.5
;

proc mixed data = aphid  Cl METHOD=TYPE3;
class var N level plot week;
model no_of_aphid = var | N |level | week / htype = 3 OUTP=ResidDATA ddfm =
KR;
Random plot (var N level);
lsmeans var  N level  week/ pdiff adjust=tukey cl;
ods output diffs=ppp;
ods output lsmeans=mmm;
ods listing exclude diffs;
ods listing exclude lsmeans;
RUN;
%include 'C:\Users\llama1\Desktop\New folder\pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
ods rtf close;
```

The mixed procedure

Model Information	
Data Set	WORK.APHID
Dependent Variable	no_of_aphid
Covariance Structure	Variance Components
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Kenward-Roger
Degrees of Freedom Method	Kenward-Roger

Class Level Information		
Class	**Levels**	**Values**
var	2	R S
N	3	0 100 200
level	3	1 2 3
plot	48	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
week	7	1 2 3 4 5 6 7

Dimensions	
Covariance Parameters	2
Columns in X	384
Columns in Z	48
Subjects	1
Max Obs per Subject	336

Number of Observations	
Number of Observations Read	336
Number of Observations Used	336
Number of Observations Not Used	0

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
plot(var*N*level)	1368.17	0.05	-2014.28	4750.62
Residual	34978	0.05	28743	43498

Fit Statistics	
-2 Res Log Likelihood	2916.8
AIC (Smaller is Better)	2920.8
AICC (Smaller is Better)	2920.8
BIC (Smaller is Better)	2924.5

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
var	1	30	17.51	0.0002
N	2	30	1.27	0.2956
var*N	2	30	0.03	0.9660
level	2	30	11.71	0.0002
var*level	2	30	2.74	0.0808
N*level	4	30	0.75	0.5669
var*N*level	4	30	1.26	0.3087
week	6	180	10.32	<.0001
var*week	6	180	8.00	<.0001
N*week	12	180	0.42	0.9551
var*N*week	12	180	0.24	0.9956
level*week	12	180	2.72	0.0021
var*level*week	12	180	0.54	0.8833
N*level*week	24	180	1.91	0.0093
var*N*level*week	24	180	1.33	0.1488

Effect=var Method=Tukey(P<.05) Set=1

Obs	var	N	level	week	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1	S	_	_	_	139.43	17.1661	0.05	104.37	174.48	A
2	R	_	_	_	37.8544	17.1661	0.05	2.7965	72.9122	B

Effect=N Method=Tukey(P<.05) Set=2

Obs	var	N	level	week	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
3		200	_	_	109.65	21.0241	0.05	66.7107	152.58	A
4		100	_	_	93.3060	21.0241	0.05	50.3690	136.24	A
5		0	_	_	62.9655	21.0241	0.05	20.0285	105.90	A

Effect=level Method=Tukey-Kramer(P<.05) Set=3

Obs	var	N	level	week	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
6		_	3	_	161.71	16.2852	0.05	128.46	194.97	A
7		_	2	_	66.3857	23.0307	0.05	19.3507	113.42	B
8		_	1	_	37.8190	23.0307	0.05	-9.2160	84.8541	B

Effect=week Method=Tukey-Kramer(P<.05) Set=4

Obs	var	N	level	week	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
9		_	_	1	248.04	29.0058	0.05	190.85	305.22	A
10		_	_	3	156.05	29.0058	0.05	98.8714	213.24	AB
11		_	_	2	122.83	29.0058	0.05	65.6422	180.01	BC
12		_	_	4	57.3611	29.0058	0.05	0.1783	114.54	BCD
13		_	_	5	28.9639	29.0058	0.05	-28.2189	86.1467	CD
14		_	_	6	4.9389	29.0058	0.05	-52.2439	62.1217	CD
15		_	_	7	2.2986	29.0058	0.05	-54.8842	59.4814	D

Effect=var*week Method=Tukey-Kramer(P<.05) Set=5

Obs	var	N	level	week	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
16	S	_	_	1	471.95	41.0205	0.05	391.08	552.82	A
17	S	_	_	2	194.18	41.0205	0.05	113.31	275.05	B
18	S	_	_	3	187.82	41.0205	0.05	106.95	268.69	B
19	R	_	_	3	124.29	41.0205	0.05	43.4175	205.15	B

Obs	var	N	level	week	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
20	S	_	_	4	83.1806	41.0205	0.05	2.3119	164.05	B
21	R	_	_	2	51.4667	41.0205	0.05	-29.4020	132.34	B
22	R	_	_	4	31.5417	41.0205	0.05	-49.3270	112.41	B
23	S	_	_	5	30.8611	41.0205	0.05	-50.0075	111.73	B
24	R	_	_	5	27.0667	41.0205	0.05	-53.8020	107.94	B
25	R	_	_	1	24.1250	41.0205	0.05	-56.7436	104.99	B
26	S	_	_	6	5.0306	41.0205	0.05	-75.8381	85.8992	B
27	R	_	_	6	4.8472	41.0205	0.05	-76.0214	85.7159	B
28	S	_	_	7	2.9500	41.0205	0.05	-77.9186	83.8186	B
29	R	_	_	7	1.6472	41.0205	0.05	-79.2214	82.5159	B

Effect=N*week Method=Tukey-Kramer(P<.05) Set=6

Obs	var	N	level	week	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
30		200	_	1	308.00	50.2396	0.05	208.95	407.04	A
31		0	_	1	232.15	50.2396	0.05	133.10	331.19	AB
32		100	_	1	203.97	50.2396	0.05	104.92	303.01	AB
33		200	_	3	201.93	50.2396	0.05	102.89	300.97	AB
34		100	_	3	182.45	50.2396	0.05	83.4065	281.49	AB
35		100	_	2	168.45	50.2396	0.05	69.4107	267.50	AB
36		200	_	2	118.42	50.2396	0.05	19.3774	217.46	AB
37		0	_	3	83.7833	50.2396	0.05	-15.2601	182.83	AB
38		200	_	4	82.7458	50.2396	0.05	-16.2976	181.79	AB
39		0	_	2	81.6000	50.2396	0.05	-17.4435	180.64	AB
40		100	_	4	65.2292	50.2396	0.05	-33.8143	164.27	AB
41		200	_	5	47.4375	50.2396	0.05	-51.6060	146.48	B
42		100	_	5	26.5625	50.2396	0.05	-72.4810	125.61	B
43		0	_	4	24.1083	50.2396	0.05	-74.9351	123.15	B
44		0	_	5	12.8917	50.2396	0.05	-86.1518	111.94	B

Obs	var	N	level	week	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
45		200	_	6	7.3583	50.2396	0.05	-91.6851	106.40	B
46		0	_	6	4.1083	50.2396	0.05	-94.9351	103.15	B
47		100	_	6	3.3500	50.2396	0.05	-95.6935	102.39	B
48		100	_	7	3.1292	50.2396	0.05	-95.9143	102.17	B
49		0	_	7	2.1208	50.2396	0.05	-96.9226	101.16	B
50		200	_	7	1.6458	50.2396	0.05	-97.3976	100.69	B

Effect=level*week Method=Tukey-Kramer(P<.05) Set=7

Obs	var	N	level	week	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
51		_	3	3	392.38	38.9154	0.05	315.66	469.10	A
52		_	3	1	298.06	38.9154	0.05	221.34	374.78	AB
53		_	2	1	268.04	55.0347	0.05	159.54	376.54	ABC
54		_	3	2	193.32	38.9154	0.05	116.60	270.04	BCD
55		_	1	1	178.01	55.0347	0.05	69.5117	286.51	ABCD
56		_	3	4	158.24	38.9154	0.05	81.5229	234.96	BCD
57		_	2	2	148.25	55.0347	0.05	39.7533	256.75	ABCD
58		_	3	5	76.4167	38.9154	0.05	-0.3021	153.14	CD
59		_	1	3	48.2833	55.0347	0.05	-60.2133	156.78	CD
60		_	2	3	27.5000	55.0347	0.05	-80.9967	136.00	CD
61		_	1	2	26.9083	55.0347	0.05	-81.5883	135.41	CD
62		_	2	4	10.1167	55.0347	0.05	-98.3800	118.61	CD
63		_	3	6	9.1167	38.9154	0.05	-67.6021	85.8354	D
64		_	2	5	6.4833	55.0347	0.05	-102.01	114.98	CD
65		_	3	7	4.4708	38.9154	0.05	-72.2479	81.1896	D
66		_	1	5	3.9917	55.0347	0.05	-104.51	112.49	CD
67		_	1	4	3.7250	55.0347	0.05	-104.77	112.22	CD
68		_	2	6	3.2417	55.0347	0.05	-105.26	111.74	CD
69		_	1	6	2.4583	55.0347	0.05	-106.04	110.96	CD

Obs	var	N	level	week	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
70		_	1	7	1.3583	55.0347	0.05	-107.14	109.86	CD
71		_	2	7	1.0667	55.0347	0.05	-107.43	109.56	CD

SORGHUM YIELD AS AFFECTED BY CULTIVAR, N RATE, AND INFESTATION LEVEL

```
dm 'log; clear; output; clear';
options nodate nocenter pageno = 1 ls=78 ps=53;
title1 'EXST7015 lab 9, Name, Section#';
ods rtf;

data aphids;
input id $ cultivar $ N $lev$ trt $ rep $ yield;
drop id;
cards;
id     cultivar     N      Level  trt    Rep    yield
1      R      0      1      R01    1      1.45
2      R      0      1      R01    2      1.78
3      R      0      2      R02    1      1.71
4      R      0      2      R02    2      1.73
5      R      0      3      R03    1      1.38
6      R      0      3      R03    2      1.71
7      R      0      3      R03    3      1.74
8      R      0      3      R03    4      1.81
9      R      100    1      R1001  1      5.03
10     R      100    1      R1001  2      4.20
11     R      100    2      R1002  1      4.58
12     R      100    2      R1002  2      4.96
13     R      100    3      R1003  1      4.89
14     R      100    3      R1003  2      4.22
15     R      100    3      R1003  3      3.19
16     R      100    3      R1003  4      5.06
17     R      200    1      R2001  1      4.04
18     R      200    1      R2001  2      5.84
19     R      200    2      R2002  1      5.84
20     R      200    2      R2002  2      5.08
21     R      200    3      R2003  1      5.64
22     R      200    3      R2003  2      5.49
23     R      200    3      R2003  3      4.91
24     R      200    3      R2003  4      5.64
25     S      0      1      S01    1      0.88
26     S      0      1      S01    2      1.37
27     S      0      2      S 02   1      0.50
28     S      0      2      S02    2      1.68
29     S      0      3      S03    1      1.23
30     S      0      3      S03    2      1.43
31     S      0      3      S03    3      1.41
32     S      0      3      S03    4      1.73
33     S      100    1      S1001  1      3.89
34     S      100    1      S1001  2      1.47
35     S      100    2      S1002  1      4.74
36     S      100    2      S1002  2      4.75
37     S      100    3      S1003  1      3.41
```

```
38      S       100     3       S1003 2       3.68
39      S       100     3       S1003 3       4.38
40      S       100     3       S1003 4       3.61
41      S       200     1       S2001 1       5.35
42      S       200     1       S2001 2       5.21
43      S       200     2       S2002 1       5.52
44      S       200     2       S2002 2       5.94
45      S       200     3       S2003 1       5.28
46      S       200     3       S2003 2       5.99
47      S       200     3       S2003 3       6.30
48      S       200     3       S2003 4       5.50
```

```
;
proc mixed data=aphids Cl METHOD=TYPE3; CLASSES cultivar N lev;
TITLE3 'FACTORIAL DONE AS @ WAY ANOVA IN PROC MIXED';
MODEL yield = cultivar | N |lev/ htype=3 OUTP=ResidDATA;
lsmeans cultivar | N |lev /pdiff adjust=tukey cl;
ods output diffs=ppp;
ods output lsmeans=mmm;
ods listing exclude diffs;
ods listing exclude lsmeans;
RUN;
%include 'C:\Users\llama1\Desktop\New folder\pdmix800.sas';
%pdmix800 (ppp,mmm,alpha=.05,sort=yes);
proc univariate data=ResidData NORMAL PLOT; VAR RESID;
ods rtf close;
```

The model procedure

Model Information	
Data Set	WORK.APHIDS
Dependent Variable	yield
Covariance Structure	Diagonal
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Residual

Class Level Information		
Class	**Levels**	**Values**
var	3	R S Cultivar
N	4	0 100 200 N
lev	4	1 2 3 Level

Dimensions	
Covariance Parameters	1
Columns in X	48
Columns in Z	0
Subjects	1
Max Obs per Subject	48

Number of Observations	
Number of Observations Read	49
Number of Observations Used	48
Number of Observations Not Used	1

Covariance Parameter Estimates				
Cov Parm	**Estimate**	**Alpha**	**Lower**	**Upper**
Residual	0.3231	0.05	0.2063	0.5773

Fit Statistics	
-2 Res Log Likelihood	67.9
AIC (Smaller is Better)	69.9
AICC (Smaller is Better)	70.0
BIC (Smaller is Better)	71.3

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
cultivar	1	30	2.90	0.0987
N	2	30	180.81	<.0001
cultivar*N	2	30	3.80	0.0338
lev	2	30	3.26	0.0524
cultivar*lev	2	30	1.22	0.3102
N*lev	4	30	0.96	0.4425
cultivar*N*lev	4	30	0.98	0.4333

Effect=cultivar Method=Tukey(P<.05) Set=1

Obs	cultivar	N	lev	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1	R			3.8378	0.1223	0.05	3.5880	4.0876	A
2	S			3.5431	0.1223	0.05	3.2933	3.7928	A

Effect=N Method=Tukey(P<.05) Set=2

Obs	cultivar	N	lev	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
3		200		5.4329	0.1498	0.05	5.1270	5.7388	A
4		100		4.1533	0.1498	0.05	3.8474	4.4593	B
5		0		1.4850	0.1498	0.05	1.1791	1.7909	C

Effect=cultivar*N Method=Tukey(P<.05) Set=3

Obs	cultivar	N	lev	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
6	S	200		5.5925	0.2118	0.05	5.1599	6.0251	A
7	R	200		5.2733	0.2118	0.05	4.8407	5.7060	AB
8	R	100		4.5750	0.2118	0.05	4.1424	5.0076	BC
9	S	100		3.7317	0.2118	0.05	3.2990	4.1643	C
10	R	0		1.6650	0.2118	0.05	1.2324	2.0976	D
11	S	0		1.3050	0.2118	0.05	0.8724	1.7376	D

Effect=lev Method=Tukey-Kramer(P<.05) Set=4

Obs	cultivar	N	lev	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
12			2	3.9608	0.1641	0.05	3.6257	4.2960	A
13			3	3.7346	0.1160	0.05	3.4976	3.9715	AB
14			1	3.3758	0.1641	0.05	3.0407	3.7110	B

Effect=cultivar*lev Method=Tukey-Kramer(P<.05) Set=5

Obs	cultivar	N	lev	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
15	R		2	3.9833	0.2321	0.05	3.5094	4.4573	A
16	S		2	3.9383	0.2321	0.05	3.4644	4.4123	A
17	R		3	3.8067	0.1641	0.05	3.4715	4.1418	A
18	R		1	3.7233	0.2321	0.05	3.2494	4.1973	A
19	S		3	3.6625	0.1641	0.05	3.3274	3.9976	A
20	S		1	3.0283	0.2321	0.05	2.5544	3.5023	A

Effect=N*lev Method=Tukey-Kramer(P<.05) Set=6

Obs	cultivar	N	lev	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
21		200	2	5.5950	0.2842	0.05	5.0146	6.1754	A
22		200	3	5.5938	0.2010	0.05	5.1833	6.0042	A
23		200	1	5.1100	0.2842	0.05	4.5296	5.6904	AB
24		100	2	4.7575	0.2842	0.05	4.1771	5.3379	ABC
25		100	3	4.0550	0.2010	0.05	3.6446	4.4654	BC
26		100	1	3.6475	0.2842	0.05	3.0671	4.2279	C
27		0	3	1.5550	0.2010	0.05	1.1446	1.9654	D
28		0	2	1.5300	0.2842	0.05	0.9496	2.1104	D
29		0	1	1.3700	0.2842	0.05	0.7896	1.9504	D

Effect=cultivar*N*lev Method=Tukey-Kramer(P<.05) Set=7

Obs	cultivar	N	lev	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
30	S	200	3	5.7675	0.2842	0.05	5.1871	6.3479	A
31	S	200	2	5.7300	0.4019	0.05	4.9091	6.5509	A
32	R	200	2	5.4600	0.4019	0.05	4.6391	6.2809	AB
33	R	200	3	5.4200	0.2842	0.05	4.8396	6.0004	A
34	S	200	1	5.2800	0.4019	0.05	4.4591	6.1009	AB
35	R	200	1	4.9400	0.4019	0.05	4.1191	5.7609	AB
36	R	100	2	4.7700	0.4019	0.05	3.9491	5.5909	ABC
37	S	100	2	4.7450	0.4019	0.05	3.9241	5.5659	ABC
38	R	100	1	4.6150	0.4019	0.05	3.7941	5.4359	ABC
39	R	100	3	4.3400	0.2842	0.05	3.7596	4.9204	ABC
40	S	100	3	3.7700	0.2842	0.05	3.1896	4.3504	BC
41	S	100	1	2.6800	0.4019	0.05	1.8591	3.5009	CD
42	R	0	2	1.7200	0.4019	0.05	0.8991	2.5409	D
43	R	0	3	1.6600	0.2842	0.05	1.0796	2.2404	D
44	R	0	1	1.6150	0.4019	0.05	0.7941	2.4359	D
45	S	0	3	1.4500	0.2842	0.05	0.8696	2.0304	D
46	S	0	2	1.3400	0.4019	0.05	0.5191	2.1609	D
47	S	0	1	1.1250	0.4019	0.05	0.3041	1.9459	D

MULTIPLE LINEAR REGRESSION: SORGHUM YIELD VS APHID NUMBER/LEAF AND NITROGEN RATE

```
dm'output;clear;log;clear';
Title1'Aphid MLR Regression';
data data1;
input Obs$ yield aphid N$;
if N = '100' then z1=1 ; else z1=0;
if N = '200' then z2=1; else z2=0;
cards;
1      828.65       593.25       0
2      1014.40      691.60       0
3      977.17       709.10       0
4      989.45       287.00       0
5      791.42       800.45       0
6      975.83       900.20       0
7      995.80       876.05       0
```

```
8     1031.51     648.20      0
9     2873.13     566.65      100
10    2398.58     1175.30     100
11    2615.63     940.45      100
12    2832.13     1090.95     100
13    2794.01     3594.15     100
14    2411.59     6310.85     100
15    1821.98     3320.45     100
16    2891.06     4140.15     100
17    2305.78     802.90      200
18    3337.53     928.90      200
19    3337.16     1043.00     200
20    2901.19     671.30      200
21    3221.84     4524.45     200
22    3136.27     13333.30    200
23    2805.85     2339.75     200
24    3222.28     3739.05     200
25    504.93      920.15      0
26    781.84      3447.15     0
27    286.34      1120.00     0
28    961.20      1041.60     0
29    700.28      8908.55     0
30    816.05      16900.10    0
31    806.17      3565.45     0
32    986.79      4150.30     0
33    2224.94     588.35      100
34    841.75      1849.05     100
35    2707.97     4268.60     100
36    2712.78     4030.95     100
37    1945.80     11974.20    100
38    2103.51     9423.40     100
39    2500.24     12947.60    100
40    2060.80     11600.40    100
41    3054.88     788.20      200
42    2978.11     2352.70     200
43    3152.50     4182.85     200
44    3395.72     8346.45     200
45    3019.31     13288.50    200
46    3425.59     8883.70     200
47    3597.87     6015.10     200
48    3142.21     12579.40    200
;
ODS HTML FILE='C:\users\llama1\Desktop\Luna aphid days v yield MLR
trial.html' style = minimal
;
proc reg data=data1 ;
model yield = aphid z1 z2 / xpx i influence ;
proc graph
run;
Proc Means data=data1;
```

The REG Procedure
Model: MODEL1

Model Crossproducts X'X X'Y Y'Y					
Variable	**Intercept**	**aphid**	**z1**	**z2**	**yield**
Intercept	48	207200.2	16	16	101217.82
aphid	207200.2	1835341755.4	77821.5	83819.55	482793717.92
z1	16	77821.5	16	0	37735.9
z2	16	83819.55	0	16	50034.09
yield	101217.82	482793717.92	37735.9	50034.09	262987932.68

Aphid MLR Regression

The REG Procedure
Model: MODEL1
Dependent Variable: yield

Number of Observations Read	48
Number of Observations Used	48

X'X Inverse, Parameter Estimates, and SSE					
Variable	**Intercept**	**aphid**	**z1**	**z2**	**yield**
Intercept	0.0716306241	-3.2066E-6	-0.056034221	-0.054832137	844.1912884
aphid	-3.2066E-6	1.1261317E-9	-2.270729E-6	-2.692891E-6	-0.001300082
z1	-0.056034221	-2.270729E-6	0.1295786899	0.0679299364	1520.6258556

X'X Inverse, Parameter Estimates, and SSE					
Variable	**Intercept**	**aphid**	**z1**	**z2**	**yield**
z2	-0.054832137	-2.692891E-6	0.0679299364	0.1314394422	2289.7501027
yield	844.1912884	-0.001300082	1520.6258556	2289.7501027	6220654.1158

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	3	43328798	14442933	102.16	<.0001
Error	44	6220654	141379		
Corrected Total	47	49549452			

Root MSE	376.00333	**R-Square**	0.8745
Dependent Mean	2108.70458	**Adj R-Sq**	0.8659
Coeff Var	17.83101		

Parameter Estimates					
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**
Intercept	**1**	844.19129	100.63315	8.39	<.0001
aphid	**1**	-0.00130	0.01262	-0.10	0.9184
z1	**1**	1520.62586	135.35007	11.23	<.0001
z2	**1**	2289.75010	136.31842	16.80	<.0001

The REG Procedure
Model: MODEL1
Dependent Variable: yield

Output Statistics									
Obs	Residual	RStudent	Hat Diag H	Cov Ratio	DFFITS	DFBETAS			
						Intercept	aphid	z1	z2
1	-14.7700	-0.0402	0.0682	1.1764	-0.0109	-0.0109	0.0032	0.0066	0.0065
2	171.1078	0.4671	0.0677	1.1524	0.1259	0.1255	-0.0350	-0.0774	-0.0757
3	133.9006	0.3652	0.0676	1.1614	0.0984	0.0980	-0.0271	-0.0606	-0.0592
4	145.6318	0.3977	0.0699	1.1615	0.1090	0.1090	-0.0354	-0.0649	-0.0633
5	-51.7306	-0.1409	0.0672	1.1732	-0.0378	-0.0376	0.0100	0.0234	0.0229
6	132.8090	0.3620	0.0668	1.1605	0.0968	0.0963	-0.0245	-0.0605	-0.0592
7	152.7476	0.4166	0.0669	1.1561	0.1115	0.1109	-0.0285	-0.0695	-0.0680
8	188.1614	0.5140	0.0679	1.1478	0.1388	0.1384	-0.0393	-0.0851	-0.0831
9	509.0495	1.4307	0.0833	0.9929	0.4313	0.0769	-0.2155	0.3000	0.0477

Output Statistics									
Obs	Residual	RStudent	Hat Diag H	Cov Ratio	DFFITS	DFBETAS			
						Intercept	aphid	z1	z2
10	35.2908	0.0966	0.0778	1.1878	0.0281	0.0044	-0.0125	0.0198	0.0028
11	252.0355	0.6947	0.0798	1.1394	0.2046	0.0340	-0.0953	0.1437	0.0211
12	468.7312	1.3091	0.0785	1.0176	0.3822	0.0616	-0.1727	0.2692	0.0382
13	433.8655	1.1988	0.0643	1.0273	0.3143	0.0189	-0.0528	0.2251	0.0117
14	54.9775	0.1495	0.0649	1.1699	0.0394	-0.0027	0.0075	0.0254	-0.0017
15	-538.5203	-1.5023	0.0652	0.9557	-0.3967	-0.0287	0.0805	-0.2849	-0.0178
16	531.6254	1.4804	0.0631	0.9592	0.3841	0.0133	-0.0371	0.2725	0.0082
17	-827.1176	-2.4232	0.0847	0.7178	-0.7369	-0.1346	0.3770	-0.0709	-0.5201
18	204.7963	0.5645	0.0834	1.1613	0.1703	0.0304	-0.0853	0.0160	0.1205
19	204.5746	0.5635	0.0823	1.1600	0.1688	0.0296	-0.0828	0.0156	0.1197

Output Statistics									
Obs	Residual	RStudent	Hat Diag H	Cov Ratio	DFFITS	DFBETAS			
						Intercept	aphid	z1	z2
20	-231.8786	-0.6407	0.0860	1.1547	-0.1965	-0.0367	0.1027	-0.0193	-0.1383
21	93.7808	0.2549	0.0631	1.1631	0.0661	0.0023	-0.0063	0.0012	0.0468
22	19.6630	0.0556	0.1363	1.2689	0.0221	-0.0058	0.0163	-0.0031	0.0067
23	-325.0495	-0.8954	0.0720	1.0972	-0.2493	-0.0323	0.0904	-0.0170	-0.1802
24	93.1997	0.2536	0.0650	1.1656	0.0669	0.0047	-0.0132	0.0025	0.0481
25	-338.0650	-0.9292	0.0667	1.0849	-0.2484	-0.2468	0.0622	0.1553	0.1520
26	-57.8697	-0.1572	0.0629	1.1672	-0.0407	-0.0368	-0.0033	0.0288	0.0287
27	-556.3952	-1.5555	0.0659	0.9428	-0.4130	-0.4092	0.0933	0.2619	0.2568
28	118.3629	0.3224	0.0662	1.1627	0.0858	0.0851	-0.0202	-0.0541	-0.0530
29	-132.3294	-0.3681	0.1039	1.2081	-0.1253	-0.0626	-0.0791	0.0824	0.0845

Output Statistics									
Obs	Residual	RStudent	Hat Diag H	Cov Ratio	DFFITS	DFBETAS			
						Intercept	aphid	z1	z2
30	-6.1698	-0.0192	0.2849	1.5330	-0.0121	-0.0015	-0.0107	0.0059	0.0063
31	-33.3859	-0.0907	0.0631	1.1692	-0.0235	-0.0211	-0.0023	0.0167	0.0167
32	147.9944	0.4030	0.0644	1.1543	0.1057	0.0908	0.0182	-0.0758	-0.0759
33	-139.1122	-0.3826	0.0831	1.1795	-0.1152	-0.0205	0.0573	-0.0802	-0.0127
34	-1521	-5.3641	0.0727	0.1523	-1.5023	-0.2012	0.5636	-1.0731	-0.1247
35	348.7024	0.9571	0.0629	1.0753	0.2480	0.0071	-0.0197	0.1754	0.0044
36	353.2034	0.9699	0.0633	1.0733	0.2521	0.0100	-0.0280	0.1793	0.0062
37	-403.4497	-1.1476	0.1194	1.1035	-0.4226	0.1042	-0.2918	-0.1575	0.0646
38	-249.0560	-0.6887	0.0859	1.1479	-0.2111	0.0393	-0.1102	-0.1043	0.0244
39	152.2558	0.4316	0.1361	1.2473	0.1713	-0.0450	0.1260	0.0569	-0.0279

Output Statistics									
Obs	Residual	RStudent	Hat Diag H	Cov Ratio	DFFITS	DFBETAS			
						Intercept	aphid	z1	z2
40	-288.9357	-0.8130	0.1136	1.1636	-0.2911	0.0697	-0.1952	-0.1132	0.0432
41	-78.0367	-0.2146	0.0848	1.1928	-0.0653	-0.0120	0.0335	-0.0063	-0.0461
42	-152.7727	-0.4178	0.0719	1.1622	-0.1163	-0.0150	0.0420	-0.0079	-0.0841
43	23.9967	0.0652	0.0638	1.1705	0.0170	0.0009	-0.0024	0.0004	0.0121
44	272.6297	0.7495	0.0734	1.1233	0.2109	-0.0290	0.0812	-0.0153	0.1162
45	-97.3553	-0.2755	0.1355	1.2592	-0.1091	0.0286	-0.0800	0.0150	-0.0334
46	303.1981	0.8367	0.0775	1.1140	0.2424	-0.0380	0.1066	-0.0200	0.1266
47	471.7487	1.3066	0.0632	1.0015	0.3393	-0.0126	0.0352	-0.0066	0.2249
48	24.6229	0.0691	0.1232	1.2498	0.0259	-0.0065	0.0182	-0.0034	0.0087

Sum of Residuals	0
Sum of Squared Residuals	6220654
Predicted Residual SS (PRESS)	7274726

VITA

Luna Lama, born and raised in Chitwan, Nepal completed her undergraduate studies in Agriculture from Tribhuvan University, Nepal in 2015. As her interest in Entomology grew during her undergraduate studies, she decided to join Department of Entomology at Louisiana State University as a graduate research assistant for her Master's study in 2016. After her graduation as intended on May 2018, she plans to work as an Entomologist in Pest Management Enterprise under the supervision of Dr. Grady Coburn.