EXPLORING SEVERAL LIMITATIONS TO THE ADAPATION OF KIWIFRUIT (ACTINIDIA CHINENSIS PLANCH. AND A. DELICIOSA A. CHEV.) AS A NEW POTENTIAL SPECIALTY CROP IN TEXAS

A Dissertation

by

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ABSTRACT

The feasibility of commercial kiwifruit (*Actinidia chinensis* Planch. and *A. deliciosa* A. Chev.) production in Texas was investigated through three applied studies focusing on perceived key limitations to the adaptation of this crop. The response of two-year-old field-grown kiwifruit plants to early autumn frost was documented with respect to species, cultivar, and propagation method. *A. deliciosa* plants sustained significantly greater damage as compared to *A. chinensis,* with a propensity for basal injury and cracking proving to be unique to the former species. Among individual cultivars, *A. deliciosa* 'AU Authur' and 'AU Fitzgerald' exhibited the most severe damage, whereas *A. chinensis* Zespri Gold™ seedlings sustained the least.

A growth chamber-based study was conducted to compare floral and vegetative responses of two pistillate kiwifruit cultivars to continuously supplied winter chilling and warm temperature interrupted chilling at six weekly chilling increments using detached fruiting canes. A. *deliciosa* 'AU Fitzgerald' demonstrated evidence of chilling negation by warm temperature interruption as indicated by reduced floral activity at the two highest chilling levels imposed, whereas *A. chinensis* 'AU Golden Dragon' did not appear susceptible to chilling negation by intermittent warm winter temperatures, as are typical in southeastern Texas. Vegetative development showed no response to type of chilling.

The response of field-grown kiwifruit plants to contrasting soil pH conditions was evaluated to assess the effect of soil alkalinity on species, cultivar, and propagation

method and to identify putative physiological and nutritional mechanisms involved. Soil alkalinity resulted in a greater incidence and intensity of visual chlorosis symptoms and reduced vigor, but was not associated with inhibited physiological responses such as photosynthesis. Chlorosis varied by species, propagation method, and cultivar, with more severe symptoms observed in *A. chinensis*, as a species and among clonally propagated plants. *A. deliciosa* 'AU Authur' and *A. chinensis* 'AU Golden Dragon' exhibited the least and most severe chlorosis symptoms, respectively among cultivars. Development of chlorosis was associated with inadequate leaf tissue concentrations of iron, manganese, and copper, with no indication of iron inactivation in shoot tissue. All factors assessed in this research are expected to pose serious limitations to the commercialization of this crop.

DEDICATION

This dissertation is dedicated to my late grandmother ("Mimi") Jeanne Newman Seale (1937-2007), who first instilled in me the love for horticulture. I would also like to dedicate this work to my grandfather ("Opa") Hilmar Valentine Hartmann for cultivating in me a passion for fruit production and the art of grafting trees. Finally, I wish to dedicate the completion of this work to my mother, Patricia Dianne Seale Hartmann, and father, Larry Hilmar Hartmann, for believing in me and never allowing me to give up on my dreams. If not for these four special people, I would not be where I am today.

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CHAPTER I

INTRODUCTION

Background

The Texas fruit industry accounts for a substantial portion of the state's agricultural economic output. While acreage for crops such as pecan, olive, and wine grapes continue to increase, much of the state's production of fresh fruit has seen a sharp decline over recent years. Production of peaches for example, which has historically been the most important temperate fruit crop in Texas, has decreased by 54% over the past 15 years (USDA, 2012). Other than wine grapes and olives, which are primarily grown as processed crops, there have been very few if any introductions of new economically significant fruit crops in Texas, therefore the timing is ideal for the emergence of a new specialty fruit crop.

Origin and Botany

Kiwifruit is a temperate fruit native to China that is produced on large deciduous vines. The genus *Actinidia* consists of 66 different species in the family Actinidiaceae. Of these, *Actinidia deliciosa* produces the largest fruit, is the most important species economically, and is the species that bears the name kiwifruit. *A. chinensis*, or golden kiwifruit, also produces large fruit and has the second highest production. Several other species are also cultivated for their edible fruit including *A. arguta* (kiwi berry or hardy kiwi) and *A. kolomikta*. All *Actinidia* species are native to Eastern Asia, with all but four

found in China (Huang et al., 2004). Plants range in geographic adaptation from being nearly tropical to cold temperate (50°N) (Huang et al., 2004). Both *A. deliciosa* and *A. chinensis* are large woody deciduous vines with dioecious flowers. These flowers are produced on one-year old growth, and are primarily pollinated by insects (Beutel, 1990). The fruit are botanically berries consisting of a fleshy ovarian tissue or inner pericarp radiating from a central columnella or core with many small, black or brown seeds surrounded by a thin, leathery exocarp with a variable amount of bristly trichomes (Crisosto and Kader, 1999). *A. deliciosa* produces essentially round to cylindrical fruit with green flesh and a brown exocarp with a large amount of fuzz, while *A. chinensis* produces typically smaller fruit with yellow flesh with less pubescence. Kiwifruit may be dried and made into confectionaries, but are most commonly consumed fresh.

History and Economics

Despite having been grown for thousands of years in its native China, development of the kiwifruit did not began until 1904 when Isabel Fraser brought seeds of *A. deliciosa*, known as "miho-tao" to New Zealand (Ferguson and Bollard, 1990). Six years later, the resulting vines began producing their first crop. In 1920 plants, referred to as "Chinese gooseberries" appeared for sale in nearby nurseries. Four years later, one of these nurserymen named Hayward Wright selected what would eventually be known as the cultivar 'Hayward' and began propagating and selling (Zespri, 1999). In 1934 the first commercial orchard was planted on the North Island near Taraunga (Huang et al., 2004). By the early 1950's England began to receive its first shipments of fruit. In 1959

the fruit was renamed “kiwi” after the New Zealand’s national bird, which bears resemblance to the fruit in an effort to increase marketing in the United States (Zespri, 1999). In 1967 the first ‘Hayward’ plantings were made in Central California, with the first commercial crop harvested in 1977 (Beutel, 1990). In 1991, the first golden kiwi (*A. chinensis*) fruit appeared in New Zealand and began to spread to other growing regions (Zespri, 1990). In addition to their novel nature, kiwifruit have historically benefited from marketing success because of their high Vitamin C content. Once known in China as the “king of fruit” for this reason, green kiwifruit typically contain two to three times as much ascorbic acid as do oranges, which has long been considered a standard for this nutrient (Huang et al., 2004).

As of 2013, worldwide kiwifruit production had reached 3.26 million metric tons, ranking 20th in total production among all fruits (FAO, 2013). As a relatively new crop, kiwifruit has seen production increased by approximately 216% over past decade and by 594% over the past 20 years worldwide. During this period, yield has seen a dramatic increase followed by a slow decrease over the past decade, which may be a result of the explosion of young orchards in China that have not reached full production yield and due to increasing disease pressure. The nations with the highest production of kiwifruit as of 2013 include: China, Italy, New Zealand, Chile, Greece, France, Turkey, Iran, Japan, and the United States, respectively (FAO, 2014). China has experienced a rapid growth in production over the past 15 years, surpassing both Italy and New Zealand, as a result of extensive efforts toward development of new varieties and more efficient production systems (Huang et al., 2004). Yield in 2013 was highest in New

Zealand (329,516 Hg/Ha), followed by Chile (230,704 Hg/Ha) and the U.S. (182,731 Hg/Ha), respectively, as compared to average yield for the world (133,733 Hg/Ha) (FAO, 2014). Approximately 98% of the kiwifruit produced in the United States is in California (California Kiwifruit Commission, 2014), with the remainder mostly taking place in Oregon.

Kiwifruit in the Southeastern United States

The green or fuzzy kiwifruit (*A. delici*osa) has been grown in the United States since 1967 (Beutel, 1990), with the vast majority of production taking place in California (California Kiwifruit Commission, 2014). During the late 1970's and 1980's several attempts were made to produce kiwifruit in Southeastern states including Alabama, Georgia, North Carolina, South Carolina, and Virginia. These plantings, which consisted almost entirely of the 'Hayward' cultivar exhibited good production for several years, but were ultimately severely damaged or killed as the result of severe freezes (Mainland and Fisk, 2006), while other plantings such as in Southern Alabama grew well, but failed to produce a crop because of insufficient winter chilling. Golden kiwifruit (*A. chinensis*), which produces yellow-flesh fruit with little or no fuzz, is a relatively new crop with the first commercial production taking place in New Zealand in the 1980's (Zespri, 2000). In the mid-1990's trials began in Central Alabama evaluating two new cultivars of golden kiwifruit from China. After ten years of successful production, they were released in 2008 as 'AU Golden Sunshine' and 'AU Golden Dragon' by Auburn University and Institute of Fruit and Tea in Hubei Province, China. Both cultivars along

with ‘AU Fitzgerald’, a new cultivar of green kiwifruit, proved to be well-suited to the warm, humid conditions of the Central Gulf Coast, where earlier attempts with other varieties ultimately failed.

Golden Kiwifruit in Texas

In 2011 approximately 30 plants of the Auburn varieties were installed as an evaluation trial on the Stephen F. Austin campus at Nacogdoches. Despite receiving very little care and minimal training, these plants grew vigorously and yielded approximately 400 kg (875 pounds) in 2015, with most of the production coming from eight ‘AU Golden Dragon’ vines. Production in 2016 was very light, which could be attributed to low winter chilling accumulation (approximately 450 chill units), a noticeable lack of bee activity, and poor synchronization between females and their respective male pollinizer plants.

The recent results in Nacogdoches along with successful establishment of commercial production in Alabama suggest that further trailing and research into this crop is warranted. Given its similarity to Alabama with respect to soil, water, and climate, East Texas appears to be especially well-suited for production. Bloom tends to be much later than other crops grown in this region such as blueberry and peach, making kiwifruit a less risky alternative with respect to spring freezes. Interest and demand for local food production and novelty fruit crops appears to be increasing, and the publicity resulting from the recent success has generated interest from commercial growers and hobby fruit growers alike. With an ever increasing focus on growing consumption of

fruits and vegetables for improving human health, kiwifruit has garnered a reputation as being considered by many to be “super food” because of its rich supply of antioxidants (Motohashi, 2014) and because of the fact that golden kiwifruit in particular typically has three-times the Vitamin C content of oranges (Zespri, 2012). Additionally, kiwifruit have relatively few pest and disease problems, suggesting that they might be well suited for organic production. These benefits along with huge yield potential and a high retail price ranging from approximately 7.00 to 10.00 USD per kilogram of fresh fruit (2015) suggest that golden kiwifruit may have potential as a new specialty crop in Texas.

Research Objectives

The objectives of this project is to evaluate the feasibility of commercial kiwifruit production in Texas. Three major perceived challenges associated with the adaption of this crop have been identified and will serve as the focus of this study:

1. Cold tolerance
2. Chilling requirement
3. Soil pH

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CHAPTER II

EVALUATING FROST DAMAGE OF YOUNG FIELD-GROWN KIWIFRUIT (ACTINIDIA CHINENSIS AND A. DELICIOSA) PLANTS

Introduction

Climatic Requirements

Kiwifruit (*Actinidia chinensis* Planch. and *A. deliciosa* A. Chev.) are known for requiring demanding climatic conditions (Ferguson, 1991; Norton, 1994). Kiwifruit plants are found in their Native China growing on the slopes of hills and mountains, in the shelter of woodland edges. (Ferguson, 1991). For optimal production, a long growing season of 225 to 240 days without frost is required (Norton, 1994). Other environmental requirements include the need for acid soil with good internal drainage, protection from wind, ample supply of water that is low in salinity, relatively high humidity, and trellising (Beutel, 1990; Himelrick and Powell, 1998; Sale and Lyford, 1990; Strik, 2005). Perhaps above all of these, limited cold tolerance is generally considered the greatest barrier to production. While vines can be severely damaged or killed by temperatures of -12.2°C or lower (Norton, 1994), kiwifruit also have a specific requirement for winter chilling in order to overcome rest and produce a sufficient crop (Snelgar, 1997), with standard cultivars such as ‘Hayward’ requiring as much as 1,150 chill units (Caldwell, 1989). Caldwell (1989), along with Dozier et al., (1992) reported that sites such as South Carolina in the southeastern United States that receive adequate chilling were also subject to serious, damaging freezes. While new growth may emerge

from damaged portions of the plant (Testolin and Messina, 1987) frequent severe injury to plants would limit the development of commercial production (Chat, 1995; Dozier et al, 1992.), since flowers and fruit are produced on previous year's canes (Brundell, 1975).

Cold Hardiness within the Genus Actinidia

The genus *Actinidia* is comprised of three distinct botanical groups (Hongwen, 2016) and as many as 54 (Hongwen, 2016) to 66 unique species (Hongwen, 2004). Additionally, members of the genus include species that range from subtropical origins such *A. indochinensis* to as far as 50°N such as *A. kolomikta*, which is hardy to -35°C (Ferguson, 1991). Indeed, substantial variation has been reported among *Actinidia* species with *A. kolomikta* and, to a lesser extent, *A. arguta* generally considered the most cold-tolerant, while *A. chinensis* and *A. deliciosa* are undoubtedly much more tender (Chat, 1995; Ferguson, 1991; Hongwen, 2016). While little information is available regarding the comparison of *A. chinensis* and *A. deliciosa* in terms of cold tolerance, they do have unique (although often times overlapping) native ranges. *A. deliciosa* is primarily found in the central portion of Southern China (Yunnan, Guangxi, Guizhou, Hunan, Jiangxi, Sichuan, Chongqing, Hubei, Gansu, Shaanxi, and Henan provinces) and at higher elevations, while *A. chinensis* is more common in the more eastern portion of Southern China (Guizhou, Guangxi, Hunan, Jiangxi, Fujian, southwest Zhejiang, Hubei, southern and western Anhui, Henan, and Jiangsu) and at lower elevations (Hongwen, 2016). Considering the broad geographic distribution and highly heterozygous nature of

both species, it would be expected that relative cold tolerance between these species would vary considerably by cultivar.

Kiwifruit are functionally dioecious (Hongwen, 2016), resulting in plants with distinct male or female flowering characteristics, implicating the need for cross-pollination (Beutel, 1990; Himelrick and Powell, 1998; Norton, 1994; Strik, 2005). Much like animal species, kiwifruit exhibit some sexual dimorphism. Male plants are generally observed to be more vigorous and tend to remain in active growth later in the season, whereas females tend to be less vigorous and terminate growth earlier in autumn (Ferguson, 1991). Not surprisingly, it has been reported that male plants tend to be less cold tolerant (Pyke et al., 1986; Strik, 1990), although Dozier et al (1992) observed that the staminate cultivars 'Tomuri' and 'Matua' fared slightly better in severe frost than did pistillate 'Hayward'. Dozier also reported that there was a greater percentage of mortality among 'AU Authur', a male, than the fruiting cultivar 'AU Fitzgerald'—both of which are included in this study. Segregation for sex within F_1 seedling populations generally occurs in a 1:1 ratio (Hongwen, 2016).

Effect of Timing and Acclimation

Damage to fruiting buds of fully-hardened canes of *A. deliciosa* 'Abbott' and 'Hayward' have been observed at temperatures of -10°C, while all buds were killed following exposure to -18°C (Hewett and Young, 1981). More severe freezes have been known to result in the damage of canes as well as trunks (Pyke et al., 1986), with entire vines reportedly killed by temperatures below -12.2°C in some cases (Norton, 1994).

Controlled environment studies subjecting plants to artificial freezing have provided more impressive estimates of hardiness. Pyke et al. (1986) reported that some one-year-old ‘Hayward’ plants were killed by temperatures between -9°C and -11°C for ‘Bruno’, with most dormant plants dying at -13°C. Cold tolerance in kiwifruit appears to improve with age. As with other subtropical crops such as fig, olive, and pomegranate, old kiwifruit vines have the ability to renew growth via suckers from undamaged underground tissues (Dozier et al., 1992). Following a record cold snap in Italy that dropped the temperature to -23°C, Testolin and Messina (1987) found that none of the plants that were surveyed were completely killed, or were at least able to grow back from the ground.

As with many woody plants, the absolute minimum temperature tolerated is heavily dependent upon other modifying factors including the age of the plant, the development stage, and the amount of conditioning or acclimation that the environment has provided (Chat, 1995; Wisniewski et al., Gusta. 2003). Kiwifruit appear to follow the same pattern observed in many plant species (Pyke et al., 1986), with maximum cold tolerance observed in mid-winter (Kim and Kim, 1986a; Kim and Kim, 1986b; Pyke et al., 1986). Frost events in the spring and autumn present a major challenge to kiwifruit production, particularly when plants are not acclimated (Massai et al., 1991). De-acclimation occurs toward the end of winter or in early spring (Pyke et al., 1986) and can occur very early in some years. As with other deciduous fruit crops, the new growth is especially susceptible to damage, with -1.1°C reportedly killing new shoot growth and consequently reducing yield (Norton, 1994). In controlled environment studies young

shoots were damaged by mild frost conditions as mild as 0.5°C (Pyke et al., 1986) and flower buds were killed by exposure to -1.5°C to 2.0°C for 30 minutes (Hewett and Young, 1981). Blanchet (1985) reported that -6°C can cause damage to trunks of mature non-acclimated vines in spring and fall.

As a temperate or warm subtropical plant, kiwifruit is able to tolerate substantial cold, provided that they are conditioned by gradually declining temperatures (Sale and Lyford, 1990). Lu and Reiger (1990) reported that decreasing photoperiod combined with cool, non-freezing temperatures are most conducive to acclimation, whereas mild temperatures leading up to hard and early autumn frost present the greatest risk for damage to the non-acclimated vines (Sale and Lyford, 1990). It is only logical that differing annual weather patterns would lead to improved or perhaps very poor acclimation and subsequent tolerance to cold (Pyke et al., 1986). The proximity of regions such as Texas to the U.S. to the Gulf of Mexico and resulting tropical influence along with the strong continental influence of the North American landmass creates the potential for violent temperature swings in spring and autumn. The degree of damage sustained is greatly influenced by amount of acclimation the plant has received (Lawes et al., 1995; Lu and Reiger, 1990), the stage of development (Hewett and Young, 1981; Pyke et al., 1986;) the intensity and duration of the frost (Pyke et al., 1986; Testolin and Messina, 1988), plant age (Pyke et al., 1986), the genetic limits of the cultivar (Dozier et al., 1992; Pyke et al., 1986), and finally perhaps even method of propagation (Massai et al., 1991). Mild frosts are a catalyst for normal abscission of foliage in autumn (Sale and Lyford, 1990), with temperatures of -3°C to -5°C proving sufficient to kill all leaves

(Pyke et al., 1986). However, temperatures between -2.8°C and -6°C can cause injury to canes and even trunks, in the absence of acclimation (Blanchet et al., 1986; Hewett and Young, 1981; Norton, 1994), with young vines reportedly killed to the ground by fall temperatures as warm as -3°C (Bullard, 1987; Krewer et al., 1988; Lu and Reiger, 1990). According to Massai et al. (1991), this problem is exacerbated when plants are still actively transpiring and photosynthesizing (Massai et al., 1991).

Damage from fall frost can also extend to the crop itself, with unharvested fruit potentially damaged (Pyke et al., 1986; Sale and Lyford, 1990), particularly after the sheltering foliage has been removed by previous frost (Sale and Lyford, 1990). The same authors reported that even minor damage can negatively impact storage life of affected fruit, as ethylene emission spikes in frost-damaged tissues. Frost injury has also been implicated in the pathogenicity of Psa (*Pseudomonas syrignae* pv. *actinidiae* (Fround et al., 2014; Ferrante and Scortichini, 2014), a devastating bacterial disease found in regions including eastern Asia, Europe, New Zealand, and Chile (McCann et al., 2017). While Psa has not been detected in the United States (including California), incidence of the less virulent and common form of bacterial canker (*Pseudomonas syrignae)*, known as "bleeding canker" is not uncommon in California, where it is also more severe where frost injury occurs (Gubler and Conn, 1994).

Assessment of Frost Injury

A wide array of laboratory- and field-based assays have been employed for the assessment of frost damage in woody plants, including electrolyte leakage, phenolic

leakage, callus assay, Triphenyl tetrazolium chloride (TTC), and differential thermal analysis (Nesbitt et al., 2002). While controlled environment-based experiments and associated assays offer the advantage of repeatability, field-based studies are considered most accurate and reliable (Li, 1984). As discussed earlier, damage resulting from mild frost includes damage of foliage, flower buds, flowers, young fruit, and young shoot tissue (Hewett and Young, 1981; Pyke et al., 1986) with affected tissues exhibiting a dark water-soaked appearance, quickly followed by wilting and rapid desiccation of the killed tissue (Sale and Lyford, 1990). More extensive damage from more severe frost injury results in the damage of canes, cordons, and even trunks, as evident by exudate discharged from buds (Chat, 1995; Pyke et al., 1986), brown discolored necrotic vascular tissue or stem browning (Pyke et al., 1986; Chat, 1995). Chat (1995) rated frost damage as the percentage of necrotic relative to total stem tissue as a function of stem length.

In woody plants, frost damage tends to occur more readily in smaller shoots and progress downward into larger-diameter shoots as severity increases. Damage to the vascular tissue of kiwifruit trunks has been well documented (Blanchet, 1986; Dozier et al., 1992; Gremminger et al., 1982; Massai et al., 1991; Pyke et al., 1986), with the resulting “ring-barked” plants failing to resume growth above the damaged trunk portion or subsequent spring growth rapidly collapsing (Lyford and Sale, 1990) due to insufficient vascular connection. Such vines usually respond with vigorous sucker growth in spring when sufficiently large root systems remain (Pyke et al., 1986; Testolin and Messina, 1987). Such trunk damage is not always, but most commonly accompanied

by the development of vertical cracking of bark or "trunk-splitting" (Dozier et al., 1992; Gremminger et al., 1982; Massai et al., 1991; Pyke et al., 1985; Sale and Lyford, 1990), which is typically restricted to the lower 10 to 15 cm above ground level (Dozier et al., 1992; Sale and Lyford, 1990). Recent field observations suggest that damage to trunks may occur even in the absence of serious shoot damage in the mid or upper canopy region.

Frost Protection Strategies

As a result, protection of kiwifruit vines from frost damage has focused on the trunk (Dozier et al., 1992; Pyke et al., 1988; Sale and Lyford, 1990), with recommendations for protecting from the ground to the cordons in areas where plants are marginally hardy (Dozier et al., 1992; Sale and Lyford, 1990). The previous authors experimented with a variety of strategies in the attempt for protecting trunks, including mircrosprinklers, 'Reese Trunk Wraps' (bi-wall Styrofoam wraps with antifreeze liquid), hay, polyurethane wrapping, and sawdust banks (Dozier et al., 1992; Sale and Lyford, 1990), with sawdust banks reportedly providing most effective defense (Pyke et al., 1988). Other techniques that have been used include under-vine sprinklers, over-vine sprinklers, in addition to various heaters and wind machines, which are only effective during radiational frost events (Norton, 1994; Sale and Lyford, 1990). Passive strategies for preventing or reducing frost damage include site selection for air drainage, positioning plantings on north-facing slopes, and the use of wind breaks (natural and artificial) (Norton, 1994; Sale and Lyford, 1990).

In addition to the timing, intensity, and duration, the type of frost has a major bearing on the feasibility and type of protection that can be used. With regard to fruit production, there are two major types of frost: advective and radiational. These different types can also result in very different symptoms of damage (Pyke et al., 1986). Radiational frost events are characterized by temperature gradients (inversions) that form under calm clear conditions. In such cases, cold air settles in a shallow layer underneath a stratified layer of warmer air. Frost damage can be mitigated by the use of wind machines, cold air drains, or other measures that facilitate mixing of air in order to "break" the inversion. The second type, advective frost, is associated with windy (>6.4 km / hour) conditions, which can occur under overcast or clear skies alike. Previously mentioned strategies are ineffective due to the lack of inversion (Snyder, 1994).

Physiology of Frost Injury

At the cellular level, frost damages plant tissue in primarily two ways. Intracellular freezing of the cytosol results in the physical cutting or puncture of phospholipid membranes and subsequent damage to organelles. Extracellular ice formation, which is more common and generally occurs prior to intercellular ice formation due to the lower solute concentration in the apoplast and xylem vessels, results in the dehydration of cells, as water is pulled out of the cell with the resulting lower water potential gradient. Tolerance to frost is accomplished by most temperate plant species, such as kiwifruit (Lu and Reiger, 1990) through cold acclimation in response to decreasing photoperiod and cool, non-freezing temperatures (Radin et al.,

2010). These include the withdrawal of water from xylem and other tissues in order to prevent freezing and cracking; increased composition of saturated fatty acids in membranes for the regulation of electromagnetic gradients through improved thermostability; and the production of cryoprotectants and antifreeze proteins, which protect protein structure and allow the intercellular contents to supercool without forming ice (Radin et al., 2010; Wisniewski et al., 2003). These processes are believed to be mediated by Abscisic Acid and Ca^{2+}signal transduction (Survila et al., 2009)

A limited number of studies have attempted to elucidate possible mechanisms responsible for frost tolerance in kiwifruit. As mentioned earlier, geographic origin is believed to play a major role in hardiness. Species from more mild regions such as *A. chinensis* reportedly have more pronounced and exposed lateral buds, whereas more inland species such as *A. deliciosa* and *A. arguta* exhibit buds that are hidden inside swollen petiole bases (Ferguson, 1991). Chat (1995) observed that less vigorous genotypes sustained less damage. However, this difference became less notable, once differences in plant size were accounted for. Massai et al. (1991) reported that micro-propagated vines were less sensitive to frost. The same publication also reported that frost tolerance was related to fewer, but larger-diameter xylem vessels. Kim and Kim (1986b) found that high levels of glucose and fructose in phloem, along with high abscisic acid (ABA) concentrations were positively correlated with improved frost tolerance, while high nitrogen concentration in the plant was associated with increased susceptibility to cold damage. Ice-nucleating bacteria have long been suggested as a catalyst for freeze damage. In growth chamber experiments on kiwifruit, sensitivity to

frost damage was reportedly related to *Pseudomonas viridiflava* (bacterial leaf spot and blossom blight) bacteria concentrations along with low relative humidity (Varvaro and Fabi, 1992).

Establishment Efforts in Texas

During the early 1980's large-scale efforts were made to establish commercial production of kiwifruit (*A. deliciosa*) in the Southeastern United States (Mainland and Fisk, 2006). Commercial production, largely based on the cultivar 'Hayward', ultimately proved unsuccessful due to lack of reproductive growth and severe freezes (Caldwell, 1989). Trialing of kiwifruit by Auburn University researchers led to the field evaluation of two *A. chinensis* selections in the mid-1990's that were developed by the Institute of Fruit and Tea, Hubei Academy of Agricultural Sciences of P.R. China. After successful field performance at the Chilton Research and Extension Center at Thorsby, AL, 'AU Golden Dragon' and 'AU Golden Sunshine' were jointly released by the Institute and Auburn University in 2018 (Spiers, unpublished).

The success in Alabama inspired the establishment of replicated trailing plantings of the two Auburn golden kiwifruit cultivars and their respective pollinizers, along with *A. deliciosa* female 'AU Fitzgerald' and male 'AU Authur', two chance seedlings that had also performed well in central Alabama. In 2011 a small observational planting of the Auburn cultivars was established on the Stephen F. Austin State University campus at Nacogdoches, TX. Successful crops in 2014, 2015, 2018, and 2019 led to more expanded trialing and the initiation of applied research related to adaptation

by Stephen F. Austin State University and Texas A&M University at College Station, TX (Creech and Hartmann, 2018).

Objective

The objective of this study was to document the response of young kiwifruit plants to autumn frost and assess the effects of species, cultivar, and propagation method.

Materials and Methods

Plant Material

Plant material used in this study included a diverse collection of clonally-propagated (male and female) selections and seed-propagated material from both *A. chinensis* and *A. deliciosa* species. A total of five clonally-propagated cultivars were used in this study. A list of plant material and associated characteristics can be found in Table 1. 'AU Authur' is a clonally-propagated *A. deliciosa* selection that was found as a chance seedling near Mobile, AL and is used as a pollinizer for 'AU Fitzgerald'. 'CK-3' (CK 03 or 'Meteor') is a clonally-propagated *A. chinensis* selection that has been widely used as a pollinizer for *A. chinensis* 'Hort16A'. 'AU Golden Dragon' and 'AU Golden Sunshine' are clonally propagated pistillate selections of *A. chinensis.* Both were developed at the Institute of Fruit and Tea, Hubei Academy of Agricultural Sciences of P.R. China and released patented by Auburn University, following successful trailing in central Alabama (Spiers, unpublished). 'AU Fitzgerald' was patented by Auburn University as a clonally-propagated *A. deliciosa*. This female cultivar originated as a

chance seedling of ‘Hayward’ near Mobile, AL and has also performed well in central Alabama.

Two seed-propagated cultivar groups were also included in the study. These include open-pollinated seedlings of *A. chinensis* Zepsri Gold™ (‘ZEZY002’) and the *A. deliciosa* cultivar ‘Hayward’. Given the dioecious flowering habit of kiwifruit, it is expected that seedlings of both groups would include plants of both male and female sex (Table 2).

Seed collected from store-bought Zepsri Gold™ (‘ZEZY002’) and ‘Hayward’ fruit were cleaned and stratified for four weeks and sown in March 2016. ‘AU Golden Sunshine’, ‘AU Authur’, ‘AU Fitzgerald’, ‘AU Golden Dragon’ and ‘CK-3’ were propagated from softwood cuttings under mist during June 2016. All plants were transplanted into 2.84 L nursery containers and grown in pine bark-based soil-less media under greenhouse conditions for the remainder of the season. Plant material was transplanted into the field nursery during June 2017 at the Texas A&M University Horticulture Research, Teaching, and Extension Center (TAMU HORT-TREC) field lab near College Station, TX.

Table 1 List of plant material and characteristics included in the assessment of young field-grown kiwifruit plants' response to frost injury.

Cultivar	Species	Propagation Method	Sex	Remarks
'AU Authur'	*Actinidia deliciosa*	Clonal	Male	Pollinizer for 'AU Fitzgerald'
'AU Golden Dragon'	*Actinidia chinensis*	Clonal	Female	
'AU Fitzgerald'	*Actinidia deliciosa*	Clonal	Female	
'AU Golden Sunshine'	*Actinidia chinensis*	Clonal	Female	
'CK-3' / 'Meteor'	*Actinidia chinensis*	Clonal	Male	Pollinizer for 'AU Golden Dragon'
'Hayward' Seedling	*Actinidia deliciosa*	Sexual	[a]Mixed	Open-pollinated seedlings
Zespri Gold™ Seedling	*Actinidia chinensis*	Sexual	[a]Mixed	Open-pollinated seedlings

[a]Open-pollinated seedlings expected to segregate in a 1:1 female to male ratio.

Field Preparation

The research plot was located at the TAMU HORT-TREC field lab, approximately 16 km southwest of College Station, TX. (30°36'N 96°18'W). The site is situated in the Brazos River alluvial floodplain at an elevation of approximately 71.02 meters above sea level. Climate is considered sub-humid warm-temperate with temperatures in nearby College Station, TX ranging from 5.1°C (ave. January min. temp.) to 35.7°C (ave. August max. temp.), with 1,017.5 mm of average annual precipitation. College Station historically receives an average of 274 frost-free days, with the average first and last day of frost occurring on November 30 and March 1 (Brazos County AgriLife). The lowest and highest recorded temperatures ever recorded for College Station are -19.4°C and 44.4°C, respectively (National Weather Service). Winter chilling accumulation generally ranges from 600 to 700 units (0°C to 7°C). The

monthly climate data, based on historical average from the Easterwood Airport Weather Station (KCLL), which is approximately 8 km from the site is also listed (Figures 1 & 2). Soil within the experimental plot is classified by the United States Department of Agriculture- Natural Resources Conservation Service (USDA NRCS) as a Westwood silt loam and moderately alkaline with a pH ranging from 7.6 to 7.9.

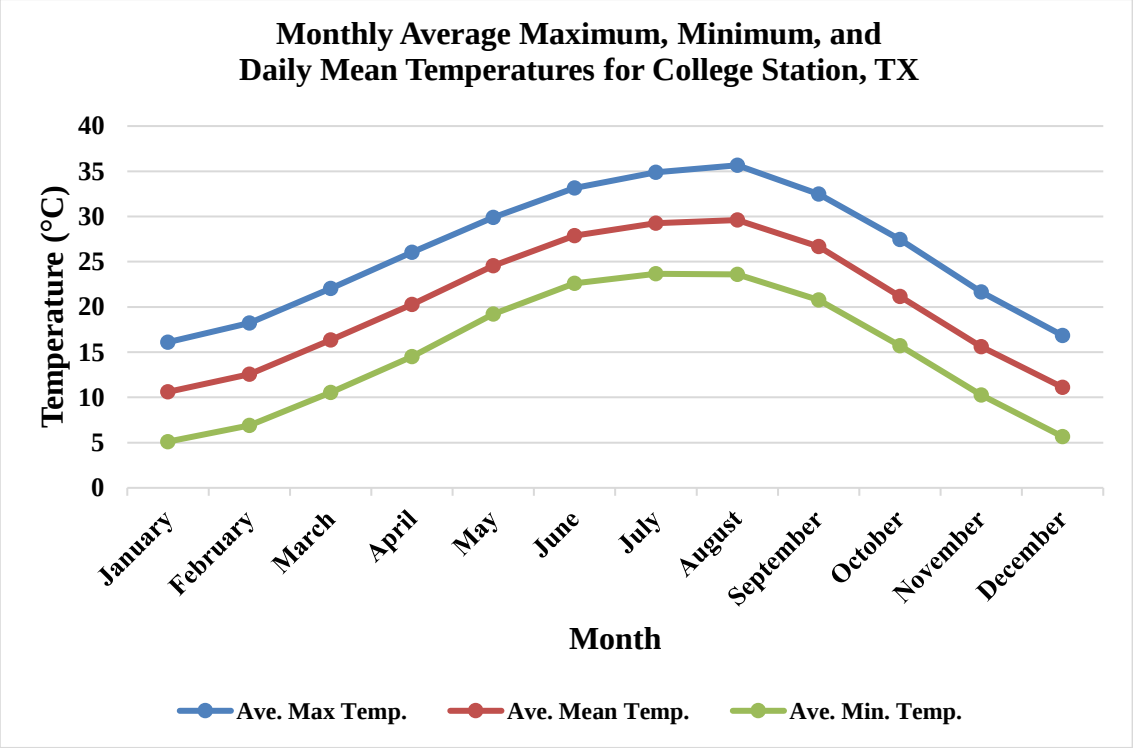

Figure 1 Average monthly temperatures for College Station, TX used in the assessment of young field-grown kiwifruit plants' response to frost injury.

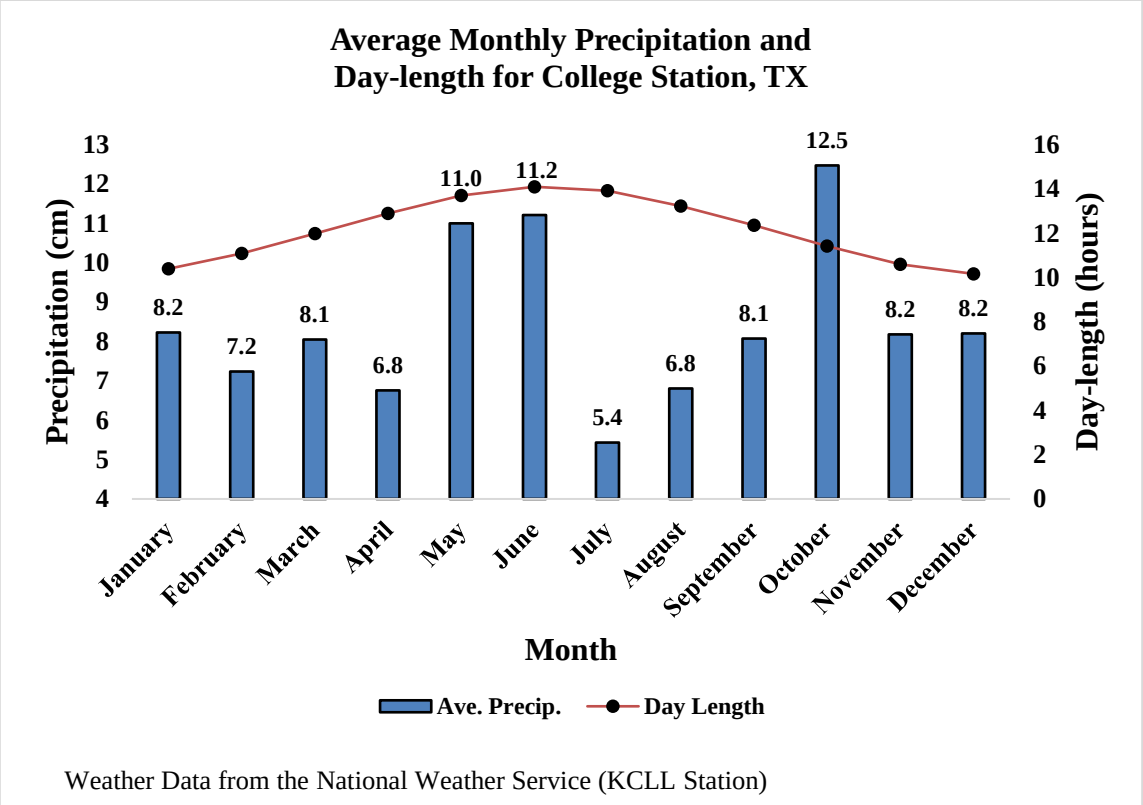

Figure 2 Average monthly precipitation and fay length for College Station, TX used in the assessment of young field-grown kiwifruit plants' response to frost injury.

Raised beds (approximately 30 to 38 cm tall by 45 to 60 cm wide) were erected using a disc-type bed-maker following the incorporation of approximately 8 cm of composted pine bark. Plants were set at 0.46 meter centers in single-row beds on 3.31 meter centers. Plants were drip-irrigated using a single line of drip tape with emitters spaced 0.46 meters apart (1.02 liters per minute). Plants were irrigated twice per week, with each plant receiving approximately 4.0 to 6.0 liters per irrigation application, in the absence of rain. Phosphorus deficiency was corrected based on soil testing via side-dressing of super-phosphate. Chelated iron, manganese, and zinc were applied as a

drench, as needed, based on visual foliar deficiency symptoms. Nitrogen was applied continuously with each irrigation at a rate of 200 mg/kg N (33-0-0) via the drip irrigation. Nitrogen fertigation was terminated near September 1st in 2017 and 2018, at which point plants received irrigation only.

Plants were allowed to grow naturally with several shoots trained to grow up a single 2.0 meter bamboo stake during 2017. Plants were cut back to approximately 30 cm in January 2018. Plants were trained to two to three shoots during the 2018 growing season, with sucker growth removed.

Experimental Design

Experimental design consisted of a randomized complete block design (RCBD) with five blocks. Rows were used as blocks, with outer rows serving as borders. Each experimental unit contained an average of five plants as subsamples. Two of the blocks were missing one or more of the seven cultivar treatments, therefore only the three complete blocks were considered for statistical analysis. A total of 113 surveyed plants were used in the analysis.

Data Collection

Assessments of freeze injury were made approximately five weeks after the freeze event from December 19 through December 22, 2018. A total of six variables were assessed to evaluate frost damage in the field (Table 2). Additionally, plant age, species, and propagation method were recorded. Assessments of freeze injury were made

visually, based on the presence of dark discolored tissue with a water-soaked appearance or based on the presence of moist necrotic tissue. A sharp knife was used to remove a thin slice (approximately 4 cm long) of bark and wood in order to expose the cork, phloem, vascular cambium, and xylem tissues. This was done, starting with the distal-most and smallest diameter wood and progressing downward toward the main shoot and base until no more injured tissue was observed. Several shoots were evaluated for each plant. The maximum diameter of shoot (MDD) exhibiting injury was recorded in mm along with the basal trunk diameter (BD) at ground-level. It is important to note that the maximum diameter of wood damaged is expected to be a representation of frost injury, but is also expected to be a function of inherent plant size.

The extent of damage to the whole shoot system was comprehensively estimated in two ways: 1.) the maximum diameter of shoot damaged (MDD) relative to base diameter (BD), expressed as a percent (PBDD); 2). percent of the entire shoot (PSD) system that was injured, which was visually estimated based on the prevalence of injured wood that was exposed, as described earlier. Percent shoot damage diameter relative to trunk diameter damaged, referred to here as percent of base diameter damaged (PBDD), provides for an assessment of the intensity of damage, while accounting for differences in size among genotypes. Estimated percent shoot damage (PSD) provides another estimate of the extent of cold damage, relative to the entire shoot system, and is also not biased by plant size.

Additionally, the lower trunk of each plant was carefully inspected for the formation of cracking or bark splitting. Frequency of cracking / splitting was reported in

a binary fashion as ‘yes’ or ‘no’. Young kiwifruit plants are reported to sustain freeze damage that is often confined to the basal 5 to 25 cm of the trunk. For this reason, a thin slice of wood and bark (approximately three to four centimeters long) was also removed on four sides of the trunk, starting 25 cm above ground level to assess for injury to the trunk. In some cases damage occurred only on one side of the trunk base. Death or damage of trunk base, as evident by dead phloem, vascular cambium, and in some cases primary xylem, was reported as completely damaged = 1.0; partially damaged = 0.5; not damaged = 0. Observations were also made regarding exudation of sap, rotting, sucker growth from the crown, and subsequent bud break. Parameters used for the assessment of frost damage, along with abbreviations and units, are listed in Table 2.

Included in the experimental plot area were also over 100 rootstocks grafted to approximately 20 different accessions of *A. chinensis* and *A. deliciosa.* Plants were grafted during July, 2018 at a height of approximately 15 to 35 cm. The same protocol was also employed to assess cold damage for this material, with the base of scion portion immediately above the graft considered for PBDD, rather than the rootstock base.

Table 2 List of parameters used to assess response to frost injury in young field-grown kiwifruit plants.

Parameter	Abbreviation	Unit	Remarks
Base Diameter	BD	mm	At ground level
Maximum Diameter Damaged	MDD	mm	Assessed visually using "knife test"
Percent of Base Diameter Damaged	PBDD	Percent	(MDD / BD) x 100
Percent Shoot Damaged	PSD	Percent	Assessed visually using "knife test"
Base Damage	DB	Binary (yes=1; no=0)	Assessed visually using "knife test"
Base Cracking	CB	Binary (yes=1; no=0)	Assessed visually using "knife test"
All assessments were made approximately five weeks after frost event.			

Statistical Analysis

All statistical analyses were performed using JMP software, Version 14.0, SAS Institute Inc., Cary, NC. As discussed earlier, three blocks were considered for analysis. Each block contained seven cultivar groups, with an average of five plants as subsamples per cultivar. The total number of plants included in the analysis was 113. Base diameter (BD), maximum diameter of shoot damaged (MDD), percent of base diameter damaged' (PBDD), and estimated percent shoot system damage (PSD) were treated as continuous response variables, while cracking base (CB) and damaged base (DB) were treated as binary (nominal) response variables.

Average base diameter (BD), maximum diameter of shoot damaged (MDD), percent of base diameter damaged (PBDD), and estimated percent shoot system damage (PSD) by cultivar response was analyzed by analysis of variance (ANOVA) at the 0.05 alpha level. Mean separation by cultivar was estimated using Tukey's HSD test. Likelihood and Pearson's Chi Square tests were used to compare effects of species and cultivar on nominal variables DB and CB. Frequency of DB and CB within individual cultivars and between species for comparison was done based on the frequency of affected individual plants for each cultivar or species.

The Student's t-test (0.05 alpha level) was used to compare the effect of propagation method (clonal and seedling) and species (*A. chinensis* and *A. deliciosa*) on BD, MDD, PBDD, PSD, DB, and CB. For species, the test was carried out for all cultivar groups (seedlings and clonal plants) and then separately for seedlings of each species exclusively.

Principle Component Analysis (PCA) on correlations was performed on six variables used to assess damage, along with four nominal / ordinal variables: species, propagation method, and cultivar. Determination of variable retention from PCA was based on eigenvalue of 1.0 or greater for non-rotated factors. Eigenvalue score plots were used to estimate the effect of vectors on total variance. Rotational factor analysis (Principle Components factoring method and Principle Components prior communality) with orthogonal Varimax and oblique Promax were used to potentially reduce the number of variables by grouping those with similar characteristics. For each rotation,

three factors were used. Factors that had a significant loading factor of <0.30 were considered non-significant.

Correlations between response variables were estimated using the Row-wise method. Correlation strengths were categorized based on correlation probability as * = P<0.05; ** = P<0.01; *** = P<0.001; **** = P<0.0001.

Results

Summary of Weather Data

On the morning of November 14, 2018 College Station was hit by an unusually hard frost. The minimum temperature of -4.1°C (12.8°C below average) served as the first frost of the season, tying a record low set in 1916. Weather conditions during the 60 days prior to the frost were comparatively normal to mild in relation to average. Only 25 of the 60 days prior to the frost recorded above average mean daily temperatures and 24 of the 60 days prior recorded above average daily maximum temperatures, with daily maximum and daily mean temperature averaging 23.°C and 1.4°C cooler compared to average, respectively (Figure 3).

The period before the November 13-14 frost was characterized by relatively mild night temperatures. 35 of the 60 days prior to the frost recorded above average minimum temperatures, with 35 of the 60 days prior being reporting below average daily lows and averaging 0.9°C above average for that period. More importantly, only seven days recorded minimum temperatures below 10°C during the 60-day period prior to the frost, with all but two of those days occurring during the week leading up to event (Figure 4).

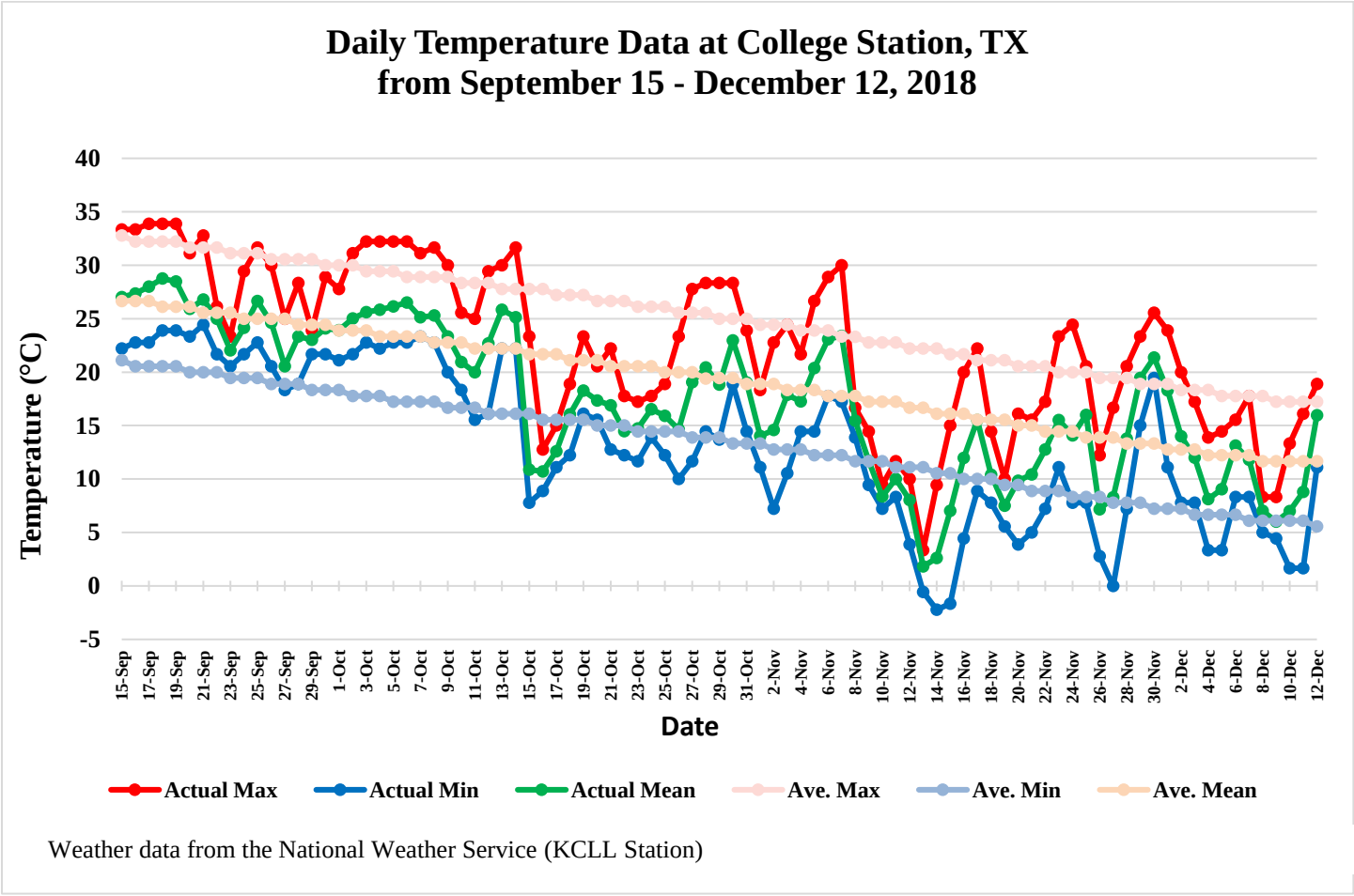

Figure 3 Daily temperature data at College Station, TX from September 15 – December 12, 2018 used in the assessment of young field-grown kiwifruit plants' response to frost injury.

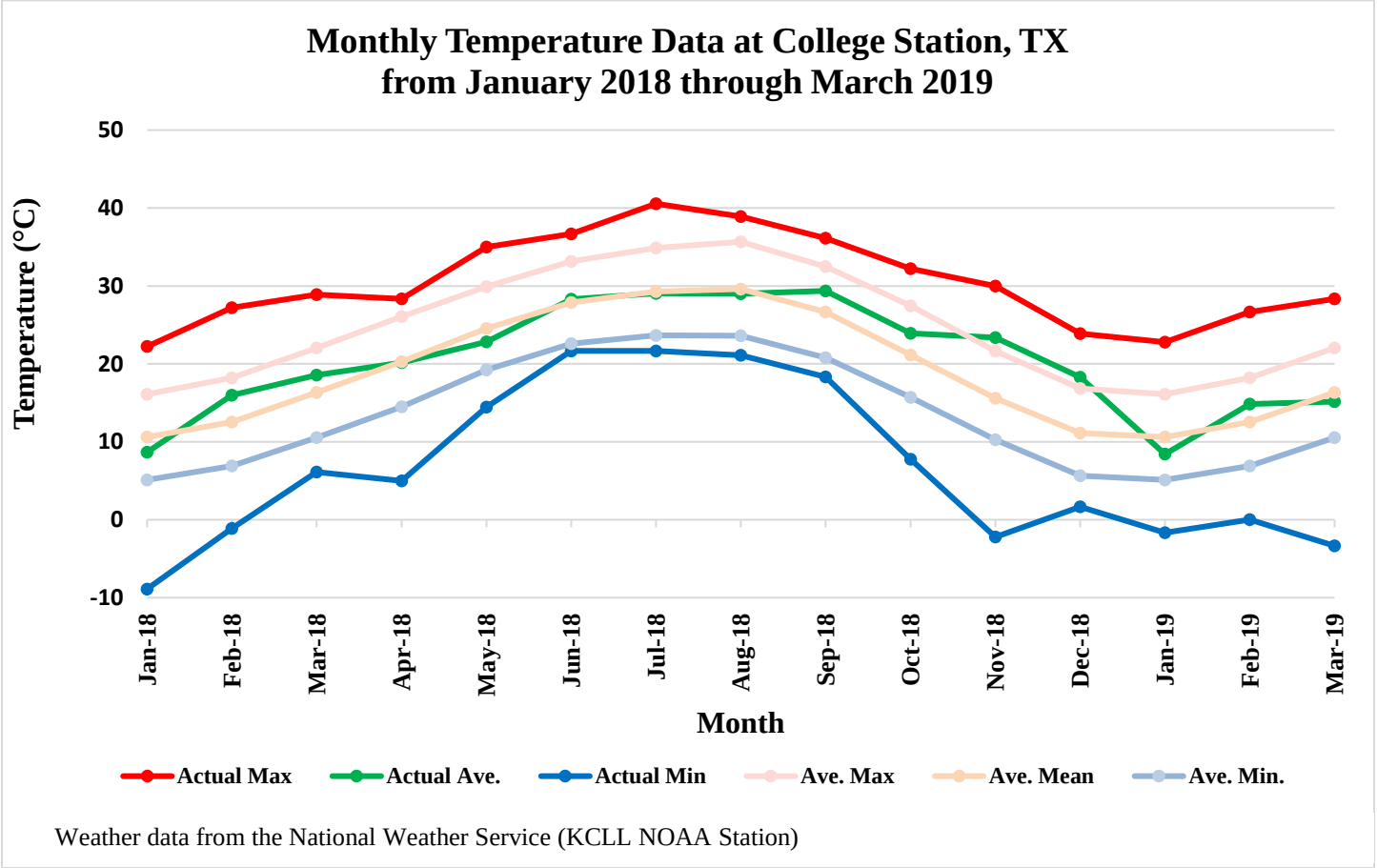

Figure 4 Monthly temperature data at College Station, TX from January 2018 through March 2019 used in the assessment of young field-grown kiwifruit plants' response to frost injury.

In fact, the coldest temperature recorded before the frost was 7.2°C, which occurred only 11 days prior. At the time the frost occurred, the nearby weather station at College Station had recorded a total of 28, 28, -27, 89 chill units for the 7°C, 0°C - 7°C, Utah Model, and Positive Utah Model, respectively. The fall of 2018 was also relatively wet, with 639 mm of rain falling during the 60-day period, compared to an average of 280.

Despite the generally mild temperature trend leading up to the frost, mean daily temperatures were well below (-6.5°C) average for the five days leading up to the frost, as a result of the passage of an earlier cold front. This likely played a role in allowing the temperature drop far below average on the nights of November 13-14 (Figure 3). At approximately 11:00 AM November 12, an advective air mass descended upon southeast Texas. Winds blew from the North-Northwest with gusts above 48 km/hour, with the temperature dropping to 14.1°C by midnight under cloudy skies. The advective conditions continued into the day on November 13 with approximately 55.9 mm of rain falling during the day, until wind speeds dropped below 16 km/hour by approximately 7:00 PM. The relatively calm conditions, along with clearing skies just after sunset, allowed for the transition to radiational cooling conditions, which allowed temperatures to drop rapidly along with relative humidity. By approximately 8:00 PM the temperature dropped below 0°C with wind speed approximately 14.4 km/hour. The temperature continued to decline before reaching a low of -4.1°C just after daybreak, which was recorded by a weather station located approximately 150 meters away (-2.8°C in College Station). Temperatures remained below freezing for a total of approximately 13 hours.

Wind speeds averaged 11.3 km/hour during this period. While a small amount of frost was observed on some leaf surfaces, the dew point remained approximately 1.7°C below the actual air temperature at the coldest point (Figure 3).

Calm sunny conditions allowed for maximum solar gain, causing the temperature to rise to a daytime high of 9.4°C later in the day November 14, with the average dew point remaining at -4.0°C. The dry, calm, and clear conditions prevailed into the night of November 14, with the temperature dropping below freezing again to a low of -1.7°C. Mean daily temperatures continued to trend below normal for the week following the November 13-14 frost by an average of -6.4 through the seven-day period (Figure 3).

The remainder of the 2018-2019 winter was relatively mild, in terms of frost. Ironically, temperatures did not drop below that of the November 13-14 frost until March 5 (-3.3°C at College Station), which proved to be the coldest temperature of the season (Figure 4). Winter chilling accumulation was relatively normal, with 706, 686, 554, and 1178 units (<7°C, 0°C -7°C, Utah, and Positive Utah Models, respectively).

Plant Observations

All surveyed plants were in an active state of growth at the time of the frost event, as evident by presence of new growth with expanding leaves. All foliage exhibited a dark water-soaked appearance, followed by rapid wilting and desiccation later in the day on November 14 after temperatures warmed. Within two days, knife-testing of affected wood revealed dark discolored phloem and cambial tissue. Longitudinal cracks in the bark on lower trunks became visible within one to two weeks

and continued to become more evident as the damaged and exposed phloem tissue continued to dry out.

Formal assessments of frost injury were made December 19 through December 22, 2018 (36 to 40 days after the frost event). By this time, frost-killed foliage had completely dried, but had not abscised. Damage to the above-ground shoot portions of plants appeared in two distinct patterns: 1.) beginning from the smaller-diameter distal shoots progressing downward toward the trunk; 2.) at the basal portion of the trunk, but not necessarily in conjunction with damage to the more distal shoots. Typical symptoms associated with the observed frost damage are depicted in Figure 5.

Figure 5 Damaged 'CK-3' shoot revealed by "knife test" with dark discolored phloem, vascular cambial, and primary xylem tissue and necrotic lateral bud (left). Primary shoot exhibiting basipetal injury trend with undamaged lateral buds in the apical region attempting to resume growth (right).

Base Diameter and Maximum Diameter Damaged

Base Diameter by Cultivar

Base diameter (BD) (mm) was surveyed at ground level as a reference of plant vigor and to provide a baseline for estimating PBDD. BD varied significantly among cultivars (P<0.0001), with individual plants ranging from 6.8 mm to 25.5 mm. Mean BD separated into three significantly distinct groups with increasing diameter based on Tukey's HSD: 1). 'CK-3' / 'AU Golden Sunshine'; 2.) 'AU Golden Dragon' / Zespri Gold™ seedling/ 'AU Authur'; 3.) 'Hayward' seedling / 'AU Fitzgerald' (Figures 6 & 8).

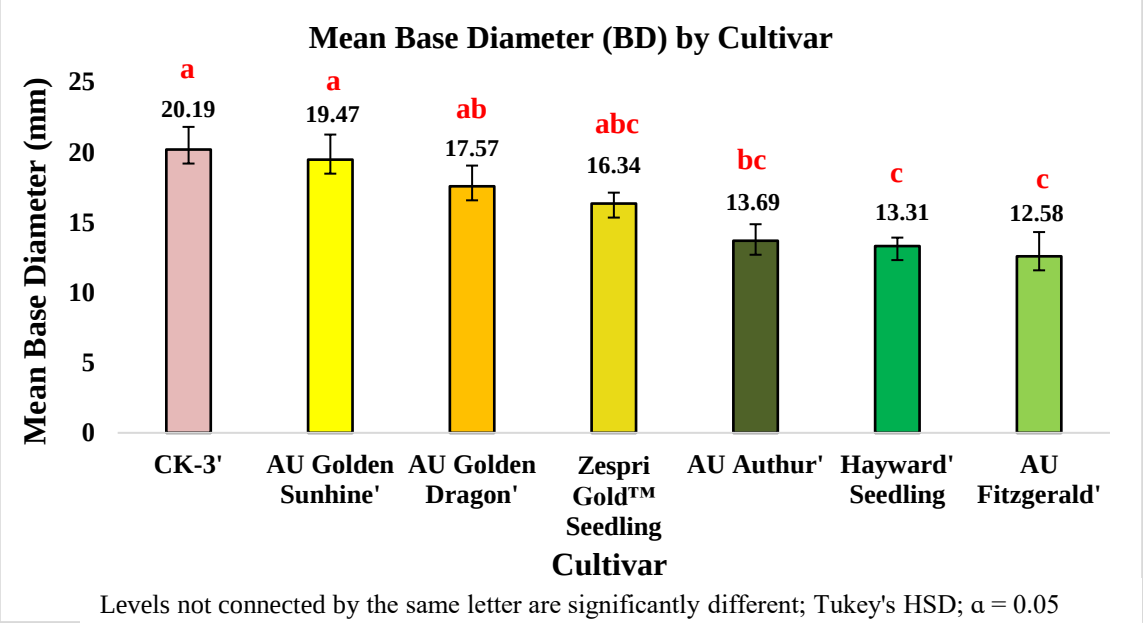

Figure 6 Mean base diameter (BD) by cultivar assessed in the response of young field-grown kiwifruit plants to fall frost.

Maximum Diameter Damaged by Cultivar

Maximum diameter of shoot system damage (MDD) was recorded on the largest shoot exhibiting damage following the removal of the bark and a thin slice of wood with a sharp knife. Damage was evident by dark discolored or brown dehydrated phloem, vascular cambium, and xylem. MDD response to cultivar was highly significant ($P<0.0001$), with values for individual plants ranging from 0 mm to 25.5 mm. Cultivar means separated into three groups for MDD based on Tukey's HSD from highest to lowest diameter (mm) damaged: 1.) 'CK-3'; 2.) 'AU Authur / 'AU Fitzgerald' / 'AU Golden Dragon' / 'Hayward' seedling / 'AU Golden Sunshine'; 3.) Zepsri Gold™ seedling (Figures 7 & 8). There was a significant ($p = 0.0301$) block effect for MDD (data not shown).

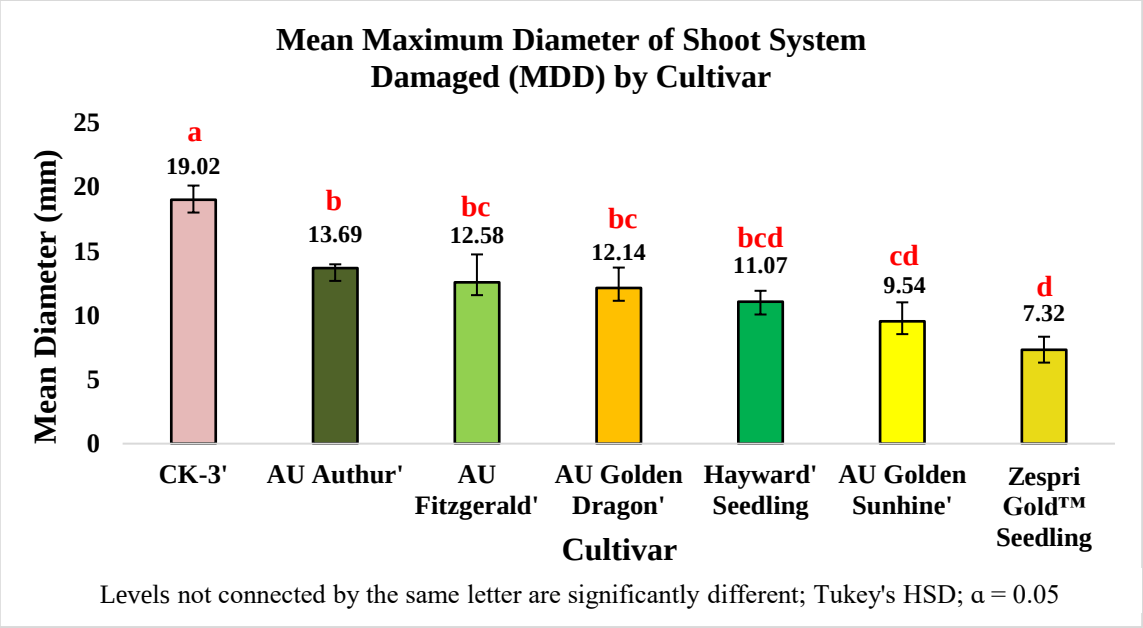

Figure 7 Mean maximum diameter of shoot system damaged (MDD) by cultivar assessed in the response of young field-grown kiwifruit plants to fall frost.

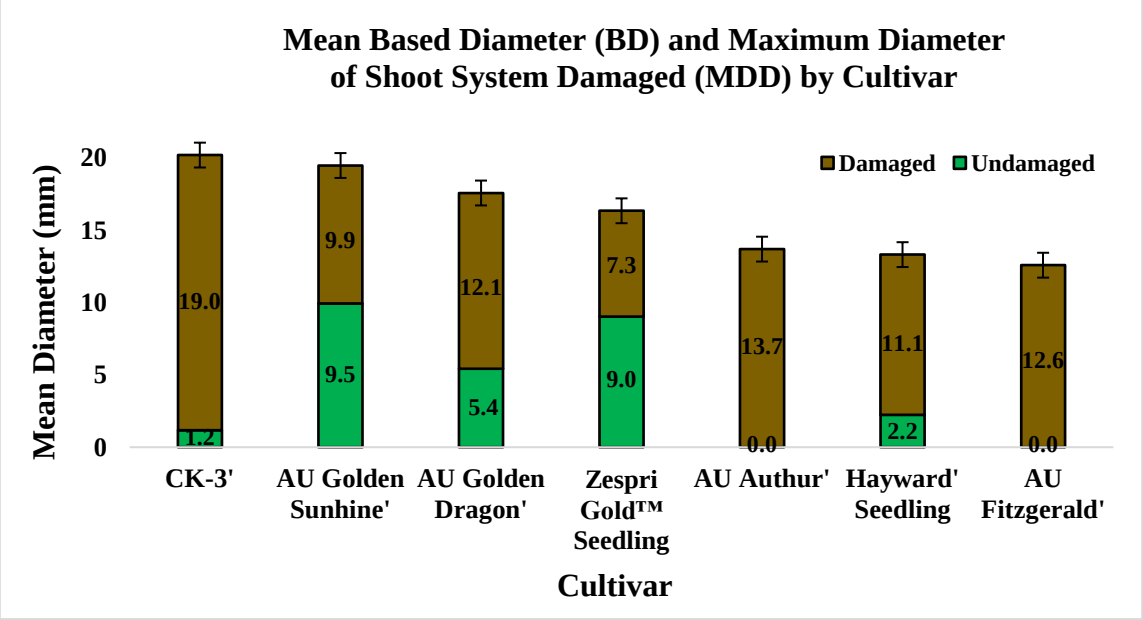

Figure 8 Mean maximum diameter of shoot system damaged (MDD) in relation to mean base diameter (BD) by cultivar assessed in the response of young field-grown kiwifruit plants to fall frost.

Base Diameter and Maximum Diameter Damaged by Species

On a species level, *A. chinensis* plants exhibited 39% greater BD (P<0.0001) than *A. deliciosa*, when all cultivars were considered. In the case of seedlings, the difference was smaller, but still significant (p = 0.02), with Zespri Gold™ seedlings averaging 23% greater BD than 'Hayward' seedlings. MDD showed no significant response to species, whether among all cultivars or between seedling groups of each species. 'Hayward' seedlings had an average of 11.1 mm maximum diameter shoot damaged, whereas Zepsri Gold™ had only 7.3 mm (Figures 9 & 10).

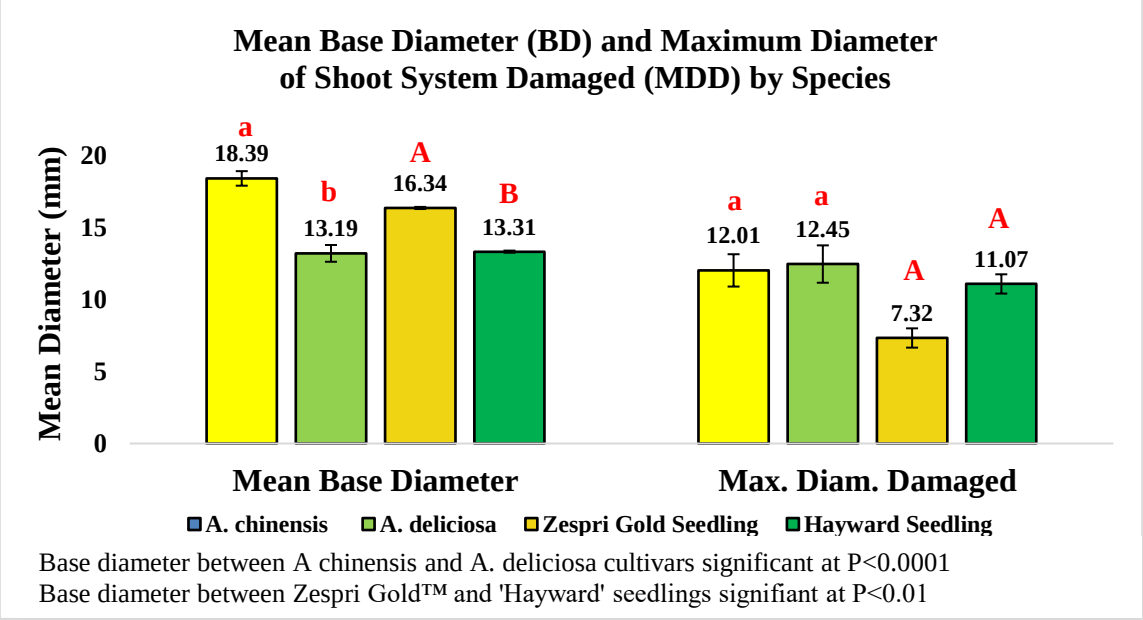

Figure 9 Mean base diameter (BD) and maximum diameter of shoot system damaged (MDD) (non-significant) by species assessed in the response of young field-grown kiwifruit plants fall frost.

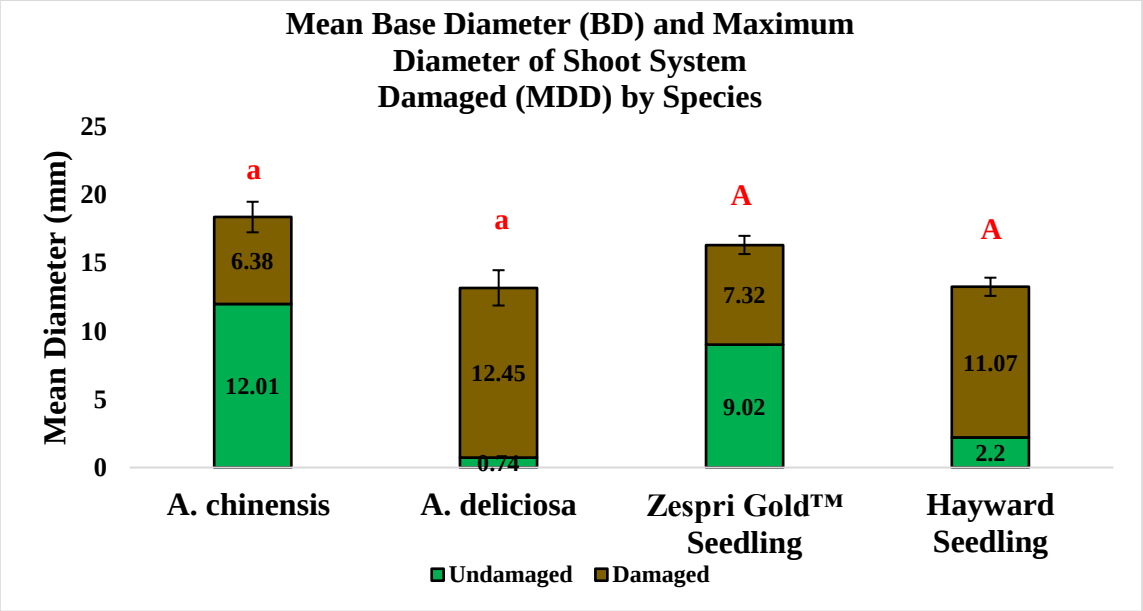

Figure 10 Mean maximum diameter of shoot system damaged (MDD) (non-significant) in relation to mean base diameter (BD) by species assessed in the response of young field-grown kiwifruit plants to fall frost.

Base Diameter and Maximum Diameter Damaged by Propagation Method

Interestingly, clonal and seedling plants were not significantly different in size for this experiment. However, method of propagation did have a significant ($p = 0.018$) effect on MDD (Figures 11 & 12), with the maximum diameter damaged for clonal material averaging 13.4 mm and seedlings averaging 9.2 mm (data not shown).

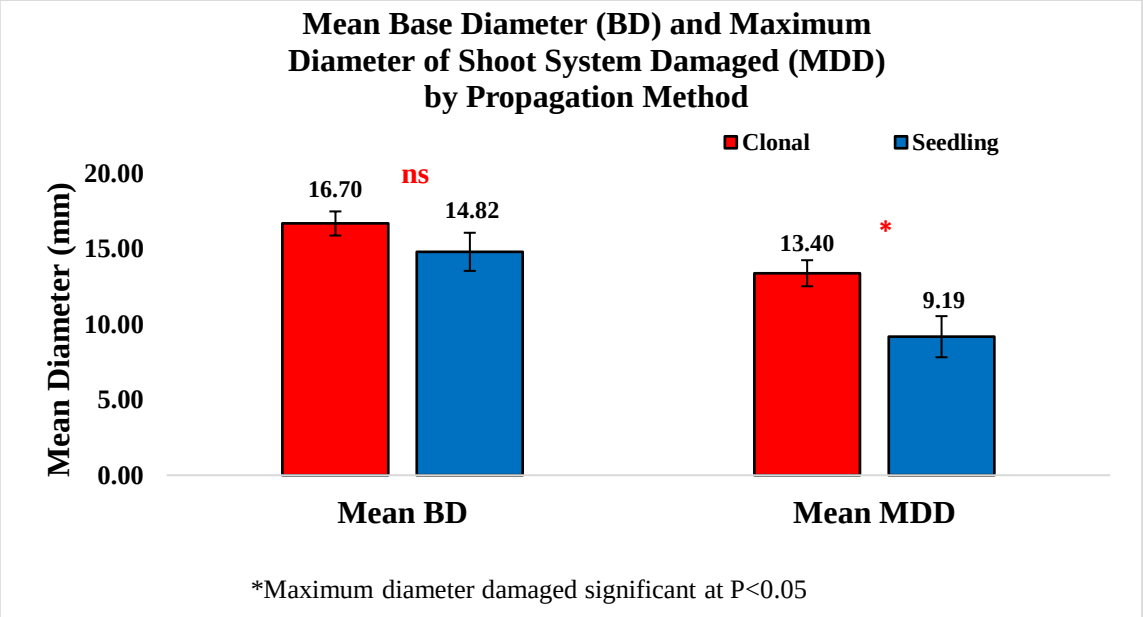

Figure 11 Mean base diameter (BD) (non-significant) and maximum diameter of shoot system damaged (MDD) by propagation method assessed in the response of young field-grown kiwifruit plants to fall frost.

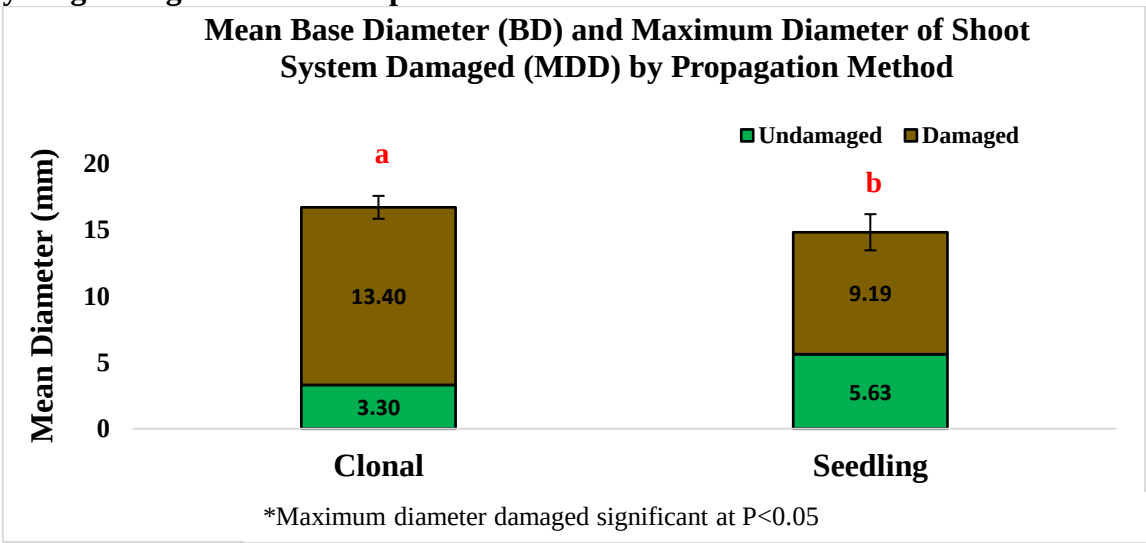

Figure 12 Mean maximum diameter of shoot system damaged (BDD) in relation to mean base diameter (BD) by propagation method assessed in the response of young field-grown kiwifruit plants to fall frost.

Percent of Base Diameter Damaged and Percent Shoot Damage

Percent of Base Diameter Damaged by Cultivar

Percent of based diameter damaged (PBDD) was calculated as the MDD relative to base diameter and expressed as a percentage. There was a highly significant ($P<0.0001$) response of PBDD to cultivar. Individually, plants varied from having 0% PBDD to 100%, as approximately 50% of surveyed plants were frozen completely to the ground (100% PBDD). Statistically, cultivar means separated into three groups. 'AU Authur, 'AU Fitzgerald', 'CK-3', and 'Hayward' seedlings sustained the most damage, 'AU Golden Dragon' had intermediate PBDD, and 'AU Golden Sunshine' and Zespri Gold™ seedlings sustained the least amount, based on Tukey's HSD (Figure 13).

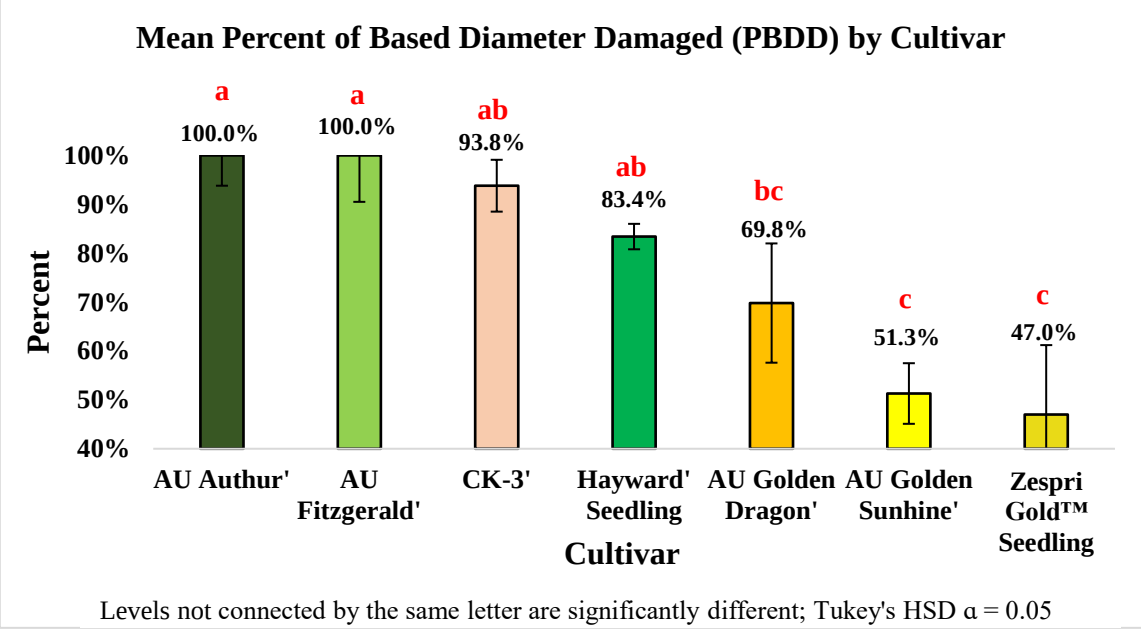

Figure 13 Mean percent of base diameter damaged (PBDD) by cultivar assessed in the response of young field-grown kiwifruit plants to fall frost.

Percent Shoot Damage by Cultivar

For plants exhibiting shoot damage in the apical or peripheral region of the canopy, injury was more severe in the in the smaller diameter wood. Removal of the bark and a thin slice of wood with a sharp knife ("knife test") revealed that the phloem, vascular cambium, and young xylem was completely dead, as evident by browning and dehydration. Injury to small (<9 mm) and larger (>9mm) shoots was irregular in some plants, with some shoots remaining unharmed and others completely killed. In most cases the previously described damage to the vascular tissue occurred in a symmetrical pattern, with necrosis completely encircling the circumference of the stem. Percent Shoot Damage (PSD) varied greatly by cultivar ($p = 0.0047$), with average percent shoot damage ranging from 79.2% for 'AU Authur' to only 19% for Zespri Gold™ seedlings. In fact, individual plants of these cultivars exhibited responses that ranged from 100% to 0% shoot damage, respectively. Separation of treatment means by Tukey's HSD partitioned cultivars into two different groups: 'AU Authur' / 'AU Fitzgerald' and 'AU Golden Sunshine' / Zespri Gold™ seedlings (Figure 14). Block effect was also significant ($p = 0.016$), as the first replication reported approximately 27% less PSD than the other two reps.

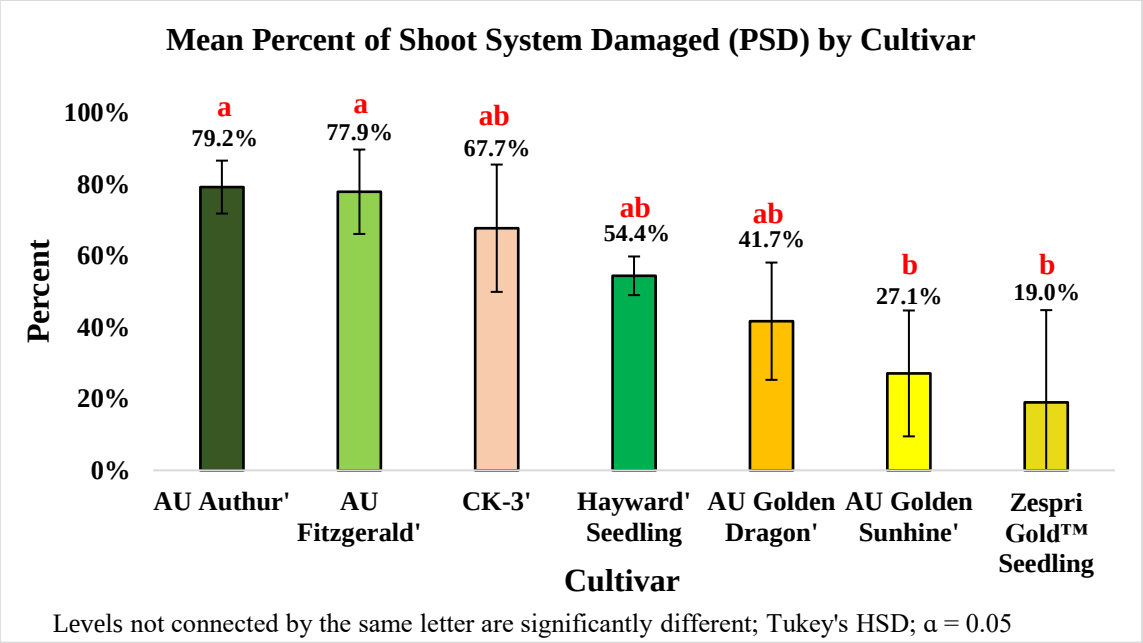

Figure 14 Mean percent of shoot system damaged (PSD) by cultivar assessed in the response of young field-grown kiwifruit plants to fall frost.

Percent of Base Diameter Damaged and Percent Shoot Damage by Species

There was a significant (p = 0.0018) response of PBDD by species. On average, *A. deliciosa* (PBDD = 94.5%) suffered 29% more damage as compared to *A. chinensis* (PBDD = 65.5%), when all cultivars were considered. This difference was even greater (36.4% margin) when only seedlings of each species were considered (p = 0.0306). *A. deliciosa* ('Hayward' seedlings) had a PBDD of 83.4%, whereas *A. chinensis* (Zespri Gold™ seedlings) sustained only 47.0% damage (Figure 15).

PSD also exhibited a significant response to species. When all cultivar groups were analyzed based on species, *A. chinensis* cultivars averaged 38.9% shoot damage, as compared to 70.5% for *A. deliciosa* (p = 0.01). When only seedlings were considered,

Zespri Gold™ sustained 19.0% shoot damage, whereas ‘Hayward’ seedlings averaged 54.4% damage (286% higher PSD) ($p = 0.04$) (Figure 15).

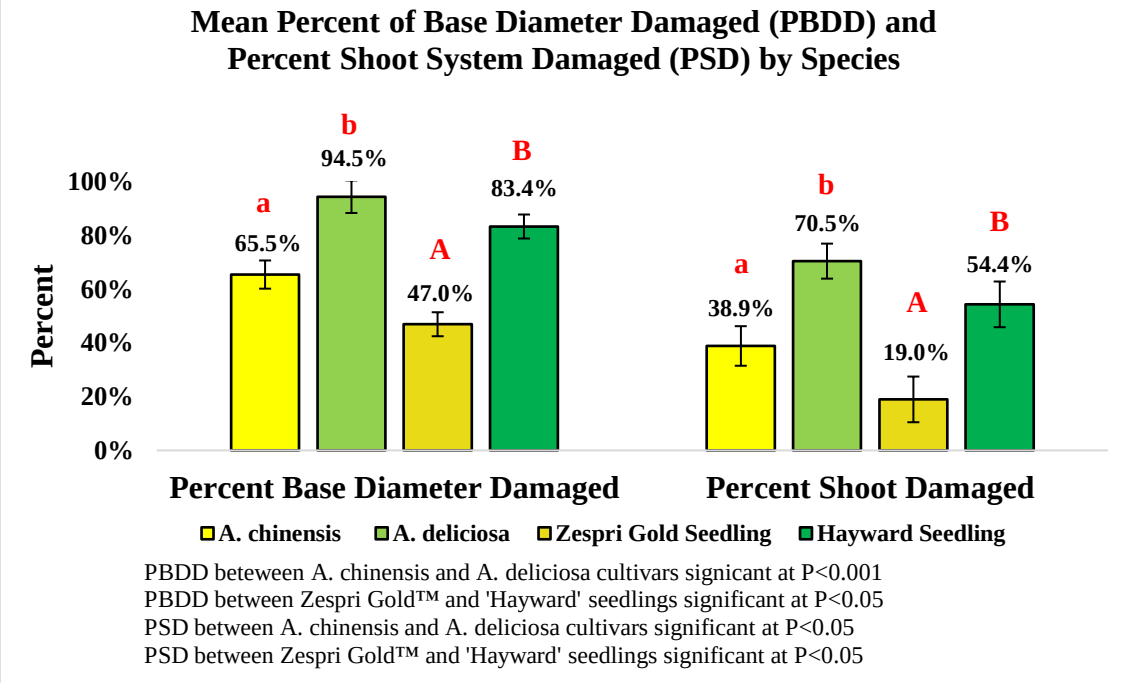

Figure 15 Mean percent of base diameter damaged (PBDD) and mean percent shoot system damaged (PSD) by species assessed in the response of young field-grown kiwifruit plants to fall frost.

Percent of Base Diameter Damaged and Percent Shoot Damage by Propagation Method

Propagation method had no significant effect on frost damage (PBDD or PSD), once plant size was accounted for (Figure 16).

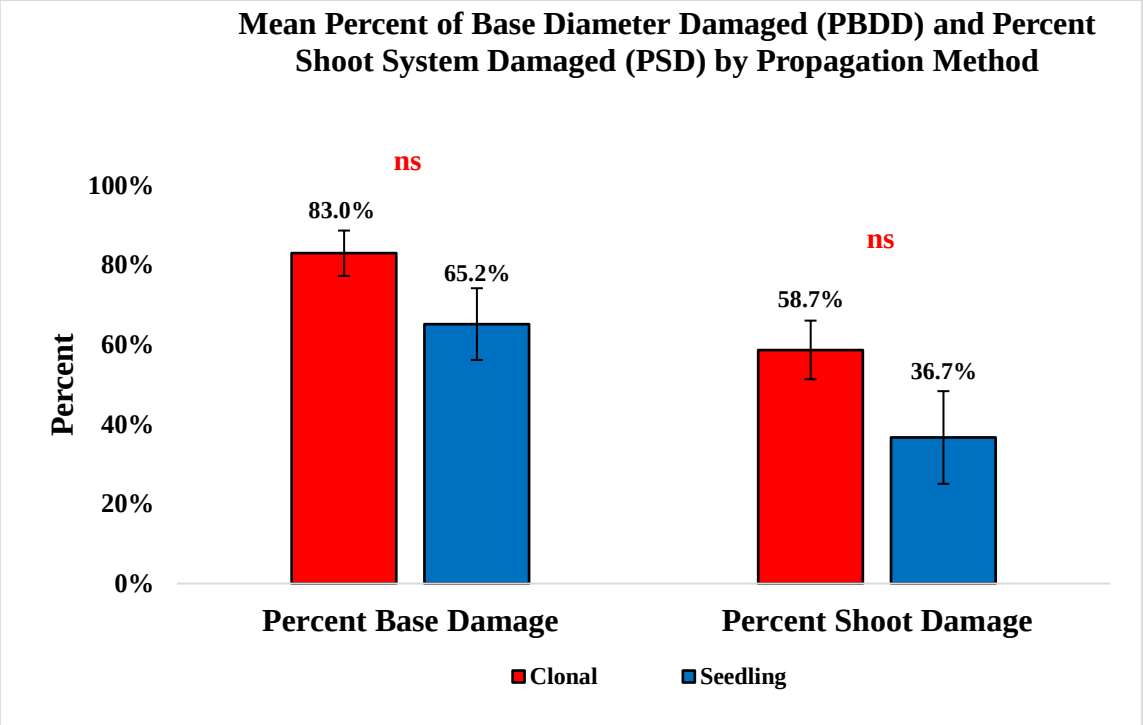

Figure 16 Mean percent of base diameter damaged (PBDD) and mean percent shoot system damaged (PSD) by propagation method (non-significant) assessed in the response of young field-grown kiwifruit plants to fall frost.

Basal Damage and Basal Cracking

Most plants generally exhibited a basipetal pattern of injury, with more apical or smaller-diameter shoots being more severely affected. However, damage that was confined to the basal portion of the main shoot was also observed, appearing seemingly independent of damage in the apical shoot portion (Figure 17). As discussed earlier, the frequency of damage to the basal region of the trunk was recorded in a binary manner. BD appeared to have a strong effect on the prevalence of base damage (DB), as evident

by a high Chi-Square value (p = 0.0006). In general, the frequency of DB tended to increase with larger plants (BD).

Vertical cracks in the bark of the basal portion of the trunk on some plants became evident within a few weeks and continued to increase in size and number as time went on (Figure 18). Base cracking (CB) appeared to trend strongly DB. As with DB, cracking was related to base diameter (Chi-Square P<0.0001).

Figure 17 Hayward' seedling with damage, as evident by sloughing bark and dark discolored primary xylem restricted to basal portion of primary shoot with apparent healthy shoot tissue above (left). Injury to basal region of 'CK-3' plant, as evident by dark discolored phloem, cambial, and primary xylem tissue (right).

Figure 18 ‘AU Authur’ plant exhibiting extensive vertical cracking of basal bark (left). ‘AU Fitzgerald’ plant with vertical cracking extending up primary shoot (right).

Incidence of Basal Damage by Cultivar

Frequency of DB varied widely by cultivar ($P<0.0001$), with cultivar means ranging between 0 and 1.0. Means separation identified four statistical groups: 1.) ‘AU Authur’ (1.00), ‘AU Fitzgerald’ (1.00), ‘CK-3’ (0.71), and ‘Hayward’ seedling’ (0.70); 2.) ‘AU Golden Dragon (0.56); 3.) ‘AU Golden Sunshine’ (0.17); 4.) Zespri Gold™ seedling (0.00) (Figure 19).

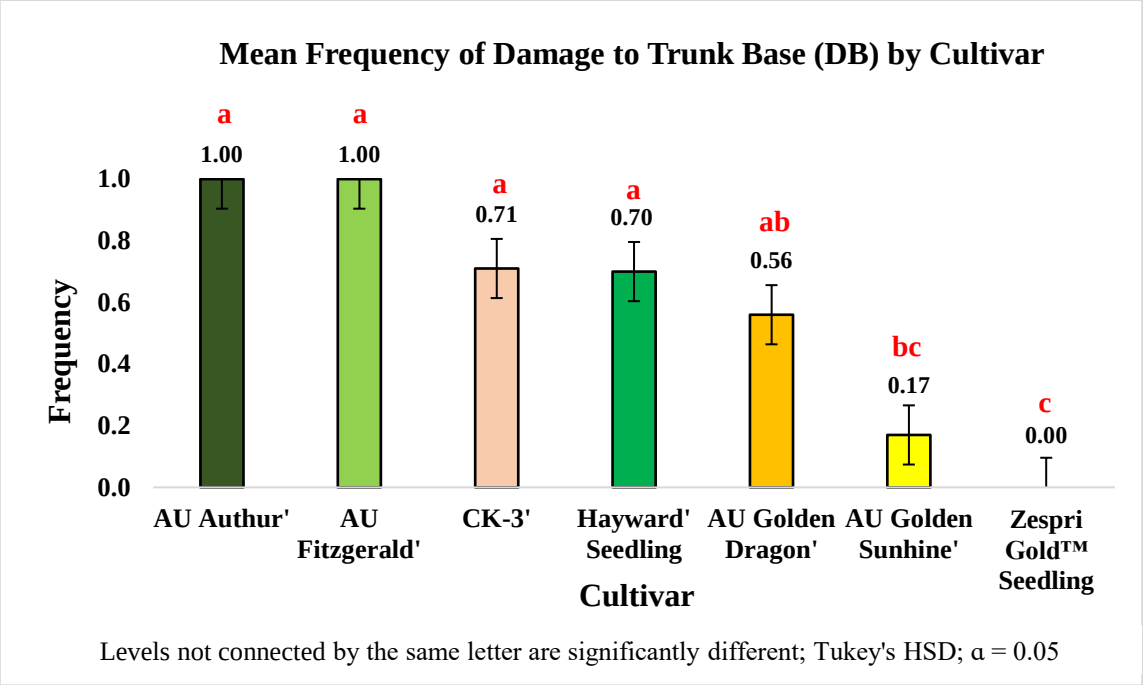

Figure 19 Mean frequency of damage to trunk base (DB) by cultivar assessed in the response of young field-grown kiwifruit plants to fall frost.

Incidence of Basal Cracking by Cultivar

Like DB, frequency of CB was strongly ($P<0.0001$) responsive to cultivar. Treatments consisted of four statistical groups: 1.) 'AU Authur' (1.00) and 'AU Fitzgerald' (0.96); 2.) 'Hayward' seedling (0.67); 3.) 'CK-3' (0.29) and 'AU Golden Sunshine' (0.25); 4.) 'AU Golden Dragon' (0.06) and Zespri Gold™ seedling (0.00) (Figure 20).

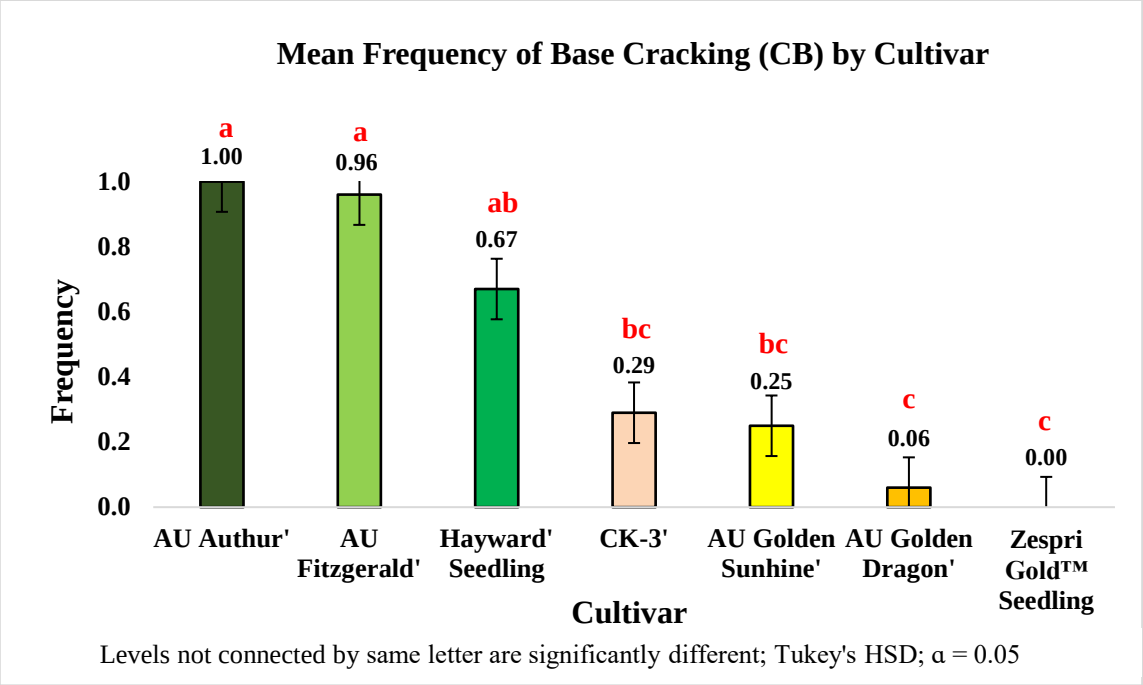

Figure 20 Mean frequency of base cracking (CB) by cultivar assessed in the response of young field-grown kiwifruit plants to fall frost.

Incidence of Basal Damage and Basal Cracking by Species

Not surprisingly, frequency of basal damage was much higher (P<0.0001) for *A. deliciosa* plants (0.82) (all cultivars included), as compared to *A. chinensis* cultivars (0.32). This trend was also significant (p = 0.017) for seedlings of each species. As mentioned earlier none of the observed Zespri Gold™ seedlings exhibited any damage to trunk bases, whereas this frequency was 0.67 for 'Hayward' seedlings. The propensity for basal damage appeared to be even higher among the two A. deliciosa clonally-propagated cultivars, with 100% of both 'AU Authur' and 'AU Fitzgerald' suffering severe damage or death to the trunk base (Figure 21).

CB was also strongly associated with species (p = 0.005), with *A. deliciosa* plants (all cultivars) exhibiting a frequency of 0.79, as compared to 0.11 for *A. chinensis* plants (all cultivars). While only 44.3% of individual plants showed visible cracking, 88% of those that exhibited cracking were *A. deliciosa* (78.6% within the species) and only 12% were *A. chinensis* (10.5% within species), for all cultivars considered. Within the seedling subcategory, none of the Zespri Gold™ seedlings displayed symptoms of cracking, as compared to 63.3% of 'Hayward' seedlings (p = 0.0285) (Figure 21).

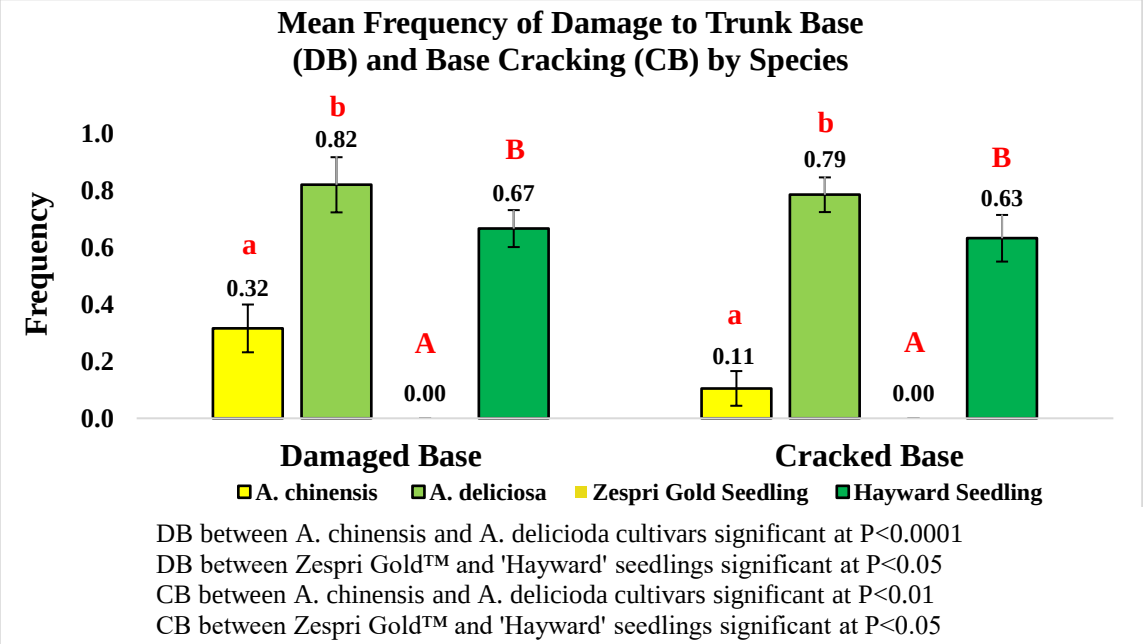

Figure 21 Mean frequency of damage to base (DB) and base cracking (CB) by species assessed in the response of young field-grown kiwifruit plants to fall frost.

Incidence of Basal Damage and Basal Cracking by Propagation Method

Propagation method had no significant effect on DB, despite the fact that clonal plants exhibited a DB frequency of 0.69 as compared to 0.35 for seedlings. Among clonal cultivars, damage to 'CK-3' exhibited a unique pattern, as damage tended to be associated with pruning wounds and other areas that had previously callused over. This difference was smaller (0.51 for clonal and 0.34 for seedling) in the case of CB and non-significant (Figure 22).

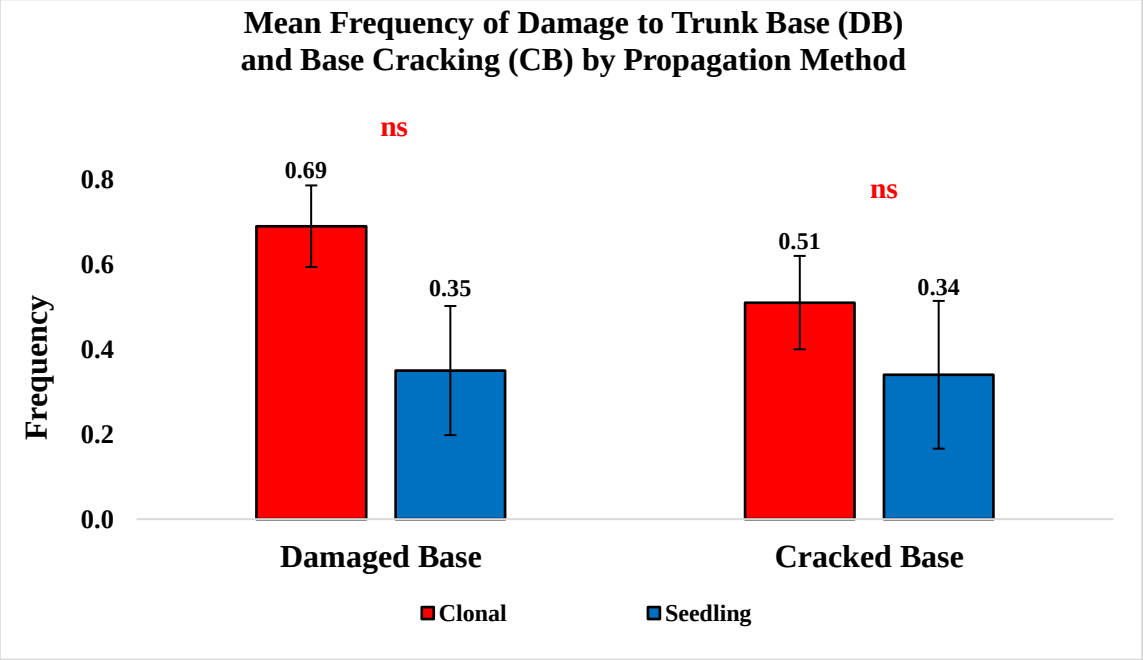

Figure 22 Mean frequency of damage to base (DB) and base cracking (CB) by propagation method (non-significant) assessed in the response of young field-grown kiwifruit plants to fall frost.

Additional Observations

It is also noteworthy that a substantial number of ‘AU Golden Dragon’, and to a lesser degree, ‘AU Golden Sunshine’ lacked the typical frozen phloem, but showed a thin dark discolored line between the phloem and primary xylem. It was surmised that frost damage was limited in this case to the vascular cambium and not the phloem, due to lower solute concentrations and a resulting higher freezing point within this tissue, as compared to the more outer-lying and more exposed phloem (Figure 26). Such damage was accounted for when considering MDD, PBDD, and PSD. As it turned out, plants displaying these unique symptoms initially resumed shoot growth, but ultimately wilted and died in late-spring, likely due to their inability to develop new xylem tissue as a result of the damaged or destroyed vascular cambium.

Figure 23 'AU Golden Sunshine' (left) and 'AU Golden Dragon' (right) shoots exhibiting apparent damage limited to vascular cambium, as evident by dark discoloration between xylem and phloem layers in the assessment of young field-grown kiwifruit plants' response to frost injury.

In addition to the replicated planting, observations of damage were also made on other material. This included a total of 16 *A. chinensis* and *A. deliciosa* cultivars, which had been grafted earlier in June 2018 (total of approximately 60 grafted plants), various seedlings and own-rooted cultivars of both species, and seven own-rooted *A. arguta* cultivars in an adjacent vineyard planting. All of these plants were approximately the same age and had received similar irrigation and nutritional management. Comparison of *A. chinensis* and *A. deliciosa* material yielded similar observations, while all but two of the plants grafted to *A. chinensis* accessions ('Bliss Red' and 'El Dorado') were killed below the graft union. Interestingly, all of the seven named *A. arguta* cultivars exhibited

death or similar damage to the phloem and cambial tissue that completely encircled the trunk bases. This severe damage to what is generally considered a cold-hardy species in Texas, emphasized the earliness and intensity of the frost event.

Correlations

The Row-wise method was used to estimate correlations between individual variables. The following correlations were considered significant, based on their probability ($P<0.05$). PBDD was negatively (-0.47) correlated with BD, but positively and strongly correlated with MDD (0.75), CB (0.76), DB (0.94), and PSD (0.92). Similarly, PSD was positively and strongly correlated with MDD (0.70), CB (0.71), and DB (0.90). CB was negatively correlated with BD (-0.63), but positively and strongly correlated and DB (0.77). Following a similar pattern, BD was negatively correlated with BD (-0.53), but positively correlated with MDD (0.63) (Table 3).

Table 3 Correlation coefficients among six parameters assessed in the response of young field-grown kiwifruit plants to fall frost.

	BD	MDD	PBDD	CB	DB	PSD
Base Diameter	1.00	0.21ns[a]	-0.47*	-0.63**	-0.53*	-0.41ns[a]
Maximum Diameter Damaged	0.21ns[a]	1.00	0.75****	0.33ns[a]	0.63**	0.70***
Percent of Base Diameter Damaged	-0.47*	0.75****	1.00	0.76****	0.94****	0.92****
Base Cracking	-0.63**	0.33ns[a]	0.76****	1.00	0.77****	0.71***
Base Damage	-0.53*	0.63**	0.94****	0.77****	1.00	0.90****
Percent Shoot Damaged	-0.41ns[a]	0.70***	0.92****	0.71***	0.90****	1.00

[a] Non-significant (P≥0.05); * Significant at P<0.05; ** Significant at P<0.01;
*** Significant at P<0.001; **** Significant at P<0.0001

Principle Component Analysis

Principle component analysis (PCA) was used to estimate relationships among responses to frost damage. PCA 1 and PCA 2 had Eigenvalues of 4.2 and 1.3, respectively, and together accounted for approximately 92.5% of the total variance associated with all six variables. PCA 3-6 collectively accounted for a total of only 7.5% of the total variance and had Eigenvalues less than 1.0, therefore were not further considered (Figures 24 and 25).

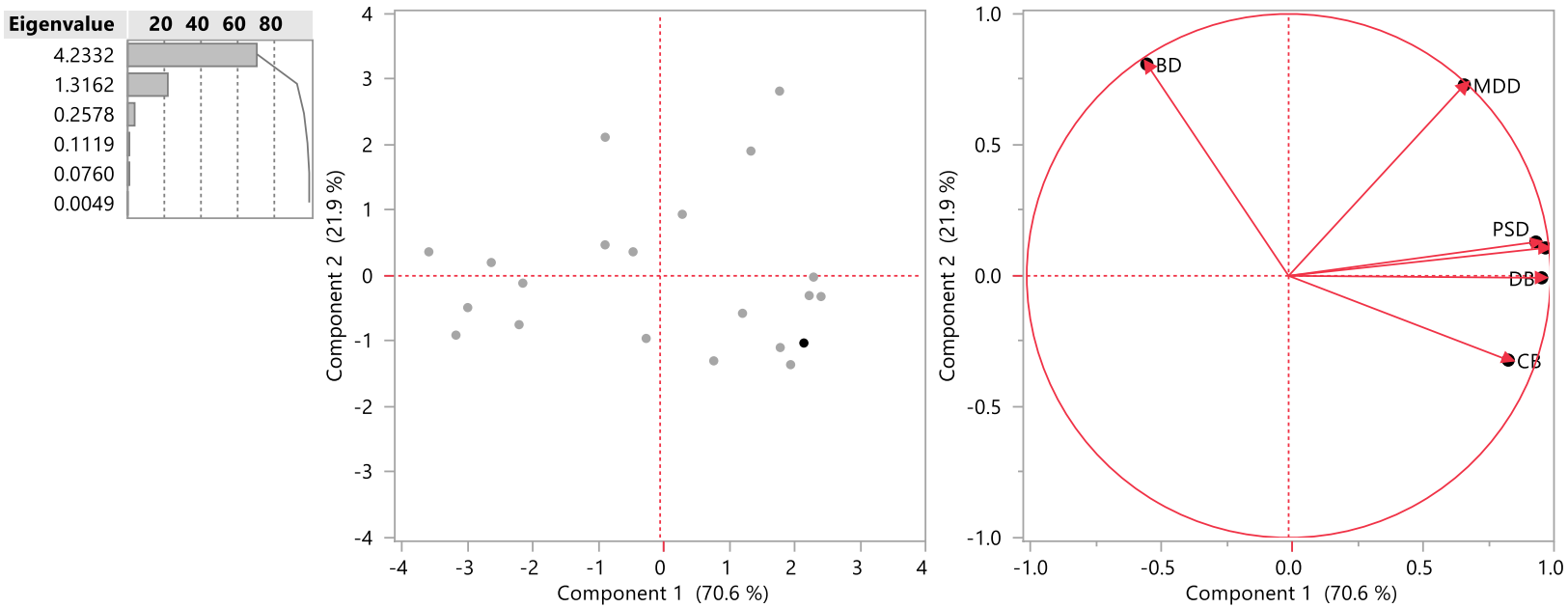

Figure 24 Principle component analysis on correlations with eigenvalues showing summary plot and score plot for PCA 1 and PCA 2 in the assessment of young field-grown kiwifruit plants response to fall frost.

Loading Matrix

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
BD	-0.54150	0.80846	0.20137	0.06052	0.09110	0.02571
MDD	0.67090	0.72865	-0.00245	-0.09988	-0.08656	-0.03849
PBDD	0.98053	0.10649	-0.07191	-0.11571	-0.07732	0.05171
CB	0.83864	-0.32187	0.43922	0.00331	-0.01103	-0.00507
DB	0.96625	-0.00767	-0.09731	-0.06058	0.23044	-0.00744
PSD	0.94415	0.12907	-0.09863	0.28491	-0.03197	0.00052

Partial Contribution of Variables

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6
BD	6.92669	49.65865	15.72680	3.27351	10.91770	13.49664
MDD	10.63291	40.33845	0.00233	8.91621	9.85691	30.25319
PBDD	22.71231	0.86158	2.00549	11.96653	7.86552	54.58857
CB	16.61461	7.87123	74.81951	0.00979	0.16014	0.52472
DB	22.05548	0.00447	3.67280	3.28067	69.85522	1.13136
PSD	21.05801	1.26561	3.77307	72.55328	1.34451	0.00552

Plot of Partial Contribution of Variables

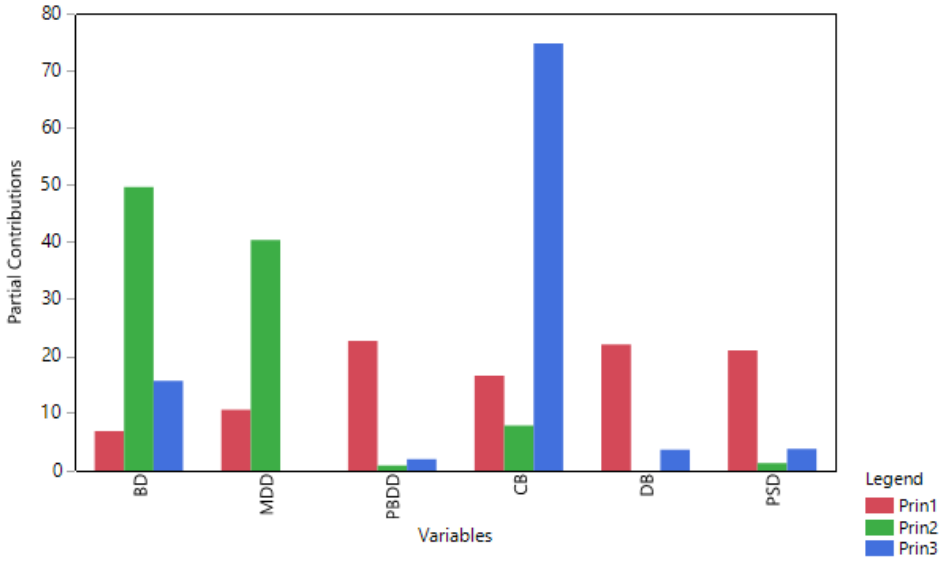

Figure 25 Loading matrix, partial contribution of variables, and plot of partial contributions of six variables used in the assessment of young field-grown kiwifruit plants response to fall frost.

PCA 1 was positively associated with MDD, PBDD, PSD, DB, and CB, while only BD was negatively connected with PCA 1. BD, MDD, PBDD, and PSD were positively associated with PCA 2, while CB was negatively associated and DB had a weak or neutral association with PCA 2 (Figure 24). Partial contribution of variables reported that PCA 1 was comprised of PBDD (22.7%); DB (22.1%); PSD (21.1%); CB (16.6%); MDD (10.6%); BD (6.9%), while PCA 2 was comprised of BD (49.7%); MDD (40.3%); CB (7.9%); PSD (1.3%); PBDD (0.9%).

Rotational factor analysis using Principle Component / Varimax reported that only BD had a significant loading factor of < 0.30, whereas the non-rotated loading matrix included all six variables. Neither Factor 2 nor Factor 3 suggested the removal of any variables relative to the non-rotated loading matrix for Factor 3 (data not shown). Rotational factor analysis using Principle Component / Promax reported that BD and CB could be removed from Factor 1, based on significant loading factors of < 0.30. Rotated Factor 2 suggested that CB could also be removed, while Factor 3 did not suggest the removal of any variables from the non-rotated analysis (data not shown). However, considering that a total of only six variables were included in the original analysis and that all variables appeared to contribute to the loading matrix (Figure 24), the decision was made to not remove any variables.

Based on the score plot for PCA 1 and PCA 2, the cultivar mean for ‘CK-3’and mean for clonal propagation were positively associated with both PCA’s. Cultivar means for ‘AU Golden Dragon’, ‘AU Golden Sunshine’, and the species mean for *A. chinensis* had a negative association with PCA 1 and a positive association with PCA 2. Cultivar

mean for Zespri Gold™ seedling and mean for seedling were negatively associated with both PCA 1 and PCA 2. Cultivar means for ‘AU Authur’, ‘AU Fitzgerald’, and ‘Hayward’ seedling were all clustered together along with the species mean for *A. deliciosa*—showing a positive association with PCA 1 and negative association with PCA 2 (Figure 26).

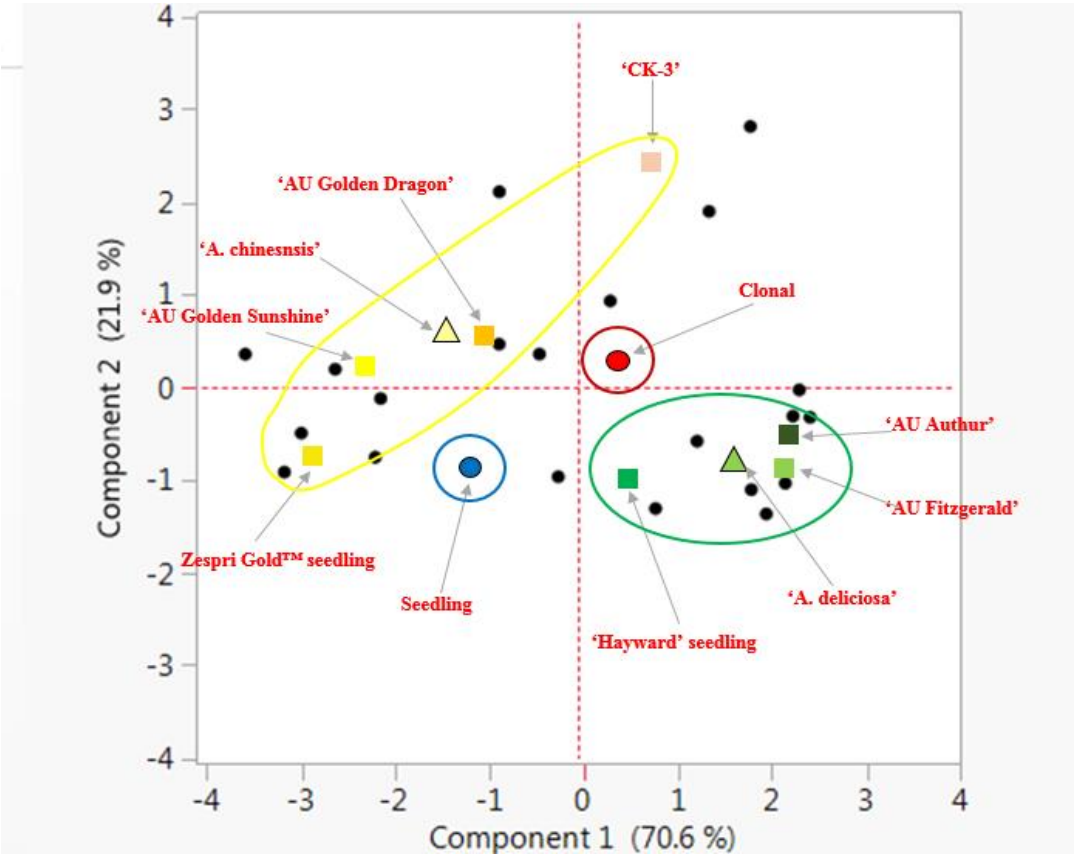

Figure 26 Principle components analysis on correlations score plot showing means for cultivar, propagation method, and species, relative to PCA 1 and PCA 2 used in the assessment of young field-grown kiwifruit plants response to fall frost with clustering indicating mean scores for *A. chinensis* and respective cultivars in yellow, *A. deliciosa* and respective cultivars in green, seedling in blue, and clonal propagation means in red.

Discussion

Base Diameter and Maximum Diameter Damaged

Base diameter (BD) varied considerably, with the largest group consisting of two *A. chinensis* cultivars, while smallest consisted of *A. deliciosa* cultivars, with the intermediate group including 'AU Golden Dragon', Zespri Gold™ seedling, and 'AU Authur'. Interestingly, both female cultivars ('AU Golden Dragon' and 'AU Fitzgerald') in this study were smaller on average than their respective male pollinizers ('CK-3' and 'AU Authur'), confirming that male plants tend to be more vigorous, as reported by Ferguson (1991). BD was higher for *A. chinensis* than for *A. deliciosa*, both among all cultivars ($P<0.0001$) and seedlings ($P = 0.02$), suggesting a clear tendency for greater vigor within the golden kiwifruit species. This trend seems logical when the natural geographic range of both species is considered, as *A. chinensis* tends to be found in more coastal regions and at lower-elevations. The fact that there was no significant difference for BD with respect to propagation method suggests that there is no inherent difference in vigor between clonally and seed propagated kiwifruit plants, at least within this study.

Maximum diameter of shoot damage (MDD) served to provide a crude quantifiable record of shoot damage—irrespective of actual plant size. Damage was highly variable by cultivar, as evident by an observed range of f 0 mm to 25 mm among individual plants. On average, 'CK-3' exhibited the greatest damage, Zepsri Gold™ seedlings least, while all other cultivars sustained intermediate damage. Despite the lack of significant species response when all cultivars were considered, Zespri Gold™ seedlings had less damage ($p = 0.01$) than 'Hayward' seedlings. Interestingly, clonal

plants received more damage than seedlings ($p = 0.02$), although this may have been an effect of 'AU Authur' and 'AU Fitzgerald's high mean MDD, as clonally-propagated plants. It is important to point out that while 'CK-3' had the highest (19.02 mm) mean MDD, it had a slightly lower (3rd) greatest damage (PBDD and PSD), when it's larger size (BD) was taken into account. While MDD proved to be a good predictor of damage, based on its correlations with PBDD and PSD (0.75 and 0.70, respectively), its implications are very much limited, unless plant size (BD) is taken into account.

Percent of Base Diameter Damaged and Percent Shoot Damage

Percent of base diameter damaged (PBDD) was used as a more comprehensive measure of damage because it takes into account the diameter of shoot damage relative to the base diameter. As such, the effect of propagation method on damage (MDD) was no longer significant (PBDD and PSD), once plant size was taken into account. The group with the highest PBDD was comprised of *A. deliciosa* cultivars 'AU Authur' and 'AU Fitzgerald', while the lowest group was comprised of *A. chinensis* 'AU Golden Sunshine' and Zespri Gold™ seedlings. The intermediate group included a mixture of golden and fuzzy kiwifruit cultivars.

The clear pattern of lower PBDD and PSD associated with all *A. chinensis,* as compared to *A. deliciosa* cultivars, occurred seemingly in spite of the greater vigor observed in the former species. Differences between these two species with respect to frost damage has yet to be reported elsewhere and was also present when seedlings were compared.

Percent shoot damage (PSD) was assessed visually and relative to plant size, but independently of PBDD. The weaker, albeit still significant, response of PSD ($p = 0.0047$) to cultivar in comparison to PBDD ($P<0.0001$) likely occurred as a result of the manner in the two assessments were made. PSD estimated total percent shoot damage, whereas PBDD tended to focus only on damage to larger diameter shoots. As a result, PSD is probably a more conservative estimation as compared to PBBD. For example, PBDD treatment means for cultivar extremes ranged from 100% to 47% for 'AU Authur' and Zespri Gold™ seedlings, respectively, whereas the range of 79% and 19% for PSD for these two cultivars was considerably narrower.

Another possible reason for this discrepancy is the different patterns of damage. Damage to cultivars with low PSD means, such as 'AU Golden Sunshine (27%) and (Zespri Gold™ seedlings (19%), was confined primarily to smaller shoots in the middle and upper canopy regions, whereas 'AU Authur' and 'AU Fitzgerald' (PBDD of 100%) also sustained frequency of 1.0 or 100% damage to base (Figure 17), while a substantial portion of the mid to upper portions of the shoot system remained unharmed. The high PBDD means for these cultivars likely inflated total variance for this parameter, since it takes into account maximum diameter of shoot damage to base diameter. Indeed, the range for PBDD was narrower (61 % to 80%) when 'AU Authur' and 'AU Fitzgerald' were excluded from the analysis ($p = 0.0025$).

Male 'CK-3' plants had higher PBDD and PSD compared to female 'AU Golden Dragon' on average, while male 'AU Authur' and female 'AU Fitzgerald' plants averaged nearly equal damage, based on PBDD and PSD. This pattern of greater damage

to male cultivars (also seen for CB and DB) might be explained by the fact that male plants reportedly tend to continue growing later in to the season (Ferguson, 1991) and are consequently less cold tolerant (Pyke et al., 1986; Strik, 1990).

While propagation method did have a significant ($p = 0.175$) effect on MDD, there was no such response for either PBDD or PSD, which are considered more accurate predictors estimators of frost damage. Based on the results here, there is no indication that cutting-grown plants are inherently less cold tolerant than seedlings.

Basal Damage and Basal Cracking

Damage to trunk bases and subsequent cracking appeared to occur somewhat independently from general damage to the remainder of the shoot system, as reported by PBDD and PSD. Some of the affected plants had relatively little damage to the smaller shoots in the middle to upper canopy region. Some of these shoots responded with bud-break (especially in apical nodes) a few weeks after the frost, suggesting that they had not yet entered endodormancy. As one might expect, such impulsive growth was ultimately killed by subsequent frost events.

Both incidence of base damage (DB) and base cracking (CB) were highly responsive to cultivar. Both clonally-propagated *A. deliciosa* cultivars ('AU Authur' and 'AU Fitzgerald') experienced the highest incidence of base damage and base cracking (1.0 and 1.0, respectively for 'AU Authur' and 1.0 and 0.94, respectively) for 'AU Fitzgerald', whereas Zespri Gold™ seedlings experienced 0% base damage and 0% cracking. 82% of *A. deliciosa* plants observed sustained basal damage and 79% had

visible cracking, compared to 35% and 11%, respectively for plants within the *A. chinensis species* (data not shown). Currently, the *A. deliciosa* species (seedlings and to a lesser degree, cuttings) is the preferred rootstock for golden kiwifruit in New Zealand and Alabama. However, propensity for basal damage/cracking observed in this species on young plants could potentially impose a major limitation to their use as a rootstock in frost-prone regions.

Clonal plants were not significantly more prone to basal damage or cracking than seedlings, despite the contribution of clonal 'AU Authur' and 'AU Fitzgerald'. Observations suggested that cracking was more common in *A. deliciosa* plants and might be possibly related to differences in bark phloem, cork, and bark structure between these species.

Correlations

Several pairs of individual variables had strong correlations between them. Nearly all of these indicated positive relationships, except for those associated with base diameter. BD was negatively correlated with PBDD, CB, and DB. Analysis of only seedlings also revealed a significant correlation between BD and PSD (-0.84) along with stronger correlations for previously mentioned relationships (Appendix A. 2). This trend was surprising, considering that one would generally expect that greater vigor would contribute to reduced cold tolerance. One factor that was not studied was the rate of growth for each cultivar toward the end of the season. While some cultivars may have had larger BD, they may have also terminated growth earlier. At the very least, results

from this study seem to contradict the assertion by Chat (1995) that frost susceptibility is associated with high vigor.

Maximum diameter damaged (MDD) was strongly and positively correlated with DB, but not CB. This was not unexpected, considering that plants that exhibited basal damage to trunks would also have more damage to large diameter shoots. As mentioned earlier, basal damage did not necessarily progress into formation of vertical cracks in the bark (at least by the time damage was assessed), suggesting that simply scouting for visible cracking may not be a reliable method for assessing basal damage. Identification of basal damage proved easier in this study with more experience, based on other signs such as the occurrence of bark with a dried out and loose appearance. As one might expect, MDD was also strongly and positively correlated with both PBDD and PSD. As mentioned earlier, PBDD was calculated as the quotient of MDD and BD, respectively as percent. The slightly lower correlation strength of MDD and PSD is probably attributable to the fact that PSD was assessed independently of MDD.

Percent Base Diameter Damaged (PBDD) was strongly and positively correlated with basal damage, and to a lesser degree, base cracking. Individual plants with damaged bases invariably received PBDD of 100% because the largest shoot diameter damaged occurred at the base, which was generally the largest shoot diameter measured on the plant. For demonstration purposes, analysis with ‘AU Authur’ and ‘AU Fitzgerald’ (cultivars with a 100% incidence of DB and very high CB) removed from the analysis, the correlation strengths between PBDD and DB as well as PBDD and CB dropped to 0.90 and 0.55, respectively (data not shown). Again, while incidence of CB generally

occurred only in the case of DB, it did not always necessarily follow, which might explain the lower correlation strength between PBDD and CB. PBDD and PSD were employed as the primary assays for quantifying freeze damage to whole shoot systems in this study. This strong positive correlation was impressive, considering that the two variables were assessed completely independently of each other, based on different parameters. Based on this data, it is also suggested that assessments could be employed interchangeably for evaluation of frost damage in young kiwifruit plants.

Basal cracking was strongly correlated with basal damage. As discussed earlier, cracking occurred following basal damage, but was not was not always present, particularly on *A. chinensis* cultivars. Base cracking was observed on 74% of individual plants with damaged bases (data not shown). Exclusion of *A. chinensis* genotypes from the analysis resulted in a much stronger (0.98) correlation between these two variables (data not shown). Cracking was also strongly correlated with PSD, although not as strongly as CB and PBDD. Plants that had greater damage (both PSD and PBDD) also tended to be more likely to exhibit cracking. However, as mentioned earlier, cracking was not observed on some badly damaged plants.

Basal damage was strongly and positively correlated with PSD. Plants that were severely damaged also tended to have greater incidence of basal damage. The stronger correlation between DB and PSD as compared to CB and PSD again can likely be attributed to the fact that only 74% of plants with basal damage also exhibited cracking.

Principle Component Analysis

Principle component analysis provided additional insight into the comprehensive relationships among the six variables assessed. Approximately 92.5% of the total variance observed was explained by only two principle component analyses: PCA 1 (70.5%) and PCA 2 (21.9%). The strong partial contributions of PBDD, DB, and PSD (22.7%, 22.1%, and 21.1%, respectively), suggest that these variables were important in partitioning the variance explained by PCA 1, while CB, MDD, and BD (16.6%, 10.6%, and 6.9%, respectively) were also influential. For PCA 2, only BD (49.7%) and MDD (40.3%) appeared to contribute strongly, while CB (7.9%) and PSD (1.2%) appeared to play more minor roles.

The positive association of MDD, PBDD, and PSD with both PCA 1 and PCA 2 (along with strongly positive correlations between these variables) suggest that these three variables had similar and positive effects on frost damage, particularly MDD. MDD accounted for 10.6% of the variance explained by PCA 1 and 40.3% of PCA 2. PBDD and PSD had very similar characteristics, as evident by the correlation strength of 0.92 between the two variables. Together, they accounted for 43.8% of the variance explained by PCA 1. Treatment means for ‘CK-3’ and clonal propagation were also positively associated with PCA 1 and PCA 2, suggesting that they were more closely associated with MDD, PBD, and PSD. ‘CK-3’ ranked highest (19 mm) for MDD, third for PBDD (94%), and third for PSD (68%). On average, clonal plants had higher MDD (10.6 mm greater) damage and higher PBDD and PSD (17.8% and 22%, respectively) greater damage, as compared to seedlings.

BD was positively associated with PCA 2, but negatively associated with PCA 1. BD accounted for only 6.9% of the variance explained by PCA 1, but 49.7% for PCA 2. BD was negatively correlated with PBDD, CB, and DB, which would also suggest that it did not trend with MDD, PBDD, and PSD, which were positively associated with PCA 2. Cultivar means for 'AU Golden Sunshine', 'AU Golden Dragon', and the mean for *A. chinensis* species were also positively associated with PCA 2, but negatively associated with PCA 1. 'AU Golden Sunshine' and 'AU Golden Dragon' ranked second and third for (19.5 mm and 17.6 mm, respectively) BD, while the *A. chinensis* (all cultivars) as a species, was averaged 39% larger than *A. deliciosa* plants. Conversely, 'AU Golden Sunshine' and 'AU Golden Dragon' ranked fifth and fourth (out of seven) (9.5 mm and 12.1 mm, respectively) for MDD, while *A. chinensis* was slightly (not significantly) lower (12.0 mm) than *A. deliciosa* plants (12.5 mm), on average. Finally, 'AU Golden Sunshine' and 'AU Golden Dragon' ranked sixth (51.3%) and fourth (69.8%), respectively for PBDD and sixth (27.1%) and fifth (41.7%), respectively for PSD. *A. chinensis* plants (all cultivars) sustained 28.0% and 31.6% less damage (PBDD and PSD, respectively), on average, as compared to *A. deliciosa*.

None of the six variables used were negatively associated with both PCA 1 and PCA 2. However, both treatment mean for Zespri Gold™ seedling and mean for seedling were, which would imply that these treatments were negatively associated with or least affected by MDD, PBDD, and PSD, but also not negatively associated with CB. Indeed, Zespri Gold™ seedlings, on average, had less damage in terms of MDD, PBDD, and PSD (3.8 mm, 36.4%, and 35.4%, respectively), compared to 'Hayward' seedlings,

on average. Similarly, seedlings (both species) had less injury with respect to MDD, PBDD, and PSD (10.6mm, 17.8%, and 22.0%, respectively), compared to clonal (all cultivars) plants, on average.

Only CB was positively associated with PCA 1, but negatively associated with PCA 2, while DB was also positively correlated with PCA 1, but had no apparent relationship with PCA 2. This was not surprising, considering that CB was negatively correlated with BD, but not significantly correlated with MDD, and positively and strongly correlated with PBDD and PSD. DB followed the same trend, although it was positively and strongly correlated with MDD. CB accounted for 16.6% of the variance explained by PCA 1 and 7.8% for PCA 2, while DB accounted for 22.1% of the variance explained by PCA 1 and 0% for PCA 2. *A. deliciosa* as a species, along with all three cultivars within that species ('AU Authur', 'AU Fitzgerald', and 'Hayward' seedlings) clustered together while indicating a positive association with PCA 1 and negative association with PCA 2. This pattern would imply that all of these groups were negatively associated with BD and somewhat positively associated with MDD, yet were positively associated with PBDD, PSD, DB, and especially CB.

As one might expect, 'AU Authur', 'Hayward' seedling, and 'AU Fitzgerald' had the lowest BD among the seven cultivars, while the *A. deliciosa* species (all cultivars) was on average approximately 28% smaller than *A. chinensis* plants. 'AU Authur', 'AU Fitzgerald', and 'Hayward' seedlings ranked second, third, and fifth, respectively for MDD, while *A. deliciosa*, as a species, averaged 0.5 mm greater damage compared to *A. chinensis*. This greater tendency for damage was much greater when size (BD) was

accounted for. Indeed, ‘AU Authur’ and ‘AU Fitzgerald’ ranked first and second, while ‘Hayward’ seedling ranked fourth in terms of both PBDD and PSD. The *A. deliciosa* species averaged 29% and 31.6% greater shoot damage as compared to *A. chinensis* in terms of PBDD and PSD, respectively.

Propensity for basal damage and especially basal cracking proved to be traits that were more unique to *A. deliciosa* plants. As was the case with PBDD and PSD, ‘AU Authur’, ‘AU Fitzgerald’, and ‘Hayward’ seedlings ranked first, second, and fourth (respectively) for DB and first, second, and third for CB. As a species, *A. deliciosa* was 256% more likely to exhibit basal damage and 718% more likely to show cracking, as compared to *A. chinensis* cultivars.

Other Considerations

Bud death/survival was not extensively surveyed in this study. However, limited assessment via “knife test” suggested that lateral buds had better survival than did stem tissue. Nearly 100% of the plants that were observed (including understocks of grafted plants) ultimately proved “root-hardy”. Plants that sustained little damage to the shoot system resumed growth in a “normal” fashion from shoot buds, while plants that were severely damaged or frozen to the ground were able to recover and produce vigorous new shoots from the crown or via root suckers.

High nitrogen concentration in shoot tissue during late season has long been implicated in the reduction of frost tolerance in fruit trees (Raese, 1997), including kiwifruit (Kim and Kim (1986b). While tissue analysis of nutritional status was not

sampled at the time of the frost, the same cultivars in adjacent rows that were irrigated and fertilized in the same manner were sampled during the first week of October (approximately five weeks prior to the frost). Whole leaf tissue analysis of this material reported that total nitrogen concentration was 2.89 % at that time, which would be considered above the threshold for 'high' (2.8%) N concentration (according to Smith et al., (1987), even based on mid-season recommendations. Analyses did not reveal significant differences among cultivars in the nearby rows. Furthermore, 'AU Authur' and Zespri Gold™ seedlings, which had the greatest and least damage, respectively, had comparable leaf tissue N concentrations (Appendix A. 3). Soil testing of these adjacent rows in February 2019 (approximately three months after the frost) revealed that there was an average of 2.44 mg/kg nitrate-nitrogen in the top 15 cm of the soil at that time (data not shown).

As previously mentioned, all plants were in an active state of growth prior to the frost event. Kiwifruit have a relatively high irrigation demand (ET Kc = 0.86) and high transpiration rates (Buchner et al., 1994). Therefore, it is plausible that transpirational cooling may have exacerbated frost damage by enabling shoot tissues to drop even below the ambient air temperatures.

Conclusion

This study provided a rare and unique opportunity to evaluate fall frost injury in young plants of *A. chinensis* and *A. deliciosa* by observing several representative cultivar groups. In addition to species and cultivar, propagation method and, to a limited degree,

sex were also investigated. Substantial variation was observed for all six variables with respect to cultivar. *A. chinensis* cultivars tended to have larger base diameter, less maximum diameter damaged, less percent damage relative to base diameter, and less percent shoot damage, with the exception of 'CK-3', which had greater damage. *A. deliciosa* cultivars tended to be smaller, have more damage (MDD, PBDD, and PSD), but also had much higher incidence of basal damage and basal cracking. These trends were generally true among both named cultivars as well as open-pollinated seedlings of each species. Based on these findings, it would seem that at least some genotypes of young *A. chinensis* may in fact have improved frost tolerance over those of the *A. deliciosa species,* especially with respect to susceptibility to basal damage and cracking—features that could be extremely limiting in the establishment of kiwifruit vines in marginal regions.

There was no relationship between clonal propagation and increased frost sensitivity, once plant size was accounted for. Results from this study generally agree with previous reports suggesting a tendency for greater size and frost susceptibility for males as compared to female plants. Frost damage (MDD, PBDD, PSD, DB, and DB) did not appear to increase with vigor, as previously reported.

All five variables that were used for assessment of frost injury proved to be positively, and generally strongly, correlated with one another. It is suggested that assessment of damage, merely based on shoot diameter or shoot length, has limited usefulness unless plant size is considered. Percent damage relative to base diameter (PBDD) and percent shoot diameter (PSD), which was independently rated, both proved

to be reliable estimates of whole plant damage, based on their strongly positive correlation with each other.

This study also provided an opportunity to explore relationships between six different variables used to assess frost damage—providing a better understanding to whole plant response of young kiwifruit plants to fall frost, while revisiting methodology for field assessment of frost injury in this crop. While the results of this study provide valuable insight into differences in tolerance of young plants to fall frost, the implications herein are limited by genetic range of material observed (particularly seedling populations), plant age, and the unique sequence of meteorological events associated with this fall frost.

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CHAPTER III

EVALUATING THE EFFECT OF WARM TEMPERATURE INTERRUPTION ON THE ACCUMULATION OF WINTER CHILLING IN KIWIFRUIT

Introduction

Geographic Origin

Kiwifruit (*Actinidia chinensis* Planch. and *A. deliciosa* A. Chev.) are woody vining species native to the southern portion of China where winters are cool, but relatively mild (Ferguson, 1991). *A. deliciosa* is primarily found in the central portion of Southern China (Yunnan, Guangxi, Guizhou, Hunan, Jiangxi, Sichuan, Chongqing, Hubei, Gansu, Shaanxi, and Henan provinces) and at higher elevations, while *A. chinensis* is more common in the more eastern portion of Southern China (Guizhou, Guangxi, Hunan, Jiangxi, Fujian, southwest Zhejiang, Hubei, southern and western Anhui, Henan, and Jiangsu) and at lower elevations (Hongwen, 2016) (Figure 27). A comparison of climatic conditions for the native geographic distribution of each species, represented by historical monthly winter temperatures for six major cities can be found in Table 4.

Figure 27 Map depicting approximate natural distribution of *A. chinensis* outlined in yellow and *A. deliciosa* in green in China (Hongwen, 2016), relative to provinces (adapted from [https://pixabay.com/illustrations/china-map-chinese-world-globe-1356803/)](https://pixabay.com/illustrations/china-map-chinese-world-globe-1356803/)

Table 4 Elevation, average monthly minimum, mean, and maximum temperatures during winter, and estimated average annual chilling accumulation for six major Chinese cities located in the natural geographic range of two kiwifruit species used in the assessment of two kiwifruit cultivars' response to chilling type and duration.

City	Province	Elevation	Native Range of Species	November Ave. Monthly Temperatures (°C)			December Monthly Temperatures (°C)			January Monthly Temperatures (°C)			February Monthly Temperatures (°C)			Mean Annual Chilling
				Mean / Record Min.	Daily Mean	Mean / Record Max.	Mean / Record Min.	Daily Mean	Mean / Record Max.	Mean / Record Min.	Daily Mean	Mean / Record Max.	Ave. / Record Min.	Daily Mean	Mean / Record Max.	
[2,3]Changsha	Hunan	63 m	*A. chinensis*	9.2 / (-1.1)	13.2	17.3 / (26.7)	3.6 / (-1.1)	7.2	10.8 / (22.8)	1.5 / (-5.0)	4.6	7.7 / (17.8)	3.0 / (-7.2)	6.1	9.2 / (26.1)	[4]1,344
[1]Nanchang	Jiangxi	37 m	*A. chinensis*	10.6 / (-0.8)	13.7	17.9 / (32.3)	4.9 / (-9.7)	7.9	11.9 / (26.1)	3.0 / (-7.7)	5.5	8.8 / (25.3)	5.2 / (-9.3)	7.7	11.2 / (28.7)	[4]1,245
[2,3]Wuhan	Hubei	37 m	*A. chinensis*	8.2 (-3.3)	12.3	16.5 / (31.1)	2.4 / (-10)	6.3	10.3 / (22.8)	0.3 / (-12.8)	4.0	7.8 / (21.1)	2.0 / -(10.0)	5.6	9.3 / (25.6)	[4]1,437
[2,3]Chengdu	Sichuan	500 m	*A. deliciosa*	9.4 / (0.0)	12.3	15.3 / (27.2)	4.3 / (-6.1)	7.3	10.4 / (25.0)	2.6 / (-3.9)	5.8	9.1 / (20.0)	4.4 / (-2.8)	7.7	11.0 / (22.8)	[4]1,264
[1]Chongqing	Chongqing	244 m	*A. deliciosa*	12.2 / (0.7)	14.2	17.1 / (27.2)	7.7 / (-1.7)	9.3	11.5 / (21.5)	6.2 / (-1.8)	7.9	10.3 / (18.8)	8.0 / (-0.8)	10.0	12.9 / (24.6)	[4]1,010
[1]Guiyang	Guizhou	1,275 m	*A. deliciosa*	9.0 / (-2.4)	11.8	15.9 / (28.6)	4.7 / (-6.6)	7.4	11.6 / (26.1)	2.7 / (-7.8)	5.1	8.8 / (25.8)	4.0 / (-6.6)	6.6	10.8 / (29.7)	[4]1,301

Record minimum and maximum temperatures in parentheses.

[1]China Meteorological Administration

[2]https://en.climate-data.org/asia/china/

3Record monthly temperatures from http://www.weatherbase.com/weather/weatherall.

[4]Average annual chilling accumulation estimated using December-January Monthly Mean Temp model (Byrne and Bacon, 1982)

Kiwifruit Phenology

Plants are dioecious and produce mixed buds that give rise to floral buds borne on leaf axils of the emerging shoot. Flower buds are produced on new shoots arising from dormant ("winter") buds that are found in the leaf axils of previous season's canes (Brundell, 1975). Nodes 5 through 12, beginning from the basal end, on fruiting canes are observed to have the greatest floral potential (Hopping, 1990), whereas the basal buds are typically not fruitful (Snowball and Considine, 1986). During winter pruning, wood is selectively removed to retain a limited number of healthy one-year-old canes that will be fruitful (two-year-old canes can be used if suitable one-year wood is not available) (Beutel, 1990).

Rest Requirement in Kiwifruit

Many woody plants that originate in temperate regions require exposure to cold temperature (typically between 0°C and 8°C) in order to satisfy rest requirements and resume normal growth in spring. Such plants generally exhibit reduced vegetative growth and hardening or acclimation in response to decreasing photoperiod and temperatures as they transition into a temporary and reversible state of rest, known as ecodormancy (Lu and Reiger, 1990). After exposure to a certain amount of cold, buds on these plants enter endodormancy, which is imposed internally and physiologically (Melke, 2015). In this deep state of rest, resumption of growth (and flowering) is very difficult unless the chilling requirement is satisfied (Couvillon, 1995). Common symptoms associated with inadequate chilling accumulation include delayed and reduced

budbreak, decreased flowering and abortion of flowers and fruit, and more pronounced apical dominance with respect to budbreak (Austin et al., 2002; Couvillon, 1995; Dennis, 2003). In the case of kiwifruit, chilling is required before the final phase of floral development can take place (Snelgar, 1997; Snowball and Considine, 1986). Lack of budbreak (particularly more at more basal nodes) and floral production has been, to some degree, overcome through the use of hydrogen cyanimide in kiwifruit (Austin et al., 2002), along with other temperature fruit crops.

Accumulating a sufficient amount of winter chilling is extremely important for proper development of reproductive growth in the subsequent spring, and is one of the most limiting climatic limitations for selection of temperate fruit cultivars. Such chilling requirements vary by species and individual cultivar, but may also be affected by other factors including rootstock (Couvillon, 1995). In addition to having their chilling requirement met, many species must also be subjected to a specific duration of warm temperature, particularly after the chilling period before they are able to emerge from winter dormancy. This requirement is expressed in heat units, which are often calculated by subtracting the mean daily temperature from the base temperature of 10°C. In spite of being somewhat subtropical in adaptation, kiwifruit have an extensive chilling requirement as compared to other species such as olive (*Olea europaeae*), fig (*Ficus carica*), pomegranate (*Punica granatum*), and loquat (*Eriobotrya japonica*) with similar levels of cold-hardiness. Estimates for winter chilling requirements range from 700 units for ‘Bruno’ to as much as 1150 for ‘Hayward’ (Caldwell, 1989).

Chilling Requirements for Auburn Kiwifruit Cultivars

During the early-1980's efforts to establish commercial production of kiwifruit (*A. deliciosa*) was being widely trialed in the Southeastern United States (Mainland and Fisk, 2006). Commercial production, largely based on the cultivar 'Hayward', ultimately proved unsuccessful due to lack of reproductive growth and severe freezes (Caldwell, 1989). Trialing of kiwifruit by Auburn University researchers led to the field evaluation of two *A. chinensis* selections in the mid-1990's that were developed by the Institute of Fruit and Tea, Hubei Academy of Agricultural Sciences of P.R. China. After successful field performance at the Chilton Research and Extension Center at Thorsby, AL, 'AU Golden Dragon' and 'AU Golden Sunshine' were jointly released by the Institute and Auburn University in 2018 (Spiers, unpublished).

Maximum budbreak estimates were determined for 'AU Golden Sunshine' (700 cu), 'AU Golden Dragon (800 cu), 'AU Fitzgerald (800 cu), and 900 for 'Hayward' and two male cultivars, 'Matua' and 'AU Authur' by Wall et al., (2008). These estimates were based on the use of excised canes from previous-season's growth that were cut to six to eight nodes. Cuttings were held in jars with a water solution and removed from 4°C cold treatment at 50-hour increments and moved to 25°C greenhouse conditions for observation of vegetative and floral budbreak. Canes were collected after being exposed to 572 and 160 chill units in the field in 2005 and 2006, respectively. However, further observation in the field of these cultivars in years receiving unusually low amounts of chilling suggest that these estimates might be too high.

Estimation of Chilling Requirements

Estimation of winter chilling requirements is not straightforward and is complicated by many factors including age of material, whether or not the material is attached to or excised canes (Snowball, 1997) or potted plants (Stanley et al., 1995). Studies on kiwifruit chilling requirements have also proven inconclusive as well (Guerriero et al., 1990). It has been suggested that temperatures as high as 10°C are effective for chilling in kiwifruit (Lionakis and Schwabe, 1984), with temperatures of 13°C proving too warm (McPherson et al., 1995). The same authors have proposed that abscisic acid (ABA) and gibberellins are probable hormonal mediators in kiwifruit, with the former imposing dormancy via high concentrations in the bud scales, although other product such as ethylene, cytokinins, and brassinosteroids have also been implicated (Melke, 2015). Whole, excised 16 to 20 node-cuttings reportedly behave similar (but not identically) to attached canes in the field (Snowball, 1997), provided that they are allowed to defoliate naturally prior to collection (Snelgar et al., 1997). Snelgar et al. (1997) and Austin et al. (2002) also concluded that shorter canes were suitable for predicting fruitfulness, but not appropriate for modeling budbreak and flowering because they tended to underestimate chilling requirements. Snowball and Smith (1996) reported that cane cuttings should be no less than 12g in weight, 150 mm in length, and 6 mm in diameter, as starch stores could be insufficient in smaller material. It has also been reported that chilling accumulation does not become completely effective until leaf abscission in other species such as apple (Chandler, 1960) and peach (Reeder and

Bowen, 1978; Walser et al., 1981). Excised cuttings have been successfully forced at temperatures ranging from 16°C (McPherson et al., 1995) to 25°C (Brundell, 1976).

Chilling Negation by Warm Temperature

One area relating to chilling accumulation as it applies to kiwifruit that has not been explored is the effect of warm temperature interruption during winter chilling conditions and the potential effect of negation on accumulation. While the occurrence of this phenomenon is widely accepted, only several studies have provided convincing evidence or quantification of these effects. Periods of temperatures as low as 20°C at different times during continuous chilling were sufficient to delay budbreak in Asian pear, particularly toward the end of the chilling phase (Tamura et. al, 1995). Exposure to a period of 30°C in different sequences of continuous chilling resulted in budbreak and shoot growth in apple as was also reported in peach (Couvillon and Erez, 1985; Overcash and Campbell, 1956), especially when the warm temperature was applied prior to chilling (Young, 1992). The extent to which chilling is negated is dependent on the length of cycle in which warm temperature is applied in addition to the temperature and length of the warm temperature interruption (Couvillon and Erez, 1985; Erez et al., 1979), but it has been concluded that chilling negation by warm temperature is limited to the 20 to 40 chill hours immediately prior to the warm temperature treatment (Erez et al., 1979).

Models Used for Quantifying Chilling Accumulation

While the standard <7.2°C ("Old 45°F Model") (Weinberger, 1950) and the 0°C to 7.2°C ("New 45°F Model") (Weinberger, 1967) are still most commonly used for most temperate crops, other models such as the Utah Model or Richardson Model, which attempt to account for chilling negation by warm temperature, have been proposed. However, the Utah Model has proven unreliable for mild regions that receive less chilling and are frequently exposed to negating temperatures. The dynamic model quantifies chilling in terms of 'chilling portions', which cannot be reversed once they have been accumulated (conversion of the intermediate product) (Erez et al., 1990), has proven a more reliable model for estimating chilling in mild areas (Allan et al., 1993). Other methods include the mean monthly temperature model (Byrne and Bacon, 1992) and the Positive Utah Model, in which negation is not considered (Linsley-Noakes and Allan, 1974).

While it has been argued that it does not provide a satisfactory explanation of chilling accumulation (Austin et al., 2002; McPherson et al., 1995), the Richardson Model (Utah Model) is still widely considered to be the most effective means for estimating chilling in kiwifruit. This model allows for partial accumulation (at 0.5 units increments), negation by warm temperature, and it considers temperatures as high as 12.4°C effective (at least partially) for chilling accumulation. Richardson et al., (1974) model included a negative accumulation effect in which each hour of temperatures between 16°C and 18°C and >18°C resulted in -0.5 and -1.0 chill units, respectively (Table 5).

Table 5 Expected chill unit contributions for select temperature ranges based on the Richardson chilling model used in the assessment of two kiwifruit cultivars' response to chilling type and duration.

Temperature Range (°C)	Temperature Range (°F)	Expected Chill Unit Contribution
< 1.4	< 34.0	0
1.5 – 2.4	35.0 – 36.0	0.5
2.5 – 9.1	37.0 – 48.0	1.0
9.2 – 12.4	49.0 – 54.0	0.5
12.5 – 15.9	55.0 – 60.0	0
16.0 – 18.0	61.0 – 65.0	-0.5
> 18.0	> 65.0	-1.0
All expected chill unit contributions based on Richardson et al., 1974		

To date, no published research has been conducted to explore the effects of warm temperature interruption on winter chilling accumulation in kiwifruit. The potential for chilling negation is a major concern in regions with highly dynamic winter temperatures such as the Southeastern United States, particularly for a crop with marginal cold hardiness and comparatively high chilling requirement like kiwifruit.

Objective

The objective of this study was to assess the potential for negation of winter chilling accumulation in as a determined by vegetative and floral response in green and golden kiwifruit, based on dynamic winter temperatures observed in southeastern Texas.

Materials and Methods

Plant Material

One-year-old fruiting canes were collected from own-rooted plants of the pistillate cultivars *A. deliciosa* 'AU Fitzgerald' and *A. chinensis* 'AU Golden Dragon' that were established at the Auburn University Chilton Research and Extension Center near Thorsby, AL in 1985. Collection commenced shortly after leaf abscission on December 15, 2017 and on November 30, 2018. Selection of canes was based on size, uniformity, light exposure, and apparent fruiting potential. Approximately 300 canes were collected per cultivar, bulked together by cultivar, bundled and tied, and trimmed to approximately one meter from the distal end to facilitate transportation. Basal ends were placed in 18.9 L buckets with bases immersed in tap water immediately in order to prevent cavitation during transit back to Texas A&M University, College Station, TX. Immediately upon arrival, canes were placed in a in a walk-in cooler and held at approximately 8.9°C for three to five days until processing. Exposure to chilling conditions during storage was accounted for in the base chilling calculation. Base chilling included a combination of field-supplied chilling (estimated based on data from weather station located approximately 8.0 km away) and exposure to storage conditions

after collection. Base chilling was estimated to be approximately 334 Richardson units (265 units, 0°-7.2°C) in 2017 and 360 Richardson units (179 units, 0°-7.2°C) in 2018.

After removal from storage, all canes were trimmed to exactly ten nodes after the removal of the basal five nodes (with a 45° angle cut). Material for each cultivar was then graded systematically into three groups based on relative cane diameter. Any canes outside of a range of 8 mm to 15 mm in diameter or 35 cm to 66 cm in length (after trimming) were not used for the experiment. A single cane was randomly selected from each of the three groups and placed in a 946 mL Ball® "regular mouth" (Ball Corporation, Westminster, CO) fruit jars to minimize the effect of cane diameter. Jars were filled with reverse-osmosis water such that the basal two to three nodes were completely immersed.

The distal ends of the canes were sealed with Buddy Tape (Aglis Corporation, Yame City, Japan.) grafting material to reduce desiccation. Initial moisture loss (noted by loss in cane weight), likely due to xylem occlusion, which was observed during the first week necessitated the re-cutting of all canes by removing an additional one cm from the base.

Experimental Design

The experimental design consisted of a 2 x 6 factorial with two levels of chilling type (continuous and interrupted), six levels of chilling exposure (including base-chilling) at weekly intervals (168-Richardson Unit increments). A randomized complete block (RCBD) with four blocks was used. Each experimental unit consisted of one jar

containing a sub-sample of three canes per cultivar. The experiment was conducted for two years with ‘AU Golden Dragon’ and ‘AU Fitzgerald’, with data from each cultivar analyzed separately. For brevity, continuous chilling will be abbreviated as C.C. and warm temperature interruption will abbreviated as W.T. for the remainder of the text.

Treatments consisted of simulated winter chilling applied at one-week (168-hour) increments, either continuously or with alternating treatments of warm temperature interruption (Table 6). All treatments were finally exposed to mild forcing temperatures, as to simulate spring conditions conducive to budbreak and flowering. Selection of W.T. treatment conditions were imposed to reflect the dynamic temperatures observed in the Southeastern United States (particularly southeastern Texas) during dormancy, and also account for temperatures that result in chilling negation in other crops such as peach and pear (Couvillon, 1995; Erez et al, 1979; Tamura et al., 1995).

Following preparation, jars were placed in one of three respective growth chambers, according to treatment. The first chamber, which simulated chilling environment, was maintained at 7.2°C day (45°F) and 4°C (39°F) night temperature with 70% to 85% relative humidity and 8 hour / 16 hour day/night photoperiod via fluorescent lights producing approximately 400 to 550 µmol / m^2 / sec^{-1} (cane height range). These conditions were expected to provide one chill unit per hour of exposure, based on both the Richardson (Richardson et al., 1974) and 0-7.2°C models (Table 5). Fluctuation of day/night temperatures were used to create a more realistic simulation of natural field conditions. At this rate, chilling accumulation rate would be expected to occur at a rate of 168 units per week.

Table 6 List of treatments for continuous chilling (C.C.) and warm temperature interruption (W.T.) chilling type used in the assessment of two kiwifruit cultivars' response to chilling type and duration.

Continuous Chilling (C.C.)	Warm Temperature Interruption (W.T.)
Base (field-supplied chilling)	Base (field-supplied chilling) with three days intermittent W.T.
One week C.C.	One week chilling with six days intermittent W.T.
Two weeks C.C.	Two weeks chilling with nine days intermittent W.T.
Three weeks C.C.	Three weeks chilling with twelve days intermittent W.T.
Four weeks C.C.	Four weeks chilling with fifteen days intermittent W.T.
Five weeks C.C.	Five weeks chilling with eighteen days intermittent W.T.

The second chamber, which was used to impose W.T., was maintained at 25°C (77°F) day and 17.2°C (63°F) night with and 65% to 75% relative humidity (same photoperiod and light intensity). This temperature range reflects the natural occurring periods of intermittent warmer temperatures incurred in southeastern Texas (College Station, TX) and hypothetically would result in negative 16 Richardson units within a 24-hour period (Tables 5 & 6).

The third chamber, which consisted of a retrofitted walk-in cooler, was used to for forcing and modified to simulate a spring-like environment. Forcing chamber conditions consisted of 13 hour/11 hour day/night photoperiod via overhead LED

lighting at an intensity of approximately 150 to 275 µmol / m^2 / sec^{-1} (cane height range) and temperatures ranging from 22.8°C (73°F) to 26.0°C (79°F) and. Indoor humidifiers were used to maintain relative humidity at an approximate range of 80% to 90%. For the second year, diurnal temperatures ranged from 19.4°C (67°F) night to 23.9°C (75.0°F) day with 220 to 400 µmol / m^2 / sec^{-1} (cane height range) and the same relative humidity range and photoperiod. WatchDog® Micro Station 1000 Series (Spectrum Technologies, Inc., Aurora, IL) loggers were placed in each chamber to record temperature at 15-minute intervals.

For the second year (2018/2019), a fourth environment was included for observational purposes. This environment consisted of another growth chamber programmed to impose warm temperature interruption at a higher temperature treatment regime of 30.6°C (87°F) day and 23.9°C (75°F) night (same photoperiod / light intensity and comparable relative humidity). This temperature range is exceptionally warm and does not reflect normal winter temperature patterns even in regions such as along the Gulf of Mexico. However, the purpose of this observational treatment was to determine if chilling negation was achievable at a higher temperature regime. Warm temperature treatments under these warmer conditions followed the same sequence as the original warm temperature treatments. Data from this non-replicated phase was used for comparison purposes only. In order to distinguish between the previously mentioned W.T. treatments, exposure to this higher temperature regime will be referred to as 'high temperature interruption' (H.T.) from hereon.

Treatments for C.C. consisted of exposure to weekly (168 Richardson / 0°C – 7.2°C unit) increments in simulated chilling conditions provided by the first growth chamber. C.C. treatments ranged from base level chilling (no additional chilling) up to a maximum of 934 or 960 units (depending on year) at the five weeks treatment. As previously mentioned, base level chilling was estimated at 334 Richardson units (265 units, 0-7.2°C) for the first year and 360 Richardson units (179 units, 0-7.2°C) in the second year. After receiving their respective chilling treatment, samples were moved to the forcing (third) chamber.

The warm temperature interruption treatments received the same amount of chilling exposure as C.C. at the same level, however, each period of chilling exposure was followed by exposure to 72 hours in the second (warm) growth chamber. This was accomplished by physically moving the material into the second growth chamber, providing a hypothetical amount of as much as negative 40 Richardson units. For this experiment, the C.C. exposure served as the control at each level of chilling (Tables 7 & 8).

At the end of the sequence, each treatment was moved to the third (forcing) chamber, where they were arranged in a randomized complete block design (RCBD). Jars were placed within squares formed by galvanized wire panels in order to prevent them from falling over.

Table 7 Sequence of chilling and warm temperature interruption (W.T.) treatments and expected net chilling accumulations used in the assessment of two kiwifruit cultivars' response to chilling type and duration for 2017-2018.

Base chilling	Base chilling / W.T.	1 week C.C.	1 week chilling / W.T.	2 weeks C.C.	2 weeks chilling / W.T.	3 weeks C.C.	3 weeks chilling / W.T.	4 weeks C.C.	4 weeks chilling / W.T.	5 weeks C.C.	5 weeks chilling / W.T.
334 chill units	334 chill units	334 chill units	334 chill units	334 chill units	334 chill units	334 chill units	334 chill units	334 chill units	334 chill units	334 chill units	334 chill units
Forcing	Warm treatment	168 chill units	Warm treatment	168 chill units	Warm Treatment	168 chill units	Warm treatment	168 chill units	Warm treatment	168 chill units	Warm treatment
	Forcing	Forcing	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units
			Warm treatment	Forcing	Warm Treatment	168 chill units	Warm treatment	168 chill units	Warm treatment	168 chill units	Warm treatment
			Forcing		168 chill units	Forcing	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units
					Warm Treatment		Warm treatment	Forcing	Warm treatment	168 chill units	Warm treatment
					Forcing		168 chill units		168 chill units	Forcing	168 chill units
							Warm treatment		Warm treatment		Warm treatment
							Forcing		168 chill units		168 chill units
									Warm treatment		Warm treatment
									Forcing		168 chill units
											Warm treatment
											Forcing
334 chill units expected	294 chill units expected	502 chill units expected	462 chill units expected	670 chill units expected	550 chill units expected	838 chill units expected	678 chill units expected	1,006 chill units expected	806 chill units expected	1,174 chill units expected	934 chill units expected

Estimated chilling values based on 0-7.2°C model and Richardson model (Richardson et al., 1974); Negative chilling values based on Richardson model
Warm temperature interruption as exposure to 72 hours of warm temperature conditions
Extent of chilling negation estimated at 40 Richardson chill units, based on reported limitations in apple (*Malus spp.)* (Couvillon, 1995; Erez et al., 1979)
Base chilling estimate of 334 chill units includes field-supplied and accumulation during storage

Table 8 Sequence of chilling and warm temperature interruption treatments and expected net chilling accumulations used in the assessment of two kiwifruit cultivars' response to chilling type and duration for 2018-2019.

Base chilling	Base chilling / W.T.	1 week C.C.	1 week chilling / W.T.	2 weeks C.C.	2 weeks chilling / W.T.	3 weeks C.C.	3 weeks chilling / W.T.	4 weeks C.C.	4 weeks chilling / W.T.	5 weeks C.C.	5 weeks chilling / W.T.
360 chill units	360 chill units	360 chill units	360 chill units	360 chill units	360 chill units	360 chill units	360 chill units	360 chill units	360 chill units	360 chill units	360 chill units
Forcing	Warm treatment	168 chill units	Warm treatment	168 chill units	Warm Treatment	168 chill units	Warm treatment	168 chill units	Warm treatment	168 chill units	Warm treatment
	Forcing	Forcing	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units
			Warm treatment	Forcing	Warm Treatment	168 chill units	Warm treatment	168 chill units	Warm treatment	168 chill units	Warm treatment
			Forcing		168 chill units	Forcing	168 chill units	168 chill units	168 chill units	168 chill units	168 chill units
					Warm Treatment		Warm treatment	Forcing	Warm treatment	168 chill units	Warm treatment
					Forcing		168 chill units		168 chill units	Forcing	168 chill units
							Warm treatment		Warm treatment		Warm treatment
							Forcing		168 chill units		168 chill units
									Warm treatment		Warm treatment
									Forcing		168 chill units
											Warm treatment
											Forcing
360 chill units expected	320 chill units expected	528 chill units expected	488 chill units expected	696 chill units expected	576 chill units expected	864 chill units expected	704 chill units expected	1,032 chill units expected	832 chill units expected	1,200 chill units expected	960 chill units expected

Estimated chilling values based on 0-7.2°C model and Richardson model (Richardson et al., 1974); Negative chilling values based on Richardson model
Warm temperature interruption as exposure to 72 hours of warm temperature conditions
Extent of chilling negation estimated at 40 Richardson chill units, based on reported limitations in apple (*Malus spp.)* (Couvillon, 1995; Erez et al., 1979)
Base chilling estimate of 360 chill units includes field-supplied and accumulation during storage

Data Collection

Observations were made at two- to three-day intervals for all samples in the forcing stage. For each single jar experimental unit containing a three cane sub-sample, the number of dormant buds that gave rise to vegetative shoots was recorded on a per-cane basis. Vegetative budbreak was determined as defined by the opening of bud scales in conjunction with the first emergence of the shoot dome (Brundell, 1975). The number of visible floral buds / flowers along with relative stage of development (bud; bloom / anthesis; petal fall; senescence) as described by Wall et al. (2008) was recorded on a per-cane basis concurrently with vegetative observations. Floral and vegetative observations, as described above, were also made with respect to nodal position during the first (2017-2018) year in an effort to study the effect of chilling (type and level) on nodal response. Typically, eight nodes were exposed and included in the observations, while the basal two nodes were submerged in water. Node number / position was designated from the apical-most node downward (apical node = position 10, basal node = 1). For all observations, the average of each three-cane experimental unit was reported and used in the analysis.

Observations were terminated for each cane independently at the onset of visible signs of senescing leaves, at which point these canes were removed from the experiment. This was done in order prevent severe soiling of the water and allow neighboring canes in continue development. In some cases, individual canes were removed over two weeks earlier than neighboring canes from the same jar. Prior to discarding spent canes, the total number of vegetative shoots present was recorded, as determined by the presence of

one or more leaves. Shoot number was only assessed extensively in the second (2018-2019) year of the experiment. Cane diameter (mm) was also measured at the top of the jar height using a digital caliper.

From the data collected, a total of seven response variables were considered for statistical analysis. Floral bud number per cane (also assessed on a per-node basis for the first year) represented the maximum total number of floral buds, open flowers, and senescing flowers observed. While the date at which this maximum number was observed varied by cultivar, treatment, and block, it represented the maximum reproductive potential of each given sample. Percent vegetative budbreak was calculated as the percent of total nodes exhibiting budbreak. For simplicity and consistency, the total possible number of nodes for percent budbreak was considered to be eight (Table 9).

Shoot number was recorded as the number of nodes that exhibited vegetative budbreak and produced a shoot, characterized by the presence of at least one leaf. Multiple shoots developing from a single node were only counted as a single shoot. Based on the number of shoots (or nodes producing shoots), percent shoot development was calculated as the percentage of shoot-bearing nodes relative to a total of eight possible nodes, similar to percent vegetative budbreak. For example, for both percent vegetative budbreak and percent shoot development, a cane exhibiting budbreak or shoot emergence at eight nodes would be considered to have 100% vegetative budbreak or shoot development, respectively. Some nodes that exhibited budbreak did not progress into shoot development (Table 9).

Table 9 Response variables, methods, and units used in the assessment of two kiwifruit cultivars' response to chilling type and duration over two years.

	Variable	Method, units
1	Floral buds per cane	Visually; maximum total (including floral buds and flowers)
2	Vegetative budbreak number per cane	Visually (Brundell, 1975); (0 to 8 possible)
3	Percent vegetative budbreak per cane	Vegetative budbreak divided by total possible of eight buds
4	Shoot number per cane	Visually, evident by one or more leaves (0 to 8 possible)
5	Percent shoot development per cane	Shoot number per cane divided by total possible of eight shoots
6	Percent 'vegetative bud to shoot' per cane	Number of developed shoots per cane divided by vegetative budbreak per cane
7	Cane diameter	Digital caliper (mm)
Data for all variables reported as average of three-cane subsamples for each jar.		

In such cases, while the criteria were met for budbreak, the later bud shoot meristem did not develop further to the point of exhibiting leaf unfurling and expansion. The percentage of shoot development relative to vegetative budbreak (total of eight possible buds), referred to from here on as 'percent vegetative bud to shoot' was calculated in an attempt to quantify the frequency vegetative budbreak leading to shoot development, particularly with regard to chilling type and level. Vegetative budbreak (number and percentage), shoot development (number and percentage), and percent 'vegetative bud to shoot' collectively represented vegetative growth potential.

Statistical Analysis

All statistical analyses were performed using JMP software, Version 14.0, SAS Institute Inc., Cary, NC. Data for all variables was checked for normality using the Shapiro-Wilcox Test at the 0.05 alpha level. Floral bud number, shoot number, and percent shoot development were all successfully transformed using the square root method. The remaining variables (vegetative bud number, percent vegetative budbreak number, cane diameter, and percent 'vegetative budbreak to shoot' were not successfully transformed with any available method, therefore non-transformed data was used.

Data for 'AU Fitzgerald' and 'AU Golden Dragon' were analyzed separately in for this study. A Student t-Test (0.05 alpha level) was used to test for a year effect for all variables, except for the three shoot-related variables, which were only assessed during the second year of the experiment. Analysis of variance (ANOVA) (0.05 alpha level) was used to test for presence of year x treatment interaction (model construct included

year, treatment, year x treatment, and block as fixed effects). Where year x treatment interaction was present ($P \leq 0.05$), data for each year was analyzed separately. In the absence of a significant interaction, the combined data from both years was used for the analysis. For simplicity, the year number for the first year of the experiment (2017-2018) and the second year (2018-2019) are referred to as 2018 and 2019 from here on.

Because primary fixed effect of interest was chilling type, comparisons were made separately at each level of chilling by chilling type. Comparisons were made for all response variables using the Student-t Test (0.05 alpha level).

ANOVA (0.05 alpha-level) was used to estimate the effect of chilling exposure (level) on floral bud number and vegetative budbreak number at all levels of chilling. Chilling (level) was treated as a categorical (non-continuous) variable due to the limited number of finite levels imposed, whereas previous experiments treating chilling as a continuous variable used more numerous and smaller (50- to 100-chill unit) increments. Tests were carried out separately for each year and for each type of chilling (C.C. and W.T.). Tukey's HSD (0.05 alpha-level) was used for separation of means in order to estimate the effect of chilling at each level and to identify an upper threshold for chilling requirement based on the data.

Data for mean vegetative budbreak number and mean floral bud number on a per-node basis was assessed exclusively during the first year for estimation of nodal position effect on floral and vegetative response. Comparison of per node mean vegetative budbreak number and mean floral number for W.T. and C.C. was made at each level of chilling using Student's t-Test (0.05 alpha-level). Additionally, average

vegetative budbreak number and mean floral bud number across all chilling levels was calculated for each cultivar and chilling-type (W.T. / C.C.) combination for general comparison of nodal position effect on vegetative and floral response. The non-transformed data was used for analysis of floral bud number.

Student's t-Test (0.05 alpha-level) was used to compare differences in mean cane diameter: 1.) by year (both cultivars and all treatments); 2.) by cultivar and year (all treatments); 3.) by cultivar, year, and chilling type (across all chilling levels); 4.) by cultivar, year, chilling type, and at each level of chilling. Effect of cane diameter on all other dependent variables was estimated through linear regression. Existence of significant cane diameter effect ($P<0.05$ for β_1 and R^2) was analyzed systematically: 1.) by cultivar (all treatments); 2.) by cultivar and year (all treatments); 3.) by cultivar, year, chilling type (all levels).

Results

Year Effect on Floral and Vegetative Response

Comparison between years indicated that the mean number of floral buds per cane (all treatments) was higher, but not significantly higher for 'AU Golden Dragon' and 'AU Fitzgerald' (both cultivars combined) in 2018 (4.61 ± 0.382) than in 2019 (4.18 ± 0.382) (Figure 28 and Table 10). For individual cultivars, however, mean floral bud number was slightly lower (2.81 ± 0.393) per cane in 2018 as compared to 2019 (3.29 ± 0.393) for 'AU Golden Dragon' (Figure 28 and Table 11), whereas 'AU Fitzgerald averaged 6.41 (± 0.595) floral buds per cane in 2018 as compared to 5.08 (± 0.595) in

2019 (not significantly different) across all treatments (Figure 28 and Table 12). Vegetative budbreak number and percent vegetative budbreak were slightly higher for ‘AU Golden Dragon’ in 2019 than in 2018, but slightly lower for both variables in the second year for ‘AU Fitzgerald’. Statistical comparison (Student t-Test) did not indicate that years were significantly different for any of these variables (Figure 28).

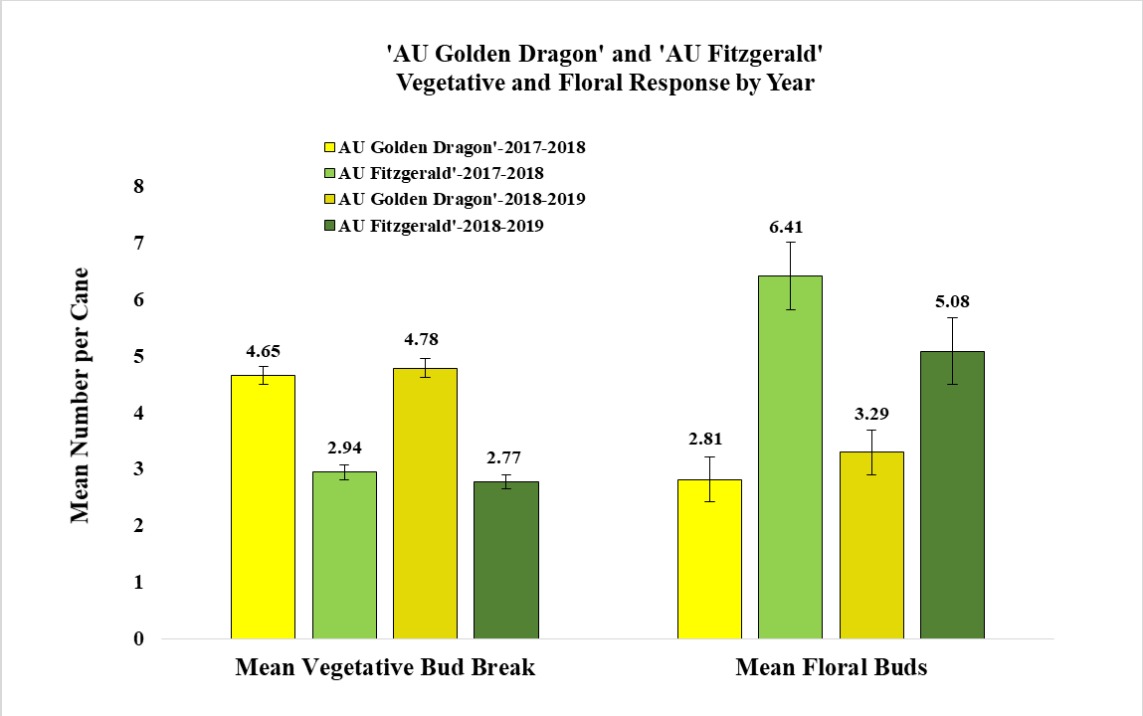


Figure 28 Mean vegetative budbreak number and mean root floral number per cane by cultivar and year (all treatments combined) (non-significant) for two cultivars over two years in the assessment of response to chilling type and duration.

Table 10 Vegetative budbreak number, percent vegetative budbreak, and floral buds per cane for 'AU Golden Dragon' and 'AU Fitzgerald' by year (both cultivars; all treatments) (non-significant) in the assessment of kiwifruit response to chilling type and accumulation.

Year	2017-2018				2018-2019			
	Mean	Standard Deviation	Standard Error	Total Observations	Mean	Standard Deviation	Standard Error	Total Observations
Vegetative buds per cane	3.80	1.39	0.142	96	3.77	1.38	0.142	96
Percent vegetative budbreak per cane	47.4%	17.35	0.018	96	47.16%	17.22	0.142	96
Total floral buds per cane	4.61	4.016	0.382	96	4.18	3.785	0.382	96
Analysis based on t-test by year Analysis of floral bud number based on square root transformation Non-transformed data presented for floral bud number								

Table 11 Vegetative budbreak number, percent vegetative budbreak, and floral buds per cane for 'AU Golden Dragon' by year (all treatments) (non-significant) in the assessment of kiwifruit response to chilling type and accumulation.

Year	2017-2018				2018-2019			
	Mean	Standard Deviation	Standard Error	Total Observations	Mean	Standard Deviation	Standard Error	Total Observations
Vegetative buds per cane	4.65	1.22	0.164	48	4.78	1.01	0.165	48
Percent vegetative budbreak per cane	58.1%	15.29	2.06	48	59.8%	12.63	2.06	48
Total floral buds per cane	2.81	3.164	0.393	48	3.23	2.084	0.393	48
Analysis based on t-test by year Analysis of floral bud number based on square root transformation Non-transformed data presented for floral bud number								

Table 12 Vegetative budbreak number, percent vegetative budbreak, and floral buds per cane for 'AU Fitzgerald' by year (all treatments) (non-significant) in the assessment of kiwifruit response to chilling type and accumulation.

Year	2017-2018				2018-2019			
	Mean	Standard Deviation	Standard Error	Total Observations	Mean	Standard Deviation	Standard Error	Total Observations
Vegetative budbreak number per cane	2.94	0.96	0.126	48	2.77	0.75	0.126	48
Percent vegetative budbreak per cane	36.8%	11.97	1.58	48	34.5%	9.40	1.58	48
Floral buds per cane	6.41	3.91	0.595	48	5.08	4.117	0.595	48

Analysis based on t-test by year
Analysis of floral bud number based on square root transformation
Non-transformed data presented for floral bud number

Floral Response to Chilling Type

For ‘AU Golden Dragon’ floral bud number, there was a strong year x treatment interaction (P = 0.00014) and significant year effect (P = 0.0034) at the 4-week level of chilling. There was also a strong year x treatment interaction (P = 0.0019), but no significant year-effect at the 5-week chilling level. Consequently, floral bud number was analyzed separately for each year. (Figure 29).

‘AU Golden Dragon’ floral response at the remaining four levels did not reveal significant differences in treatment effects nor any clear pattern. Mean floral bud number was comparable between C.C. (0.79) and W.T. (0.88) at base level chilling, one-week chilling (1.71 and 1.63, respectively), two-weeks chilling (2.25 and 2.54, respectively), and three-weeks chilling (2.96 and 3.37, respectively). Not surprisingly, none of these differences were significant at any of these chilling levels. At four-weeks chilling, W.T. produced considerably more floral buds, on average (6.62) as compared to C.C. (3.60). This pattern was also observed at the five-week chilling level, with the same types of chilling resulting in a mean of 6.92 and 3.29 floral buds per cane, respectively. However, differences at neither chilling level were significant, when the year x treatment interaction were accounted for (Figures 29 & 30).

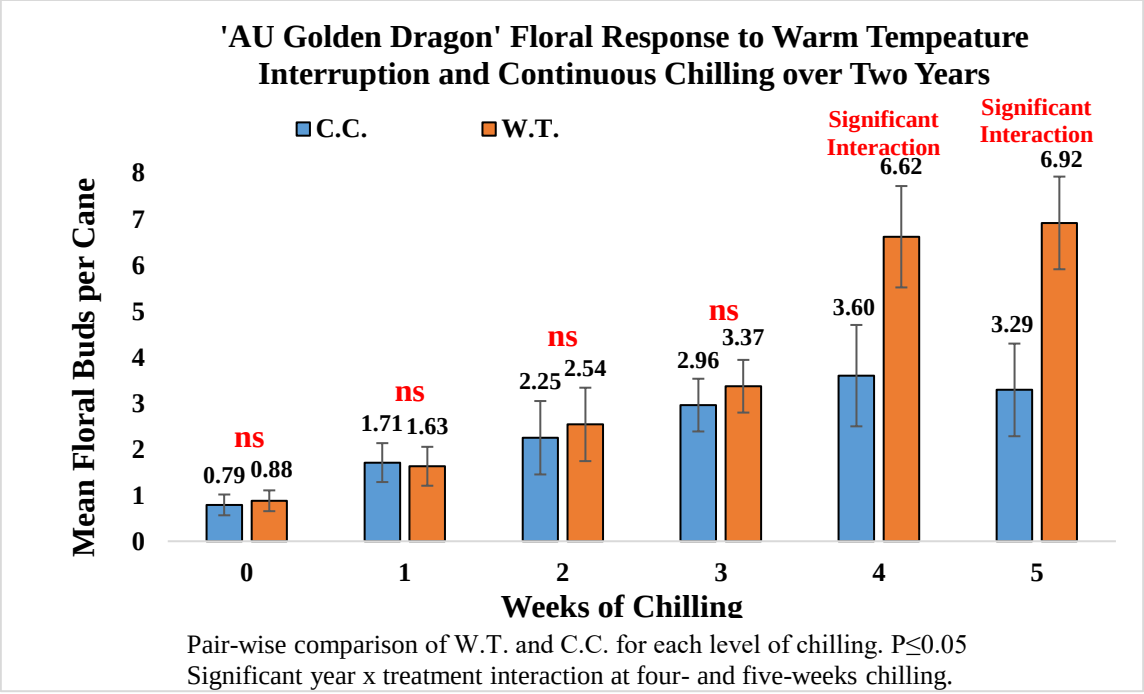


Figure 29 Histogram of 'AU Golden Dragon' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels over two years.

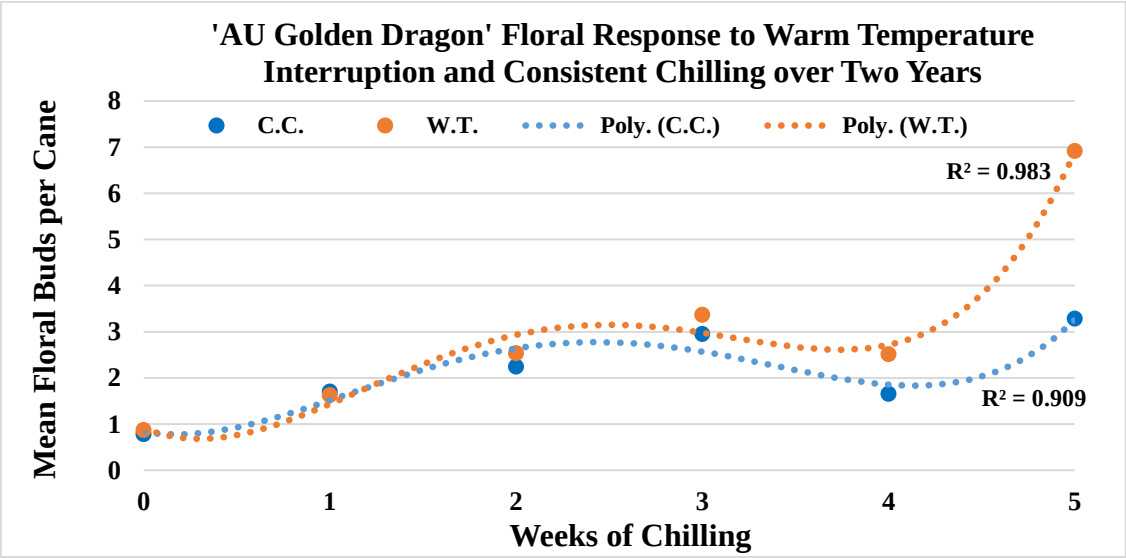


Figure 30 Scatterplot of 'AU Golden Dragon' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels over two years.

Similar to the two-year data, comparison of mean floral bud number response to C.C. and W.T. for ‘AU Golden Dragon’ for 2018 did not reveal significant differences in treatment until the two highest levels of chilling. C.C., as compared to W.T., resulted in similar average floral bud number at base chilling (0.67 and 0.75, respectively) and appreciably higher value at one-week chilling (1.58 and 0.67, respectively). At two weeks chilling, W.T. produced an average of over twice as many floral buds (2.67) in comparison to C.C. (1.00), although this difference was not significant, nor at three-weeks chilling (2.83 and 2.00, respectively). Treatment difference was significant (P = 0.0181) and much greater at four weeks chilling, with W.T. resulting in a six-fold greater (8.75) number of average values as compared to C.C. (1.38). At five weeks chilling, W.T. produced over four-times as many average floral buds (9.33) as C.C. (2.08), resulting in a more significant difference of P=0.0084 (Figures 31 & 32).

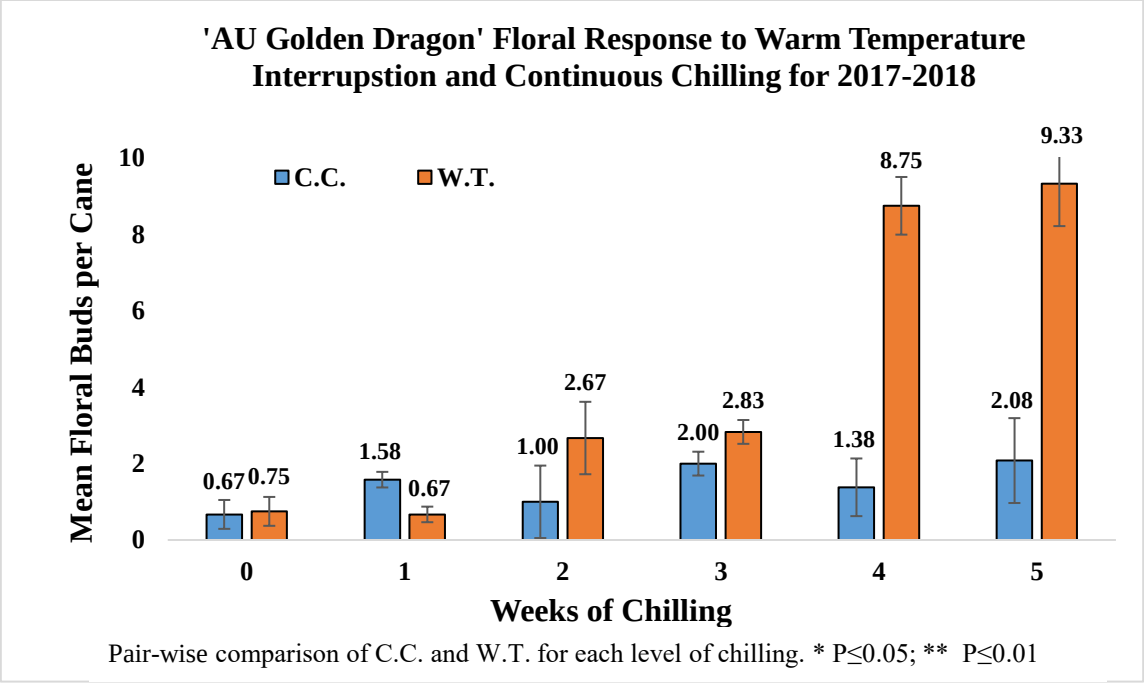


Figure 31 Histogram of 'AU Golden Dragon' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels for 2017-2018.

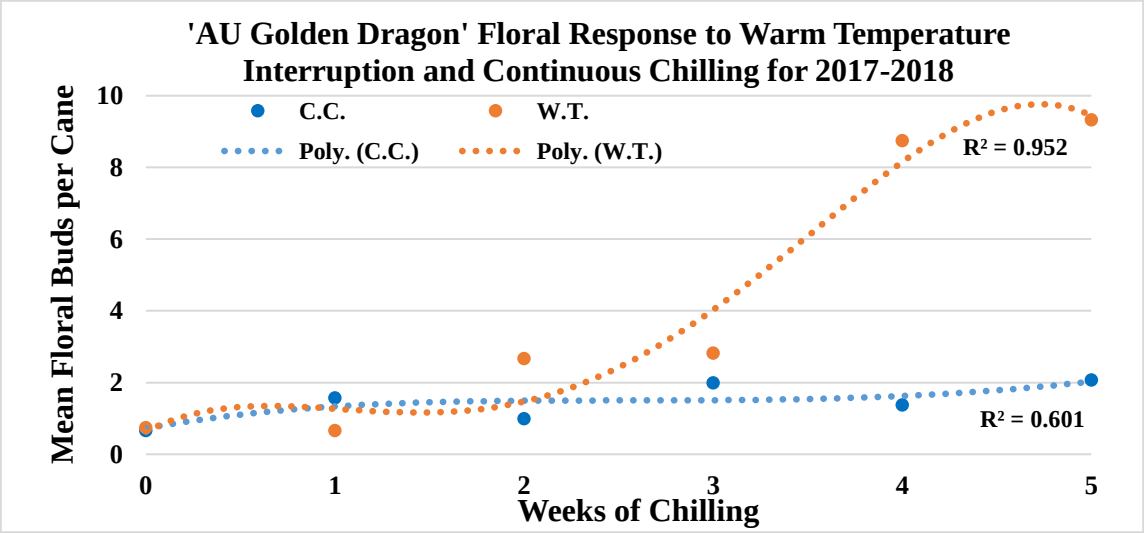


Figure 32 Scatterplot of 'AU Golden Dragon' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels for 2017-2018.

Data for the second year (2019) of 'AU Golden Dragon' floral response did not reveal significant differences at any chilling level. Average floral bud numbers for C.C. and W.T. were nearly identical (0.92 and 1.00, respectively) at the base chilling level, somewhat higher for W.T. (2.59) than C.C. (1.83) at one week chilling, but appreciably lower for W.T. (2.42) in comparison to C.C. (3.50) at the two week level. At three weeks chilling, average floral bud number was identical (3.92 for both treatments), noticeably higher for C.C. (5.83) compared to W.T. (4.50) at four weeks and identical (4.50) at the highest chilling level (Figures 33 & 34).

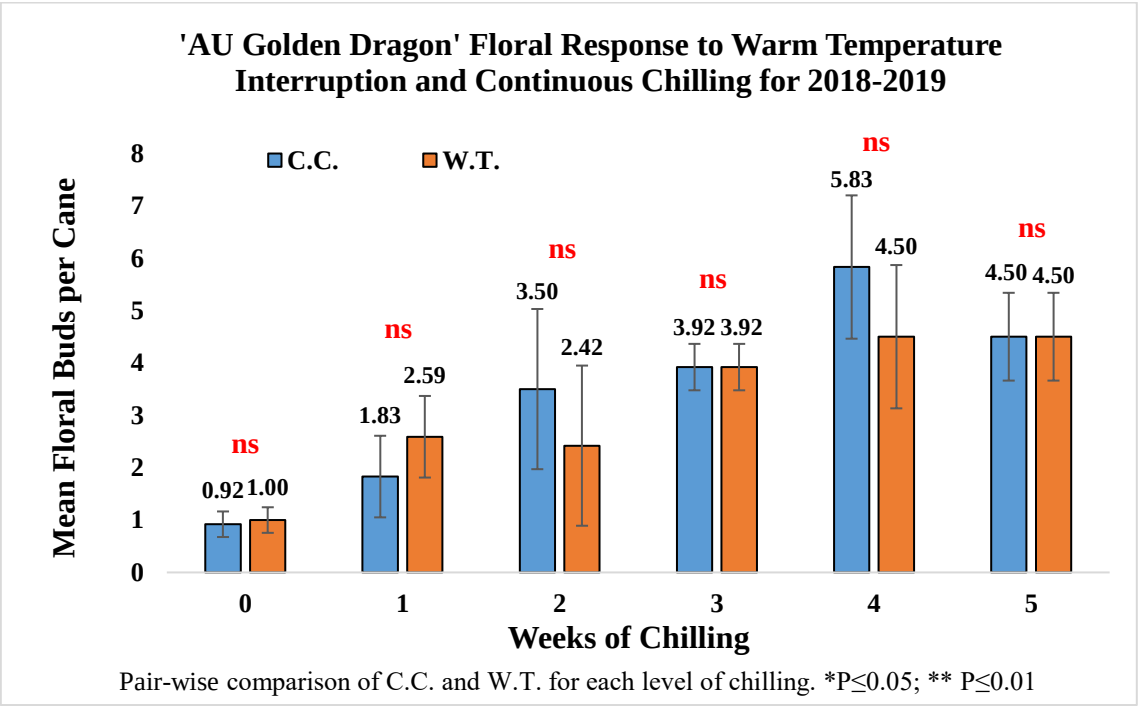


Figure 33 Histogram of 'AU Golden Dragon' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels for 2018-2019.

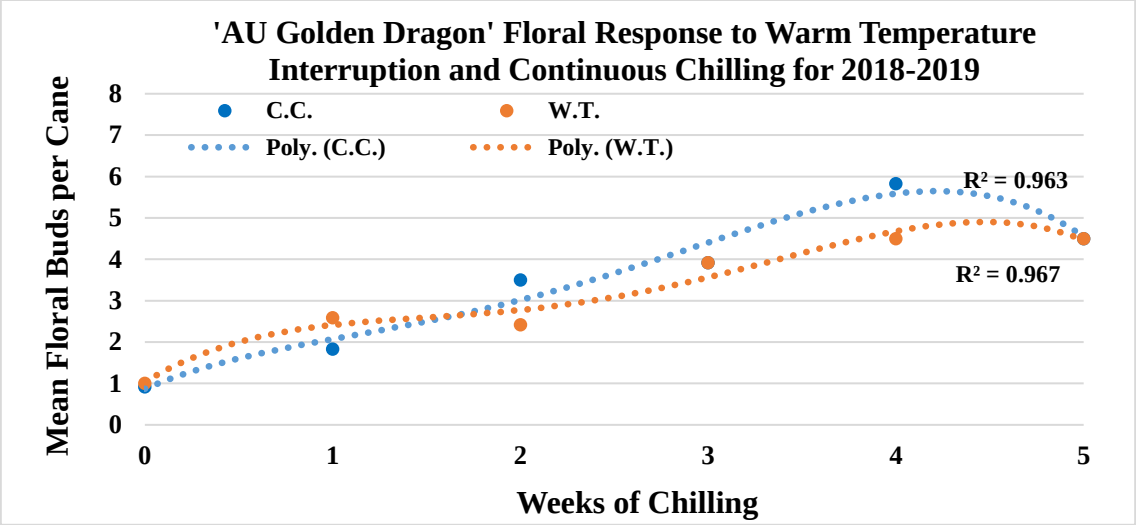


Figure 34 Scatterplot of 'AU Golden Dragon' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels for 2018-2019.

For floral response in 'AU Fitzgerald', analysis identified a significant year x treatment interaction (P = 0.0089) at the five week chilling level. Consequently, comparison of treatments was carried out separately for each year at this chilling level for floral bud number. For both years combined, W.T. resulted in appreciably greater average floral bud number per cane (3.26) in comparison to C.C. (1.29) at the base level chilling, comparable values (2.59 for C.C. and 2.42 for W.T.) at one week of chilling, and a moderately higher value W.T. (4.92) at two weeks and three weeks (7.50) chilling, as compared to C.C. treatments at these levels (3.79 and 6.87, respectively). However, at four weeks chilling, C.C. produced a significantly (P = 0.0476) greater average number of floral buds (9.08) in comparison to W.T. (7.37), once block effect (not significant), was excluded from the analysis. Average floral bud number for C.C. was much higher

(12.88) than that of W.T. (7.00), indicating a significant (P = 0.0014) treatment effect, however the strong (P=0.0089) year x treatment interaction suggests that comparisons be made separately for individual years (Figures 35 & 36).

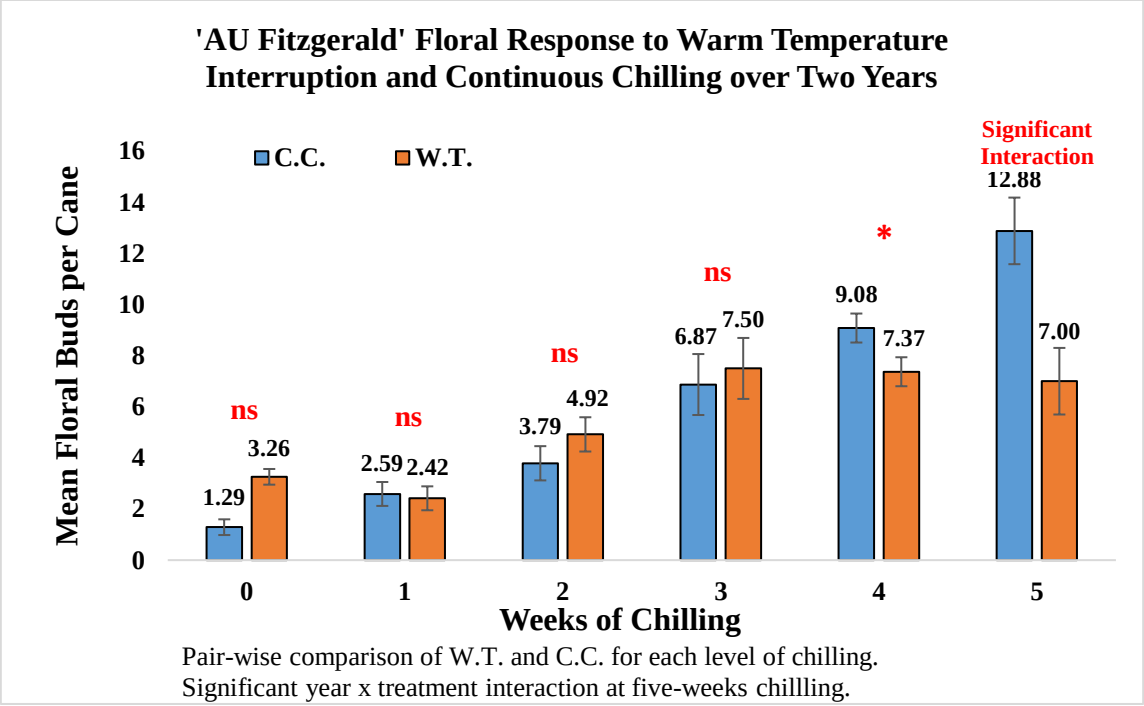


Figure 35 Histogram of 'AU Fitzgerald' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels over two years.

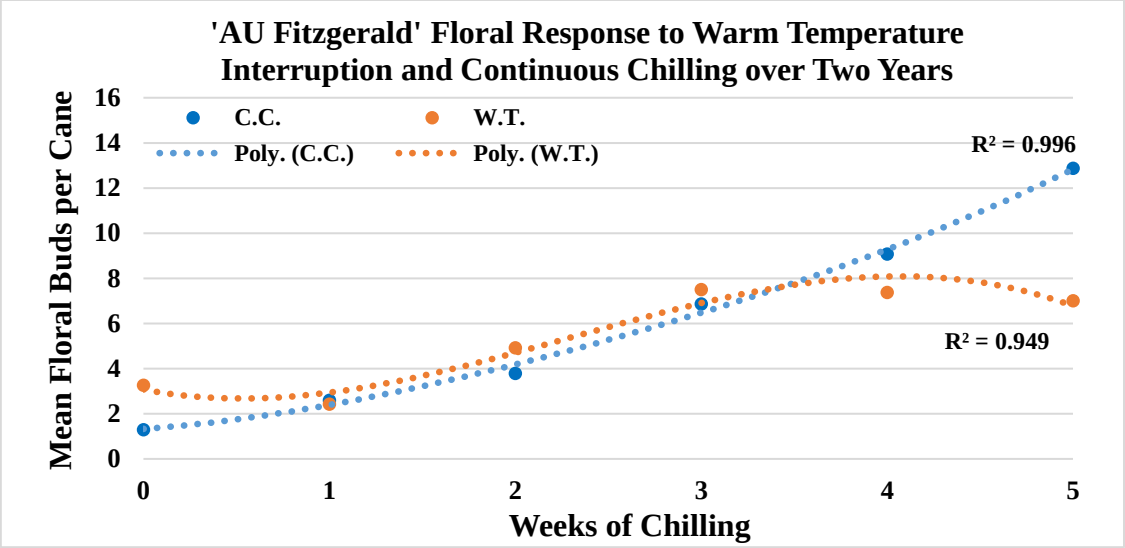


Figure 36 Scatterplot of 'AU Fitzgerald' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels over two years.

Comparison of floral response in 'AU Fitzgerald' during the first year (2018) generally yielded similar results for W.T. and C.C. at each chilling level. However, W.T. resulted in significantly (P = 0.0172) greater average floral buds (5.04) as compared to C.C. (2.17) at the base chilling level. There was also a significant (P = 0.0177) block effect at this chilling level. Average floral bud number was slightly higher for C.C. (3.42) than W.T. (2.75) at one week chilling and noticeably lower for C.C. at two weeks (4.42) and three weeks (6.50) in comparison to W.T. (5.92 and 8.25, respectively). At four and five weeks, C.C. resulted in a markedly (but not significantly) greater number of average floral buds (9.17 and 11.50, respectively) as compared to W.T. (8.00 and 9.83). Significant block effects were also observed at two weeks (P = 0.0241) and three weeks chilling (P = 0.0406) (Figures 37 & 38).

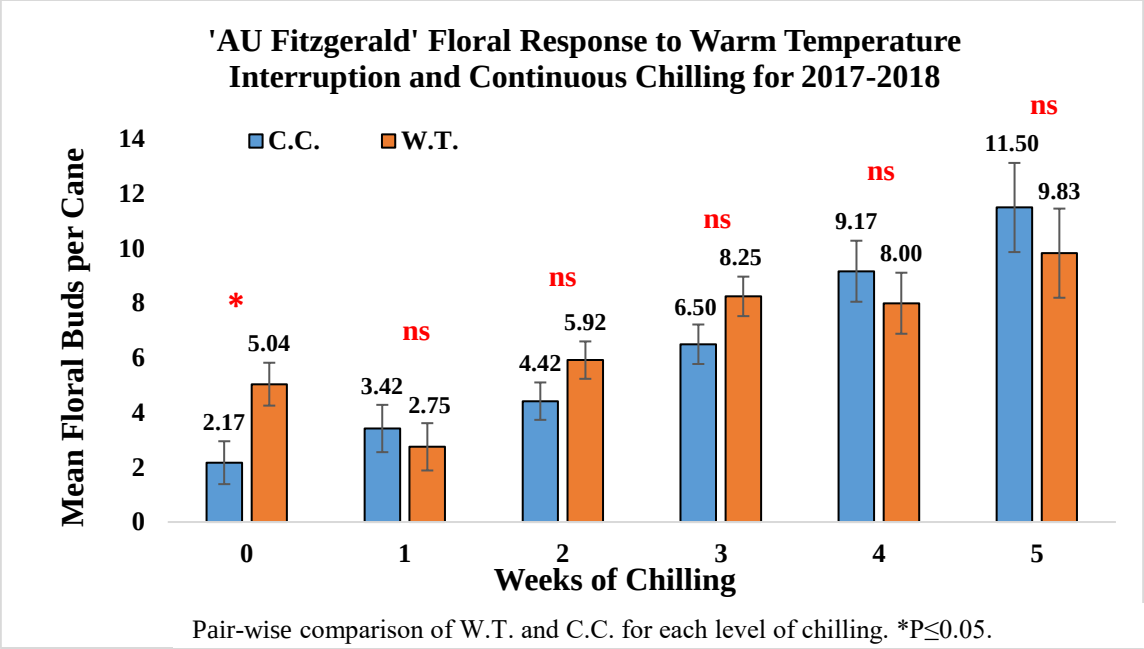


Figure 37 Histogram of 'AU Fitzgerald' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels for 2017-2018.

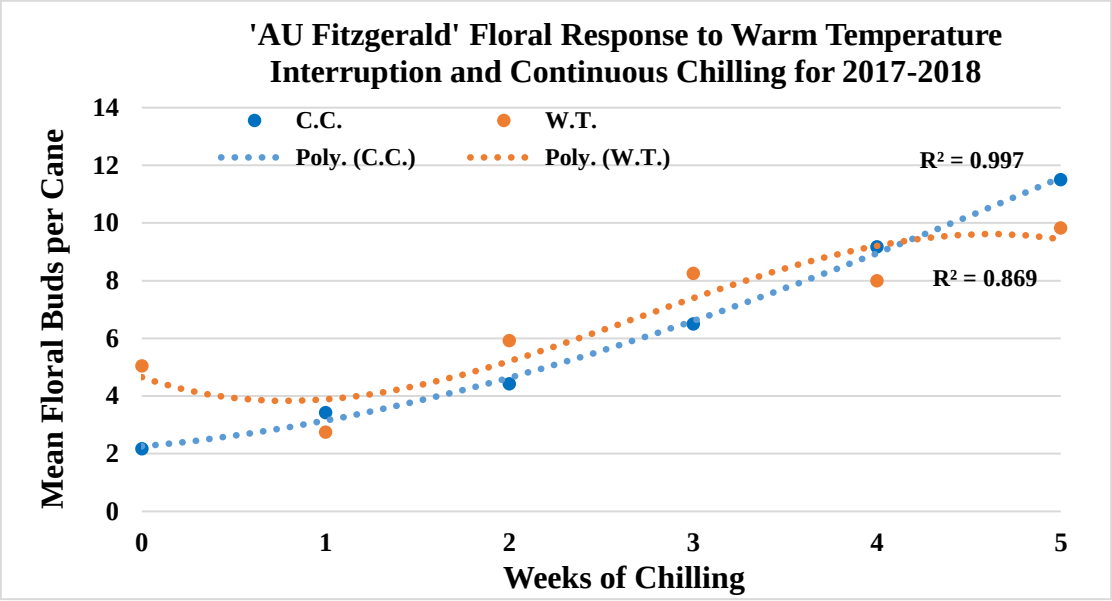


Figure 38 Scatterplot of 'AU Fitzgerald' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels for 2017-2018.

Floral response per cane followed a similar trend for ‘AU Fitzgerald’ in the second (2019) year at the lower levels of chilling. Average floral bud number per cane was appreciably (but not significantly) lower for C.C. (0.42) than W.T. (1.48) at base chilling, comparable at one week chilling (1.75 for C.C. and 2.08 for W.T.), and slightly lower for C.C. (3.17) than W.T. (3.92) at two weeks chilling. The pattern of greater floral response observed for W.T. changed at three weeks, at which C.C. produced slightly more average floral buds (7.25) than W.T. (6.25). This difference was greater, though still not significant, at four weeks chilling (9.00 for C.C. and 6.75 for W.T.). However, comparison at five weeks chilling resulted in a significant difference ($P = 0.0016$), with C.C. producing more than three-times as many floral buds on average (14.25) as compared to C.C. (4.17) at this highest chilling level. This difference was even stronger ($P = 0.0007$) when block effect (not significant) was removed from the analysis (data not shown) (Figures 39 & 40).

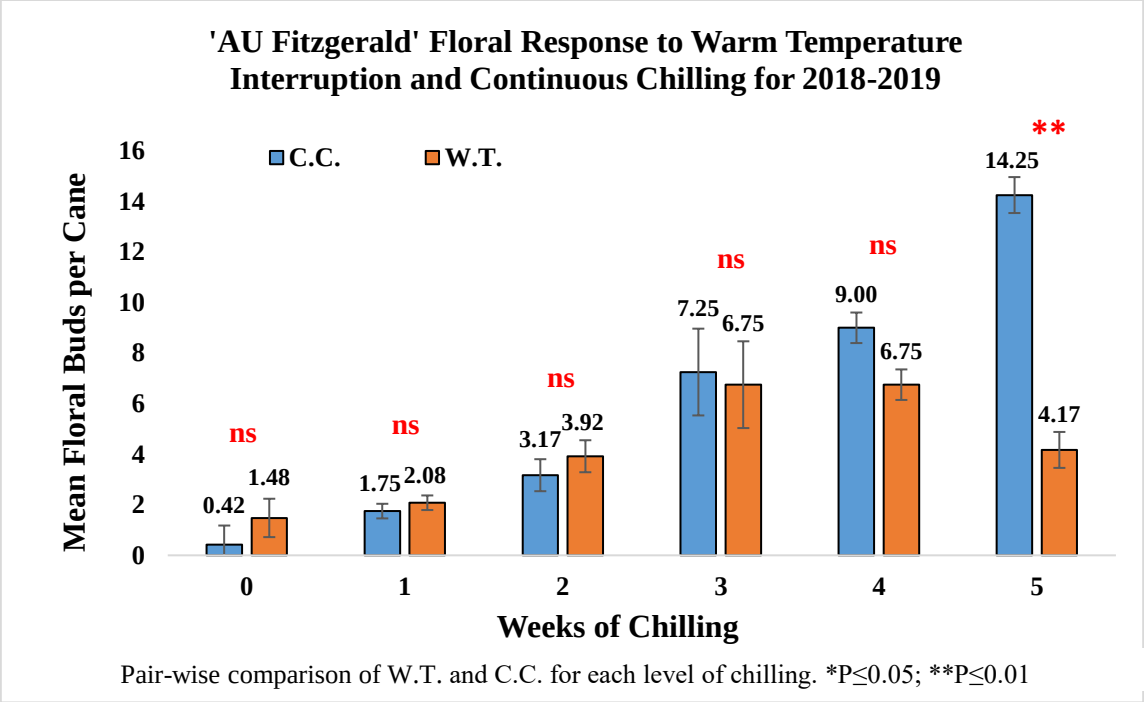


Figure 39 Histogram of 'AU Fitzgerald' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels for 2018-2019.

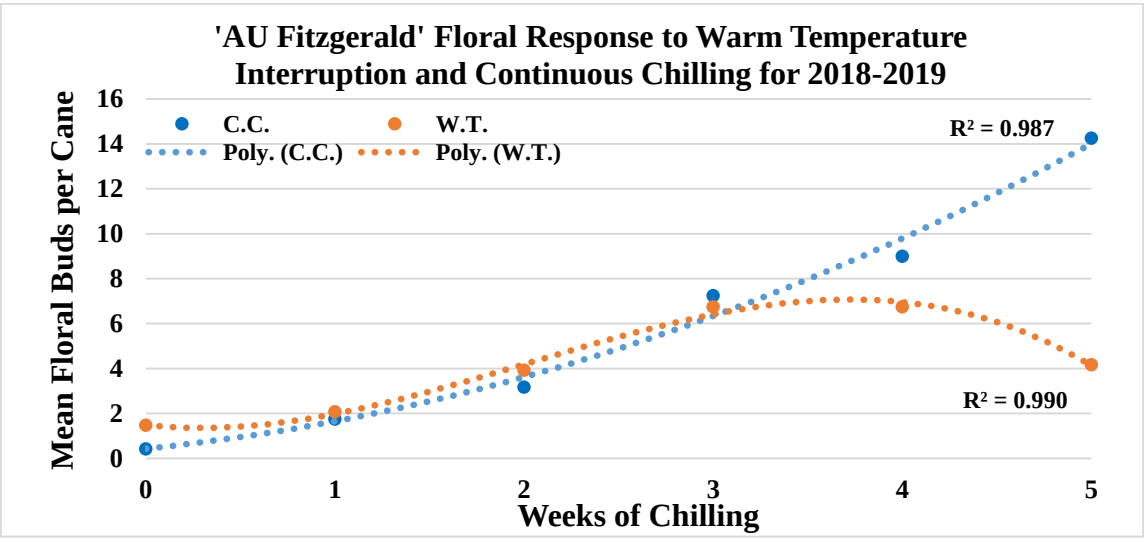


Figure 40 Scatterplot of 'AU Fitzgerald' kiwifruit mean floral bud number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels for 2018-2019.

Vegetative Budbreak Response to Chilling Type

As mentioned earlier, vegetative budbreak number per cane was not significantly different between years for either 'AU Golden Dragon' or 'AU Fitzgerald' (Figure 28). Both vegetative budbreak number (4.78 ± 0.165) and percent vegetative budbreak (59.8% ± 2.06) were slightly higher in 2019 as compared to 2018 (4.65 ± 1.22 and 58.1% ± 2.06, respectively) for 'AU Golden Dragon', on average across all treatments (Figure 28 and Table 11). Conversely, average vegetative budbreak number and percent vegetative budbreak per cane were slightly lower in 2019 (2.77 ± 1.26 and 34.5% ± 1.58, respectively) than in 2018 (2.94 ± 0.126 and 36.8% ± 1.58, respectively) for 'AU Fitzgerald' treatments (Figure 28 and Table 12).

Comparison of chilling type effect on average vegetative budbreak per cane in 'AU Golden Dragon' for both years did not result in significant differences at any chilling level. W.T. resulted in considerably greater number of buds (4.79 than C.C. (4.00) at the base level chilling. However, budbreak number for C.C. was slightly higher (4.02 compared to 3.88 for W.T.) at one week chilling. W.T. resulted in noticeably greater average budbreak per cane at two, three, four, and five weeks chilling (4.87, 5.21, 5.88, and 5.71, respectively) as compared to C.C. (4.21, 4.88, 5.00, and 4.83, respectively) at the same chilling levels. While average budbreak number was as much as 18% higher average budbreak per cane for W.T. at the highest chilling level, this difference was not significant (P = 0.0631). In this experiment, maximum vegetative budbreak number occurred at four weeks for both C.C. (5.00) and W.T. (5.88) (Figures 41 & 42).

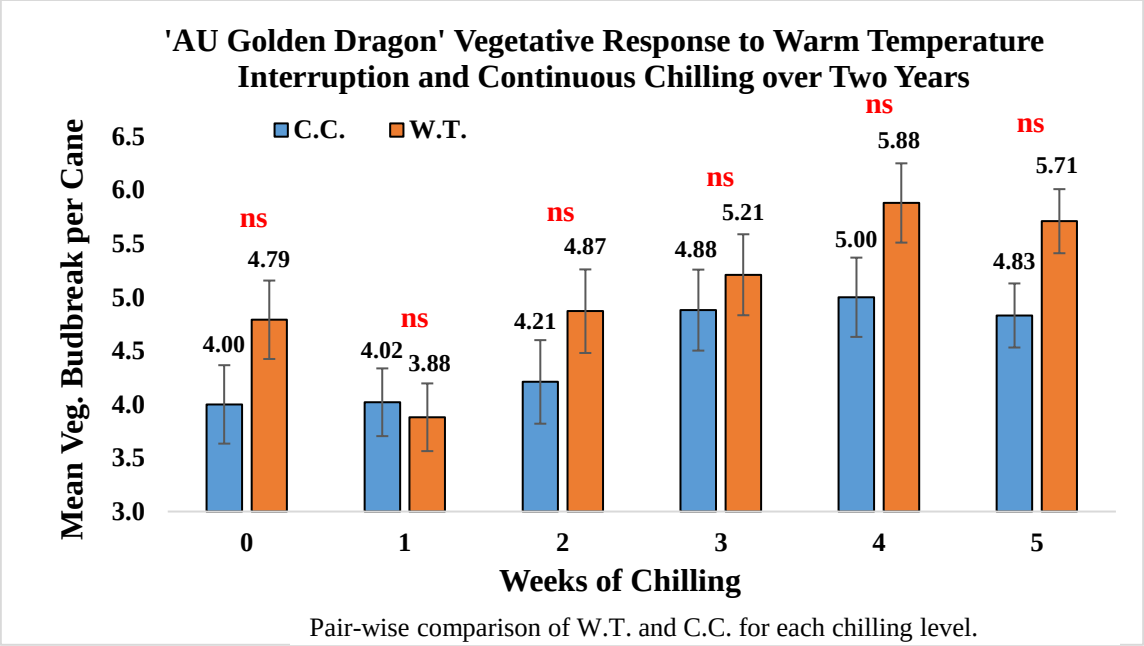


Figure 41 Histogram comparing mean vegetative budbreak number per cane response in 'AU Golden Dragon' kiwifruit to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels over two years.

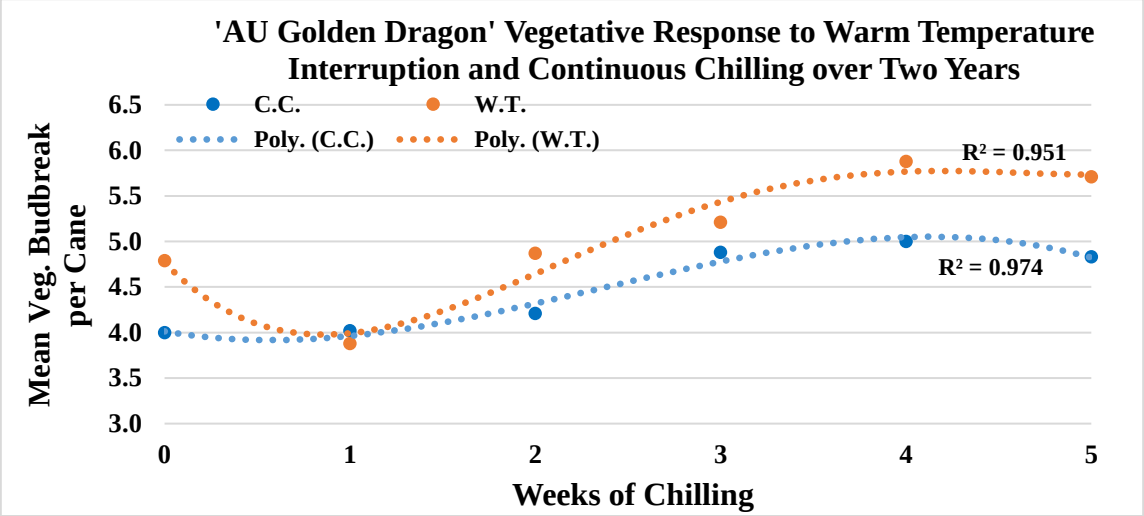


Figure 42 Scatterplot comparing mean vegetative budbreak number per cane response in 'AU Golden Dragon' kiwifruit to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels over two years.

For ‘AU Fitzgerald’, there were also no significant treatment differences in average vegetative budbreak number per cane at any chilling levels for both years. Similar to ‘AU Golden Dragon’, average budbreak was considerably higher with W.T. (3.02) compared to C.C. (2.42) at the base chilling level. Interestingly, average budbreak number was much lower following one week of simulated chilling for W.T. (2.34) and C.C. (2.21) alike. W.T. continued to result in higher values at two, three, four, and five weeks chilling (3.04, 3.42, 3.00, and 3.10 respectively) as compared to C.C. (2.79, 3.12, 2.92, and 2.87, respectively) at the same levels of chilling. The highest average vegetative budbreak for ‘AU Fitzgerald’ was observed at three weeks chilling for both C.C. (3.12) and W.T. (3.42) (Figures 43 & 44).

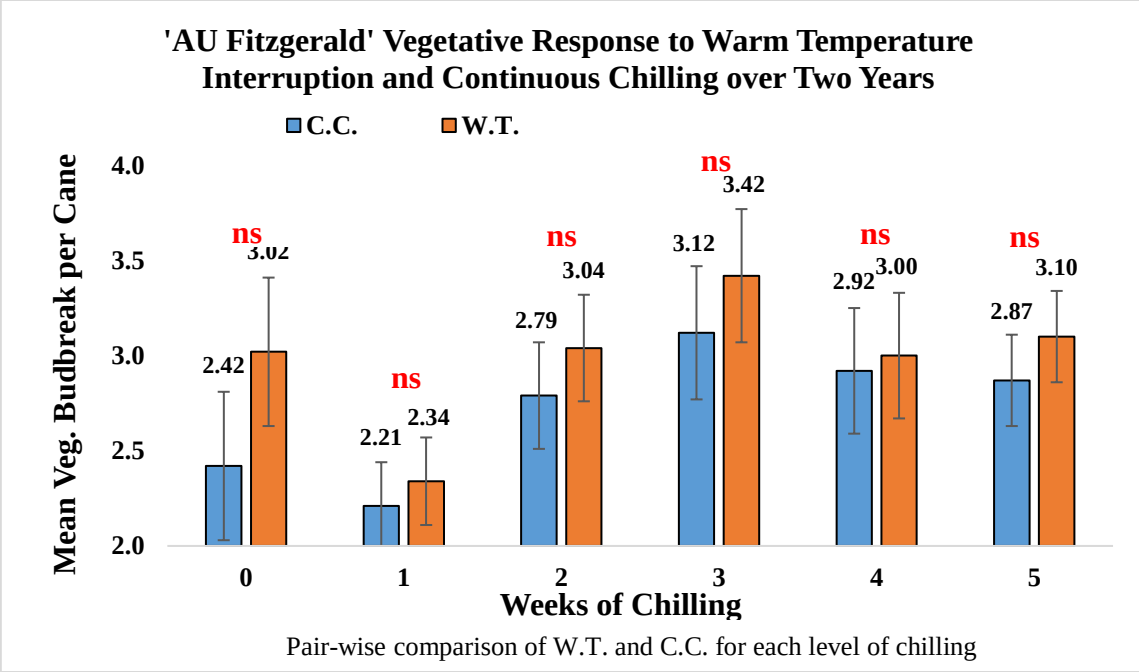


Figure 43 Histogram comparing mean vegetative budbreak number per cane response in 'AU Fitzgerald' kiwifruit to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels over two years.

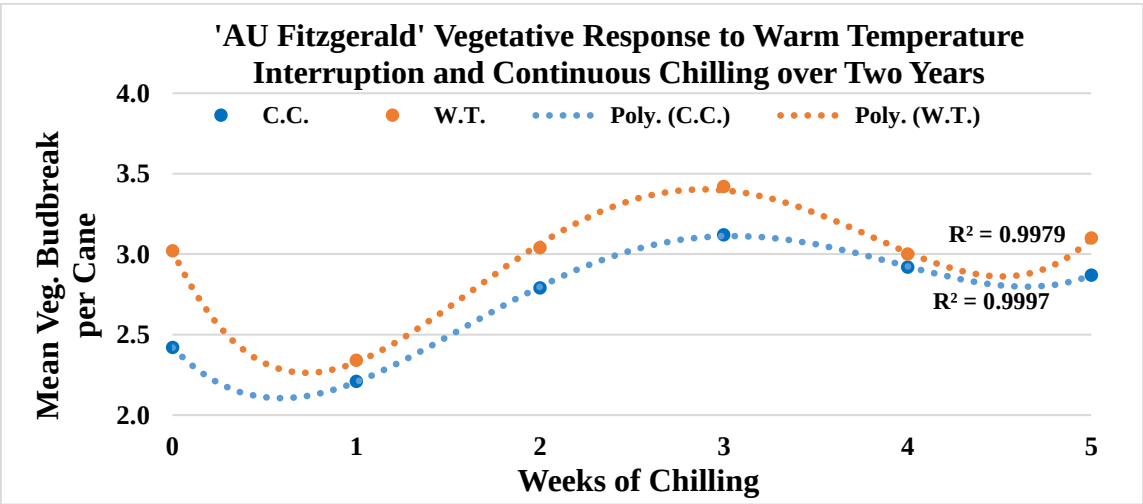


Figure 44 Scatterplot comparing mean vegetative budbreak number per cane response in 'AU Fitzgerald' kiwifruit to warm temperature interruption (W.T.) and continuous chilling (C.C.) across six chilling levels over two years.

Neither average vegetative budbreak per cane, whether expressed as a number or percentage, showed significant difference in treatment at any chilling level for either cultivar (Appendices 1 - 4). Therefore, results for individual years is not discussed here for these variables. Average percent budbreak per cane data was not presented graphically due to the absence of significant treatment effects and close similarity to that of average budbreak number per cane.

Percent vegetative budbreak per cane was expressed as the relative frequency of winter budbreak per cane, based on a possible total of eight buds. Because results for percent vegetative budbreak trended very closely with those of vegetative budbreak number, results are not presented graphically. Frequency of vegetative budbreak was comparable between W.T. and C.C. at base (51.0% and 50.0%, respectively) and one week chilling (48.4% and 50.3%, respectively) for 'AU Golden Dragon'. Exposure to intermittent warm temperature resulted in a greater (but not significantly greater) incidence of budbreak at two, three, four, and five weeks chilling (61.0%, 65.1%, 73.4%, and 71.4% respectively) than C.C. (52.6%, 60.9%, 62.5%, and 60.4%) at these same levels of chilling. As with the average number of vegetative budbreak, percent budbreak per cane was highest for 'AU Golden Dragon' at four weeks for both types of chilling, with WT. producing 73.4% budbreak (Table 13).

Average percent vegetative budbreak per cane for 'AU Fitzgerald' over two years was considerably, but not significantly higher with W.T. (37.8%) than C.C. (30.2%) at base level chilling (Table 14).

Table 13 Comparison of mean percent vegetative budbreak per cane response to continuous chilling (C.C.) and warm temperature interruption (W.T.) in 'AU Golden Dragon' kiwifruit across six chilling levels over two years.

Chilling Level	Consistent Chilling				Warm Temperature Interruption				Significance
	Days Warm Temp.	Mean	Standard Deviation	Standard Error	Days Warm Temp.	Mean	Standard Deviation	Standard Error	
Base	0	50.0%	11.71	4.58	3	51.0%	1.13	4.58	ns
1 week	0	50.3%	6.83	3.97	6	48.4%	12.29	3.97	ns
2 weeks	0	52.6%	13.49	4.88	9	61.0%	10.82	4.88	ns
3 weeks	0	60.9%	15.75	4.72	12	65.1%	5.67	4.72	ns
4 weeks	0	62.5%	14.07	4.62	15	73.4%	8.38	4.62	ns
5 weeks	0	60.4%	9.38	3.75	18	71.4%	9.44	3.75	ns

Percent vegetative budbreak based on number of lateral buds exhibiting bud burst (Brundell, 1975) divided by eight total lateral buds.
Pair-wise comparison at each level of chilling ($\alpha = 0.05$).

Table 14 Comparison of mean percent vegetative budbreak per cane response to continuous chilling (C.C.) and warm temperature interruption (W.T.) in 'AU Fitzgerald' kiwifruit across six chilling levels over two years.

Chilling Level	Consistent Chilling				Warm Temperature Interruption				Significance
	Days Warm Temp.	Mean	Standard Deviation	Standard Error	Days Warm Temp.	Mean	Standard Deviation	Standard Error	
Base	0	30.2%	14.17	4.91	3	37.8%	10.14	4.91	ns
1 week	0	27.6%	6.26	2.88	6	29.2%	8.08	2.88	ns
2 weeks	0	34.9%	8.36	3.45	9	38.0%	8.94	3.45	ns
3 weeks	0	39.1%	7.52	4.38	12	42.7%	13.57	4.38	ns
4 weeks	0	36.5%	13.75	4.10	15	37.5%	4.77	4.10	ns
5 weeks	0	36.0%	6.54	2.98	18	38.8%	8.32	2.98	ns

Percent vegetative budbreak based on number of lateral buds exhibiting bud burst (Brundell, 1975) divided by eight total lateral buds.
Pair-wise comparison at each level of chilling ($\alpha = 0.05$).

At all other chilling levels (two- through five weeks chilling), W.T. produced only slightly higher (2.4% on average) percent budbreak than C.C. across these chilling levels. The highest percent budbreak of 42.7% was observed at three weeks chilling with W.T.

Shoot Development Response to Chilling Type

As discussed earlier, shoot-related observations were only extensively made during second (2019) year of this experiment. For 'AU Golden Dragon', W.T. resulted in a higher, but not significantly higher, average per cane shoot number at every chilling level. The only exception was at three weeks chilling, at which C.C. produced a slightly higher average shoot number (4.78) than W.T. (4.33). The greatest difference observed between W.T. and C.C. occurred at five weeks chilling ($P = 0.0521$), at which W.T. resulted in 4.68 average shoots per cane, compared to 3.75 for C.C. Across all treatments, W.T. resulted in an average of 4.35 shoots per cane as compared to 3.86 for C.C. The highest average shoot number of 5.18 for 'AU Golden Dragon' was observed at four weeks chilling with W.T (4.25 with C.C.) (Figures 45 & 46).

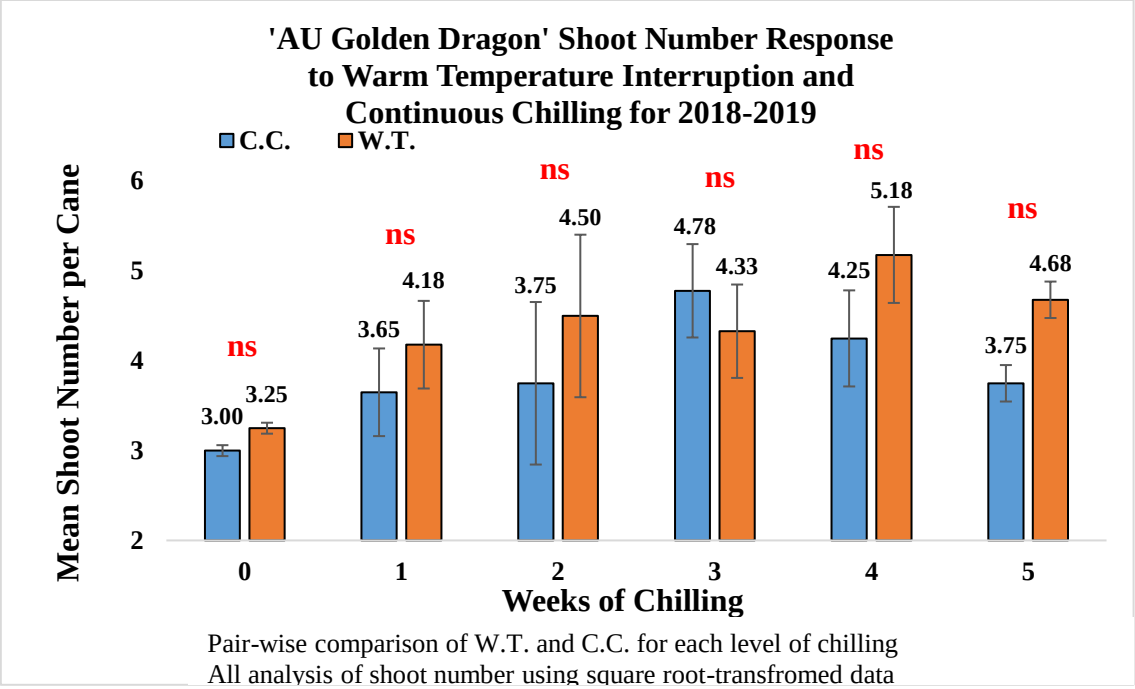


Figure 45 Histogram comparing mean vegetative shoot number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) in 'AU Golden Dragon' kiwifruit across six chilling levels for one year.

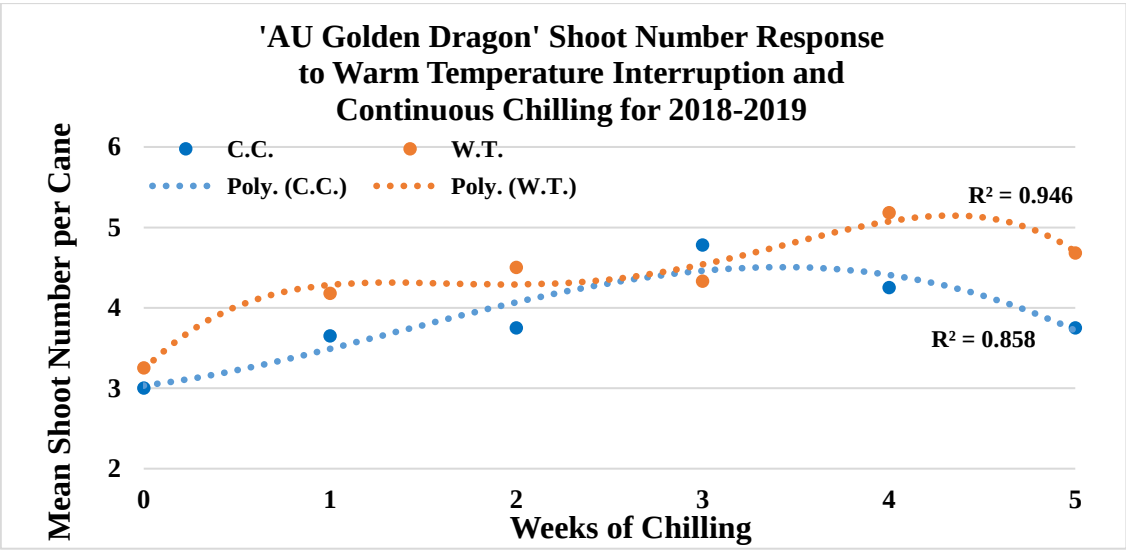


Figure 46 Scatterplot comparing mean vegetative shoot number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) in 'AU Golden Dragon' kiwifruit across six chilling levels for one year.

Average number of shoots per cane was considerably lower for the fuzzy cultivar AU Fitzgerald. As with 'AU Golden Dragon', there was no significant treatment difference at any of the six chilling levels. Shoot number closely followed the trend observed for vegetative budbreak number in this cultivar. Average shoot number was nearly identical for C.C. (2.50) and W.T. (2.58) at base chilling, slightly higher for C.C. at one week (2.03), but slightly lower (2.18) at two weeks chilling, as compared to W.T. (1.83 and 2.28, respectively) at these same chilling levels. C.C resulted in considerably greater values at the remaining chilling levels (three, four, and five weeks) (2.68, 3.00, and 2.85, respectively) as compared to W.T. (2.23, 2.60, and 2.40, respectively) for the same chilling levels. The greatest treatment difference for 'AU Fitzgerald' was observed at three weeks and five weeks chilling (tie), with a margin of 0.45 shoots (Figures 47 & 48).

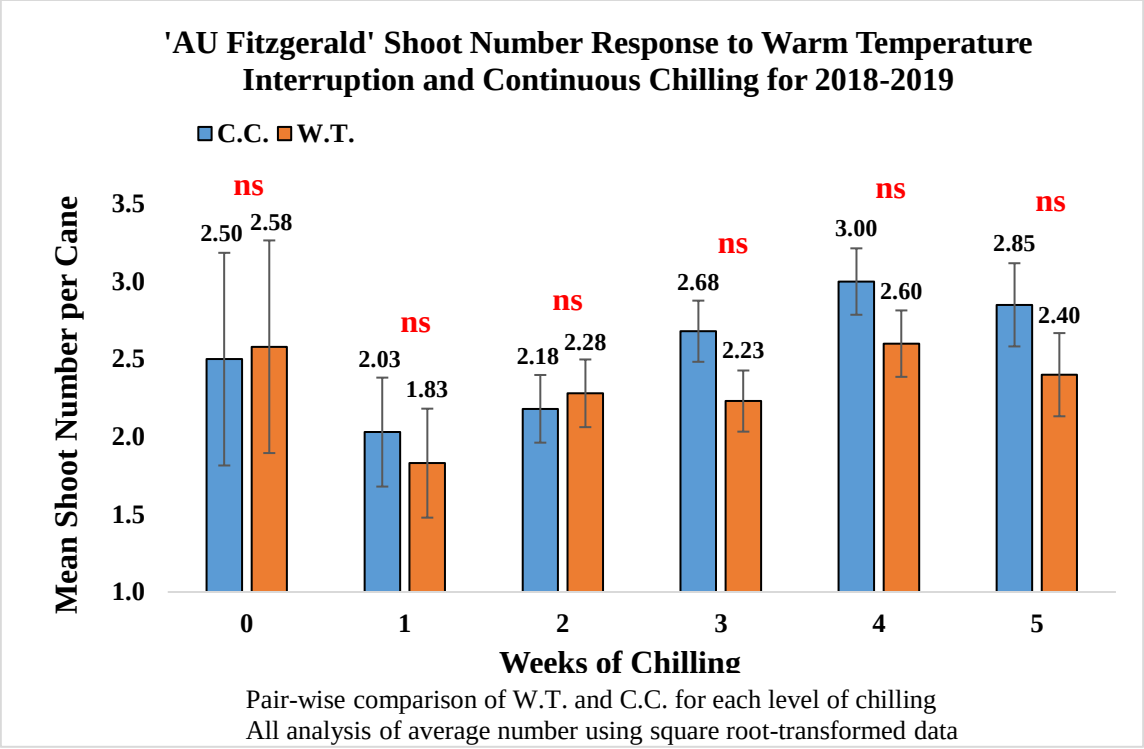


Figure 47 Histogram comparing mean vegetative shoot number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) in 'AU Fitzgerald' kiwifruit across six chilling levels for one year.

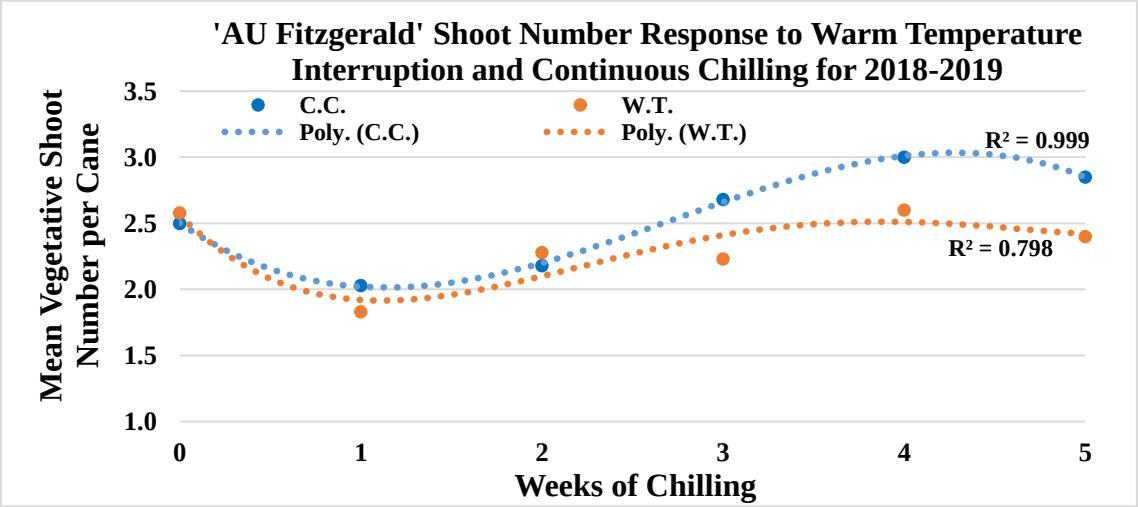


Figure 48 Scatterplot comparing mean vegetative shoot number per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) in 'AU Fitzgerald' kiwifruit across six chilling levels for one year.

Percent shoot development was calculated as percentage of the number of visible shoots relative to the total number of lateral buds or nodes (considered at eight). As with percent in relation to number of vegetative budbreak, percent shoot development trended closely with shoot number and consequently, the results are not presented graphically. For the gold cultivar AU Golden Dragon, average percent shoot development per cane was higher for W.T. at every chilling level, as compared to C.C. (average margin of 6.1% across all chilling levels), except at three weeks chilling (59.4% for C.C.; 54.2% for W.T.). While none of these differences were significant, the greatest margin of 11.5% was observed at four weeks chilling ($P = 0.0520$). There was also a significant block effect ($P = 0.00014$) at the base chilling level. The highest average percent shoot development per cane of 64.6% occurred at four weeks chilling with W.T. for 'AU Golden Dragon' (Table 15).

Similar to 'AU Golden Dragon', average percent shoot development per cane trended closely with average shoot number for 'AU Fitzgerald'. Comparable values were observed at base level, one, and two weeks chilling for C.C. (31.3%, 25.0%, and 27.1%, respectively) and W.T. (32.3%, 22.9%, and 28.1%, respectively), while four- and five-weeks chilling resulted in more noticeably higher values for C.C. (37.5% and 34.5%, respectively) as compared to W.T. (32.3% and 30.2%, respectively) at these levels of chilling. A significant block effect ($P = 0.0382$) was observed at two weeks chilling. The greatest difference in shoot development between treatments of 5.2% was observed at four and five weeks chilling (tie) and the highest value of 37.5% occurred at four weeks of chilling with C.C. (Table 16).

Table 15 Comparison of mean percent shoot development per cane response to continuous chilling (C.C.) and warm temperature interruption (W.T.) in 'AU Golden Dragon' kiwifruit across six chilling levels for one year.

Chilling Level	Consistent Chilling				Warm Temperature Interruption				Significance
	Days Warm Temp.	Mean	Standard Deviation	Standard Error	Days Warm Temp.	Mean	Standard Deviation	Standard Error	
Base	0	37.5%	1.04	0.74	3	40.6%	1.04	0.74	ns
1 week	0	45.9%	8.59	6.07	6	52.1%	8.59	6.07	ns
2 weeks	0	46.9%	15.79	11.17	9	56.3%	15.79	11.17	ns
3 weeks	0	59.4%	9.39	6.64	12	54.2%	9.39	6.64	ns
4 weeks	0	53.1%	9.37	6.63	15	64.6%	9.37	6.63	ns
5 weeks	0	46.9%	3.56	2.52	18	58.3%	3.56	2.52	ns
Average		48.3%	7.96	5.63		54.4%	7.96	5.63	

Percent shoot development based on number of shoots arising from lateral buds divided by eight total lateral buds.
Pair-wise comparison at each level of chilling ($\alpha = 0.05$).
All analysis of percent shoot development data using square root transformation
Non-transformed data for percent shoot number presented

Table 16 Comparison of mean percent shoot development per cane response to continuous chilling (C.C.) and warm temperature interruption (W.T.) in 'AU Fitzgerald' kiwifruit across six chilling levels for one year.

Chilling Level	Consistent Chilling				Warm Temperature Interruption				Significance
	Days Warm Temp.	Mean	Standard Deviation	Standard Error	Days Warm Temp.	Mean	Standard Deviation	Standard Error	
Base	0	31.3%	11.94	8.44	3	32.3%	11.94	8.44	ns
1 week	0	25.0%	6.00	4.24	6	22.9%	6.00	4.24	ns
2 weeks	0	27.1%	3.95	2.79	9	28.1%	3.95	2.79	ns
3 weeks	0	33.3%	3.56	2.52	12	28.2%	3.56	2.52	ns
4 weeks	0	37.5%	3.54	2.50	15	32.3%	3.54	2.50	ns
5 weeks	0	35.4%	4.61	3.26	18	30.2%	4.61	3.26	ns
Average		31.6%	5.60	3.96		29.0%	5.60	3.96	

Percent shoot development based on number of shoots arising from lateral buds divided by eight total lateral buds.
Pair-wise comparison at each level of chilling ($\alpha = 0.05$).
All analysis of percent shoot development data using square root transformation
Non-transformed data for percent shoot number presented

Shoot Development Relative to Budbreak

Lateral budbreak, as described by Brundell (1975) did not necessarily result in shoot development. Frequency of shoot development relative to vegetative budbreak, referred to here as 'percent vegetative bud to shoot' was surveyed during the second year (2019) to determine if either the amount of chilling or type (W.T. vs. C.C.) had any impact on lateral bud progression into shoots. Comparison between W.T. and C.C. effect on average 'percent vegetative bud to shoot' (per cane) did not reveal significant treatment differences at any chilling level for 'AU Golden Dragon'. However, notable differences were observed. Average 'percent vegetative bud to shoot' was slightly higher for C.C. (81.7%) than W.T. (78.8) at base chilling, while W.T. resulted in noticeably higher percentages at one and two weeks chilling (91.2% and 85.1%, respectively) as compared to C.C. (84.2% and 85.1, respectively). C.C. produced slightly higher values at three weeks chilling (89.3%; P=0.0632) and four weeks chilling (92.5%) in comparison to W.T. (85.3% and 87.2%, respectively) at these chilling levels, while W.T. resulted in a slightly higher (89.4% compared to 84.5% for C.C.) frequency at five weeks chilling. A significant (P= 0.0112) block effect was present at three weeks chilling. The greatest treatment difference of 5.3% was observed at four weeks chilling. Across all treatments, there was a margin of only 0.8% favoring W.T., with the highest value recorded at four weeks chilling with C.C. 'Percent vegetative bud to shoot' did not appear to be strongly affected by chilling (level) (Figures 49 & 50).

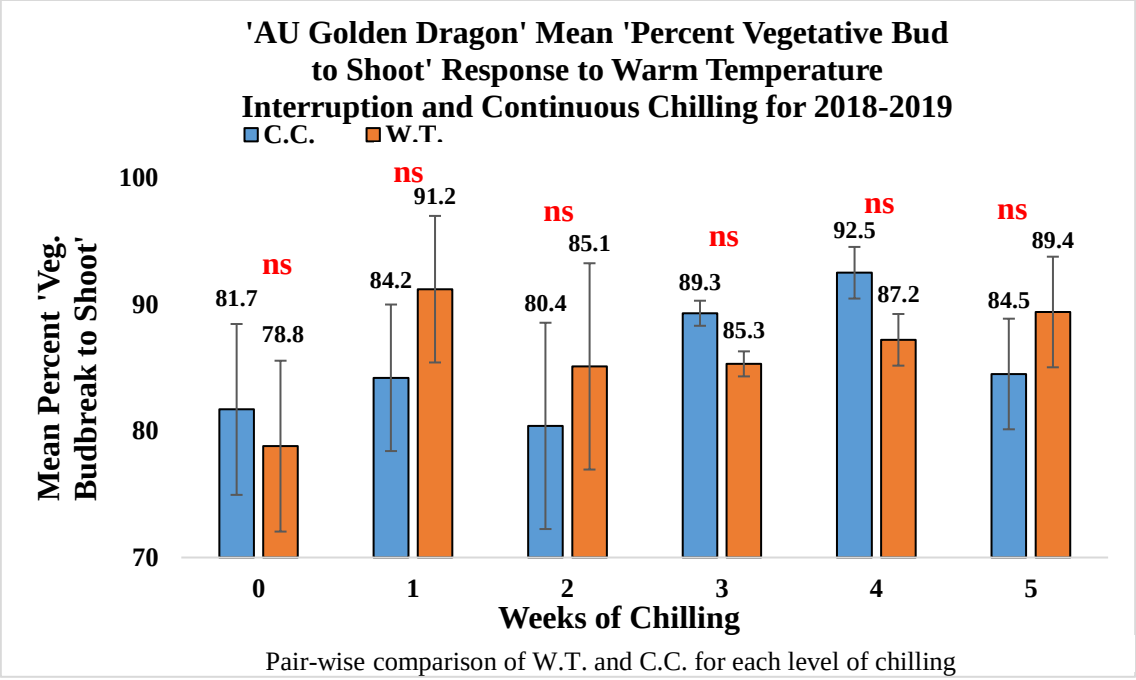


Figure 49 Histogram comparing average 'percent vegetative budbreak to shoot' per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) in 'AU Golden Dragon' kiwifruit across six chilling levels for one year.

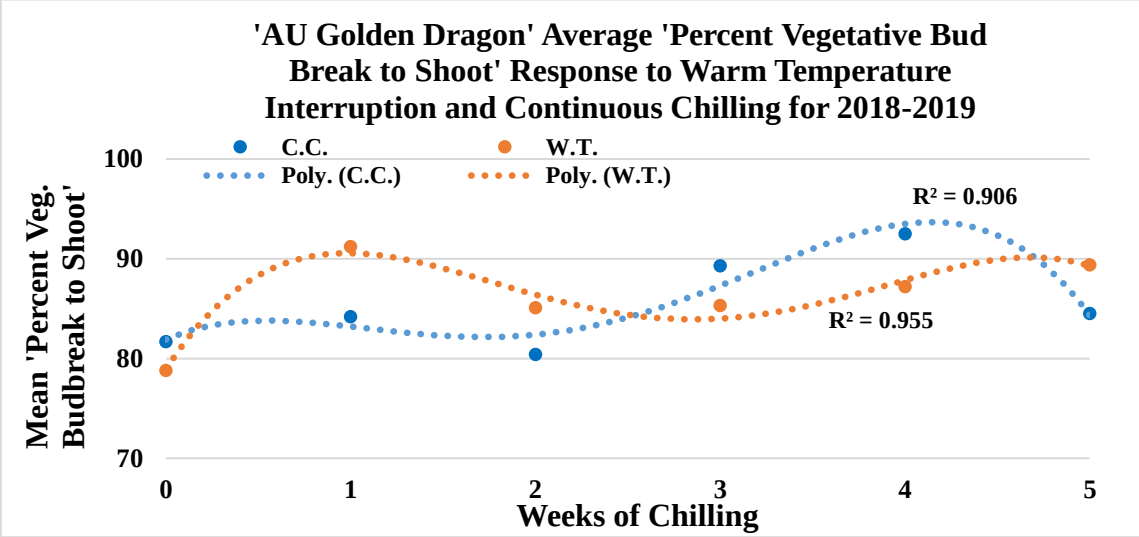


Figure 50 Scatterplot comparing mean 'percent vegetative budbreak to shoot' per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) in 'AU Golden Dragon' kiwifruit across six chilling levels for one year.

For the cultivar AU Fitzgerald, there were also no significant differences for average ‘percent vegetative bud to shoot’ per cane between treatments at any chilling level. However, C.C. resulted in a noticeably higher value (92.5%) at the base level of chilling compared to W.T. (83.1%), while W.T. produced the greatest percentage (96.8%) at one week chilling (compared to 90.3% for C.C.). Treatment values were lower at two and three weeks chilling levels for both types of chilling, with C.C. producing higher percentages (83.2 and 85.7) compared to W.T. (81.0 and 78.7) at these levels. W.T. resulted in considerably greater ‘percent vegetative bud to shoot’ (94.0% compared to 87.1% for C.C.) at four weeks of chilling, while W.T. and C.C. produced nearly identical frequencies (88.5% and 89.0%) at five weeks chilling. Across all treatments, C.C. netted an average of only 1.0% greater ‘percent vegetative bud to shoot’ per cane. As with ‘AU Golden Dragon’, this variable did not appear to be influenced strongly by amount of chilling (Figures 51 & 52).

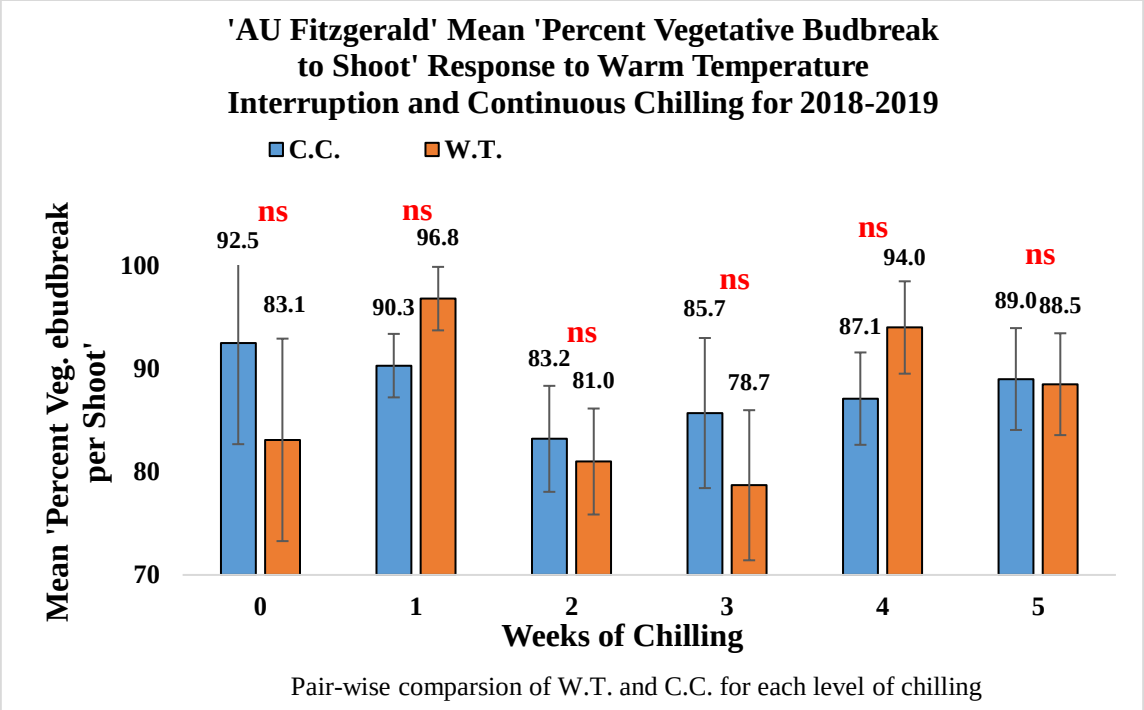


Figure 51 Histogram comparing mean 'percent vegetative budbreak to shoot' per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) in 'AU Fitzgerald' kiwifruit across six chilling levels for one year.

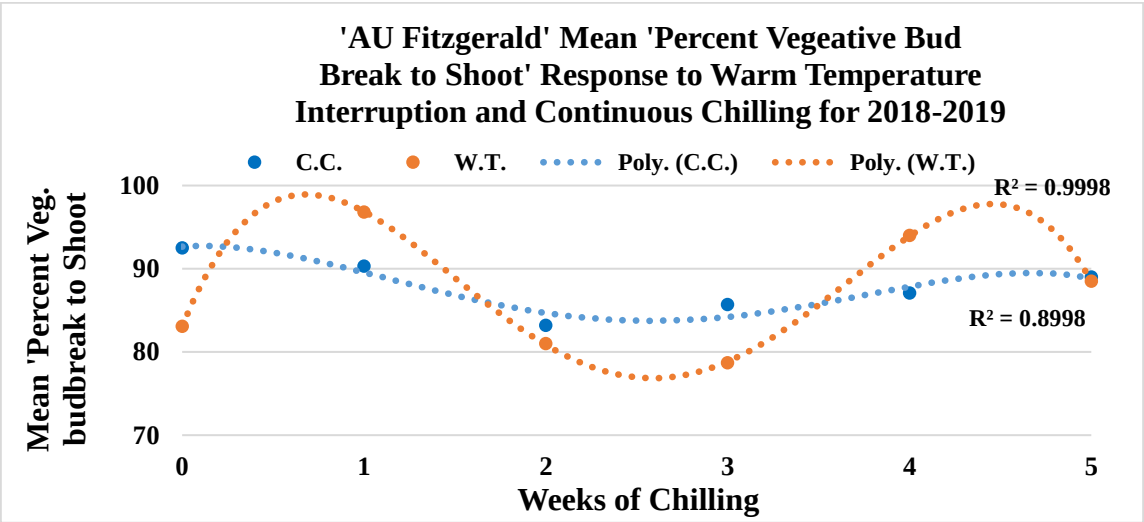


Figure 52 Scatterplot comparing mean 'percent vegetative budbreak to shoot' per cane response to warm temperature interruption (W.T.) and continuous chilling (C.C.) in 'AU Fitzgerald' kiwifruit across six chilling levels for one year.

Effect of Cane Diameter

Average cane diameter (mm) was measured at the termination of observations for each cane. As with all other variables, observations were reported as an average for each jar (three-cane subsample). While cane diameter was not intentionally included as an experimental variable, effect of cane size was surveyed, as it can influence vegetative and especially floral response (Snowball et al., 1996). Existence of significant response to cane diameter was assessed for all other dependent variables. Average cane diameter (all treatments) was significantly ($P<0.0001$) larger for both 'AU Golden Dragon' and 'AU Fitzgerald' in 2018 (12.2 ± 0.13 and 12.6 ± 0.14) than in 2019 (10.4 ± 0.11 and 9.6 ± 0.14, respectively). Average cane diameter (mm) showed ranges of 10.5 – 15.0 and 10.5 – 14.7 for these cultivars in 2018 and 9.0 – 11.5; 8.1 – 10.8 in 2019, with the first year being associated with 24% greater size, on average, for both cultivars (all treatments). However, it should be noted that for each cultivar and within each year, comparison of chilling types (W.T. and C.C.) did not reveal significant differences in average cane diameter (across all and between each chilling level) (Table 17).

Cane diameter did not have a significant effect on any of the measured variable across both years for either cultivar. However, regression analysis of 'AU Fitzgerald' data showed a strong ($P<0.0001$; $R_2 = 0.29$) negative ($\beta_1 = -0.60$) floral response to average cane diameter across all treatments during 2019. Average floral bud number per cane was significantly and negatively ($\beta_1 = -0.53$) influenced by average cane diameter

Table 17 Comparison of mean cane diameter by year, cultivar, and chilling type (W.T. and C.C.) for two kiwifruit cultivars over two years.

Comparison Parameter	Treatment	Mean	Range	Standard Error	Treatment	Mean	Range	Standard Error	Significance
Year (both cultivars)	Year 2018	12.4	10.5 – 15.0	0.10	Year 2019	10.0	8.1 – 11.5	0.10	P<0.0001
'AU Golden Dragon' by year	Year 2018	12.2	10.5 – 15.0	0.13	Year 2019	10.4	9.0 – 11.5	0.11	P<0.0001
'AU Fitzgerald' by year'	Year 2018	12.6	10.5 – 14.7	0.14	Year 2019	9.6	8.1 – 10.8	0.14	P<0.0001
'AU Golden Dragon' by Chilling Type (2018)	C.C.	12.2	10.7 – 15.0	0.26	W.T.	12.1	10.5 – 14.2	0.25	ns
'AU Golden Dragon' by Chilling Type (2019)	C.C.	10.6	9.8 – 11.5	0.10	W.T.	10.3	9.0 – 11.2	0.10	ns
'AU Fitzgerald' by Chilling Type (2018)	C.C.	12.5	10.8 – 14.0	0.20	W.T.	12.7	10.5 – 14.7	0.20	ns
'AU Fitzgerald' by Chilling Type (2019)	C.C.	9.7	8.1 – 10.8	0.18	W.T.	9.6	8.1 – 10.8	0.19	ns

Pair-wise comparison of chilling type (W.T. and C.C.) across all levels of chilling ($\alpha = 0.05$).
Average based diameter (mm) measured at top of jar, based on three-cane subsample

(P = 0.0216; R^2 = 0.30) for ‘AU Golden Dragon’ with W.T. (across all chilling levels) in 2018.

Average cane diameter had a significant effect on both vegetative budbreak number (P = 0.0313; R^2 = 0.14) and percent vegetative budbreak (P = 0.0319; R^2 = 0.14 in ‘AU Golden Dragon’ (across all treatments) during 2018. During the same year, significant responses to average cane diameter were also observed for average vegetative budbreak number (P = 0.0330; R^2 = 0.10) and percent vegetative budbreak (P = 0.0327; R^2 = 0.10) in the cultivar AU Fitzgerald across all treatments. For budbreak number and percent budbreak, this response was negative (β_1 = -0.42 and -0.05) in ‘AU Golden Dragon’ and positive (β_1 = 0.30 and 0.04) in ‘AU Fitzgerald’). In 2019 average cane diameter had a significant effect on vegetative budbreak number (P = 0.0449; R^2 = 0.17) and average vegetative percent vegetative budbreak (P = 0.0449; R^2 = 0.17) in ‘AU Fitzgerald’ for C.C. across all chilling levels. Cane diameter was not significantly different between W.T. and C.C. for this cultivar in 2019. Again, average cane diameter was not significantly different between W.T. and C.C. for any of these relationships.

Chilling Effect and Chilling Requirement Estimation

‘AU Golden Dragon’ Floral Chilling Requirements

Estimation of chilling requirement was carried out separately for chilling type (W.T. and C.C.) and for each year, due to previously discussed year x treatment interactions associated with floral response. Chilling had a significant effect on average

per-cane floral bud number (across all levels) for ‘AU Golden Dragon’ for W.T. treatments in 2018 ($P<0.0001$) and 2019 ($P = 0.0052$) and for C.C. in 2019 ($P = 0.0106$). For ‘AU Fitzgerald’, floral response was significantly affected by chilling in 2018 for C.C. ($P = 0.0015$) and in 2019 for both W.T. ($P = 0.0005$) and for C.C. ($P<0.0001$).

Average floral bud number per cane for ‘AU Golden Dragon’ in 2018 under W.T. consisted of only two statistical groups: 1.) base (0.75), one week (0.67), two weeks (2.67), and three weeks chilling (2.83); 2.) four weeks (8.75) and five weeks (9.33) chilling. There was also a significant ($P = 0.0394$) block effect associated with the data. Based on Tukey’s HSD analysis, a statistical maximum value of 8.75 was met at four weeks chilling, indicating a maximum floral chilling requirement for ‘AU Golden Dragon’ at an estimated amount of approximately 1,006 Richardson Units. (Figure 53).

Floral response to continuous chilling in 2018 showed a very unusual trend, as average floral bud number per cane increased from 0.67 at base chilling to 1.58 at one week, then dropped to 1.00 at two weeks, increased to 2.00 at three weeks, dropped to 1.38 at four weeks, before finally reaching a maximum value of 2.08 at five weeks chilling. Not only did the analysis fail to identify an upper statistical threshold for floral chilling requirement, but chilling effect was not significant across the six levels for ‘AU Golden Dragon’ with C.C. in 2018 (Figure 54).

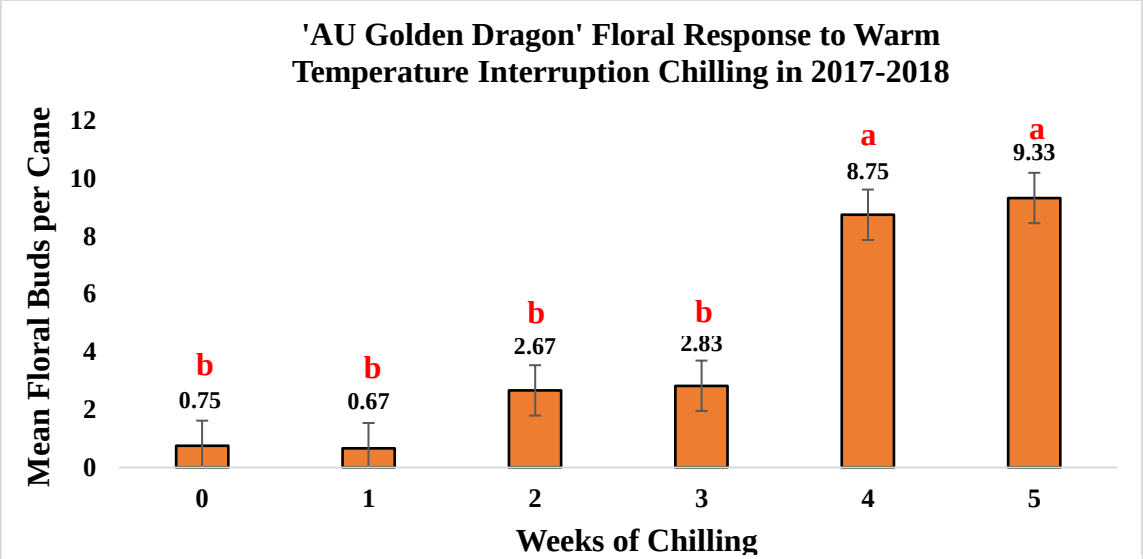


Figure 53 Histogram of 'AU Golden Dragon' kiwifruit floral (mean floral buds per cane) response to warm temperature interruption (W.T.) across six chilling levels in 2017-2018.

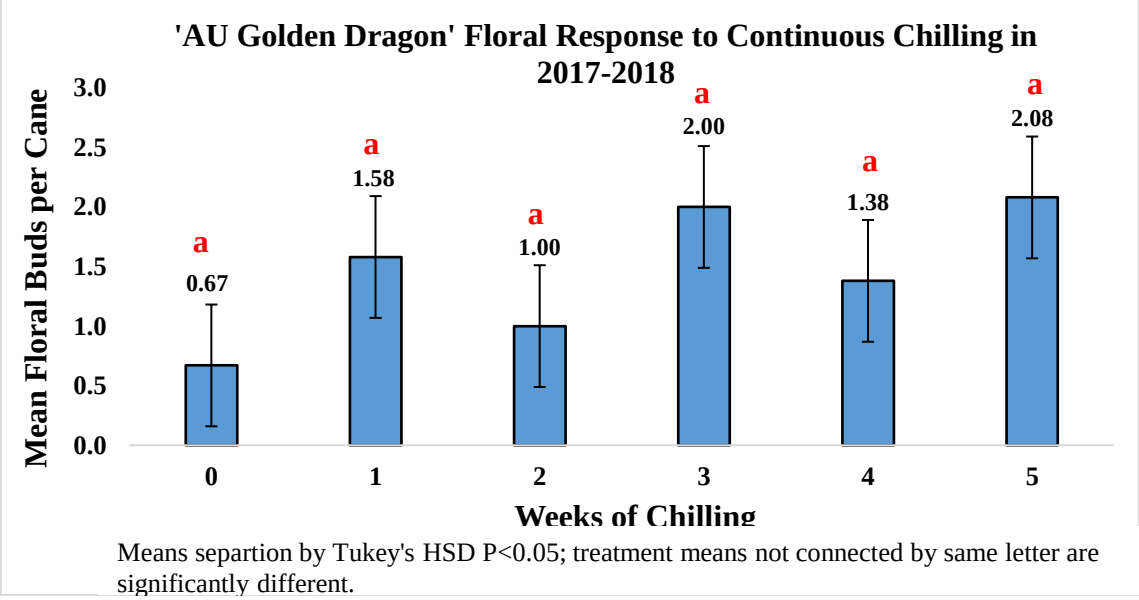


Figure 54 Histogram of 'AU Golden Dragon' kiwifruit floral (mean floral buds per cane) response to continuous chilling (C.C.) across six chilling levels in 2017-2018.

Floral response for ‘AU Golden Dragon’ under W.T. conditions in 2019 separated into three statistical groups: 1.) base chilling (1.00); 2.) one week (2.59) and two weeks (2.42) chilling; 3.) three weeks (3.92), four weeks (4.50), and five weeks (4.50) chilling. Based on Tukey’s HSD, the maximum floral chilling requirement was met at three-weeks chilling with an estimate of approximately 864 Richardson units (Figure 55).

The average floral bud number per cane for ‘AU Golden Dragon’ under continuous chilling separated into three statistical groups in 2019: 1.) base-chilling (0.92); 2.) one week (1.83), two weeks (3.50), and three weeks (3.92) chilling; 3.) four weeks (5.83) and five weeks (4.50) chilling. Based on the analysis, maximum floral chilling requirement was met at four weeks or approximately 1,032 Richardson units (Table 8 and Figure 56).

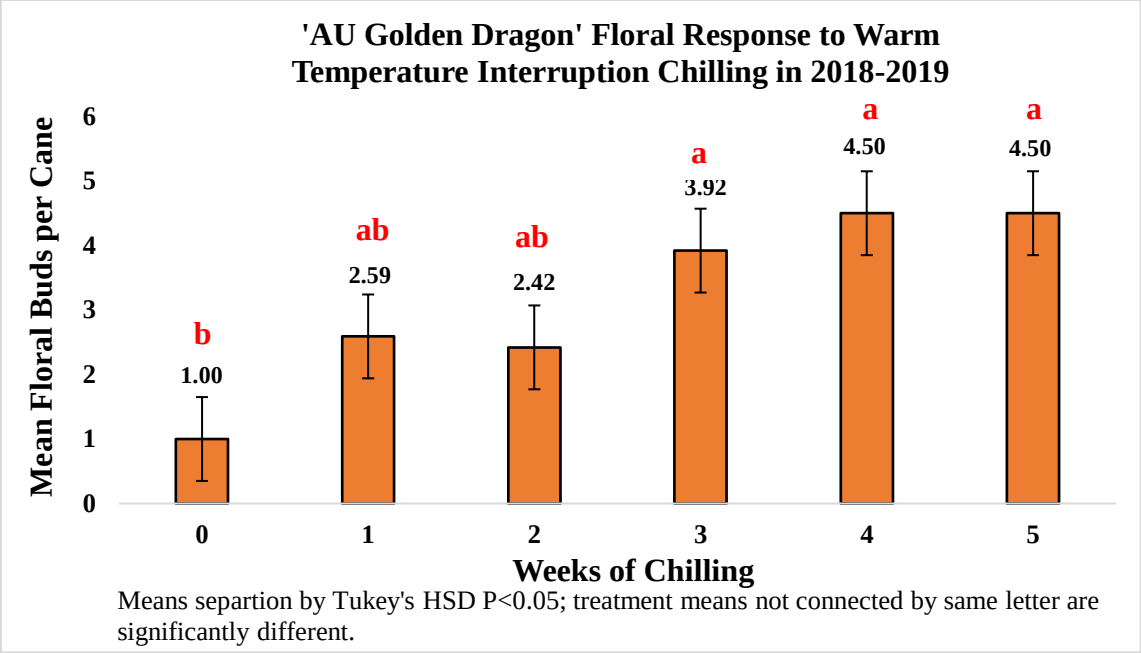


Figure 55 Histogram of 'AU Golden Dragon' kiwifruit (mean floral buds per cane) response to warm temperature interruption (W.T.) across six chilling levels in 2018-2019.

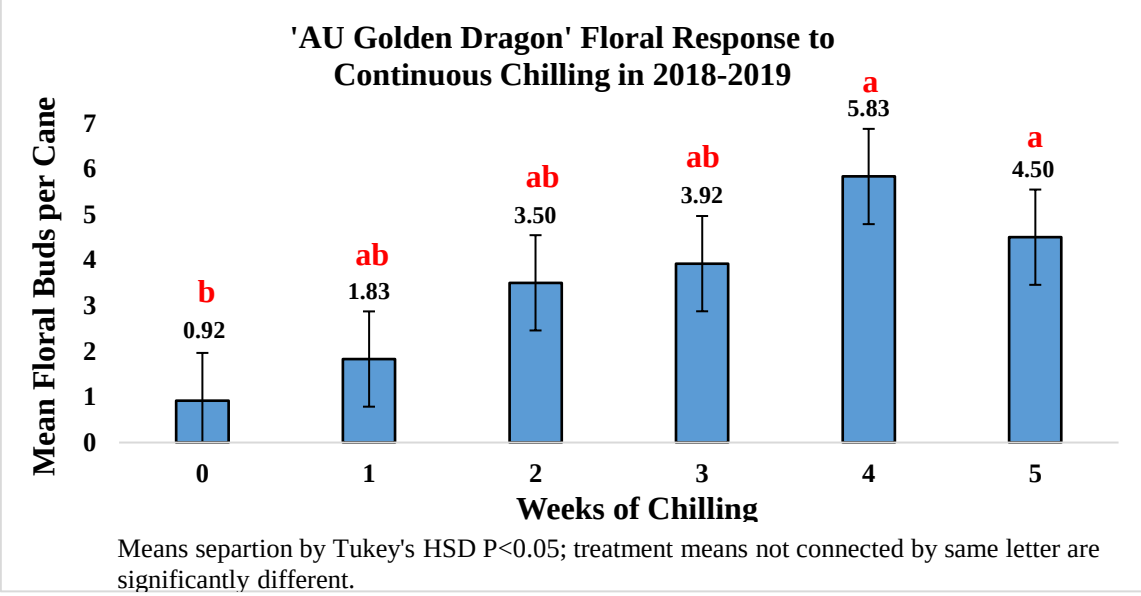
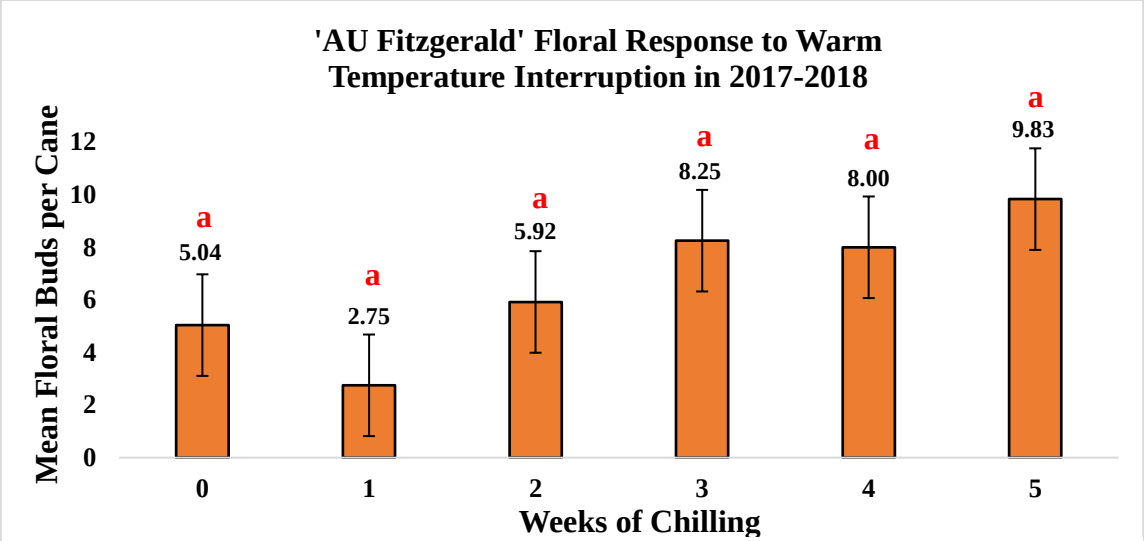


Figure 56 Histogram of 'AU Golden Dragon' kiwifruit floral (mean floral buds per cane) response to continuous chilling (C.C.) across six chilling levels in 2018-2019.

‘AU Fitzgerald’ Floral Chilling Requirements

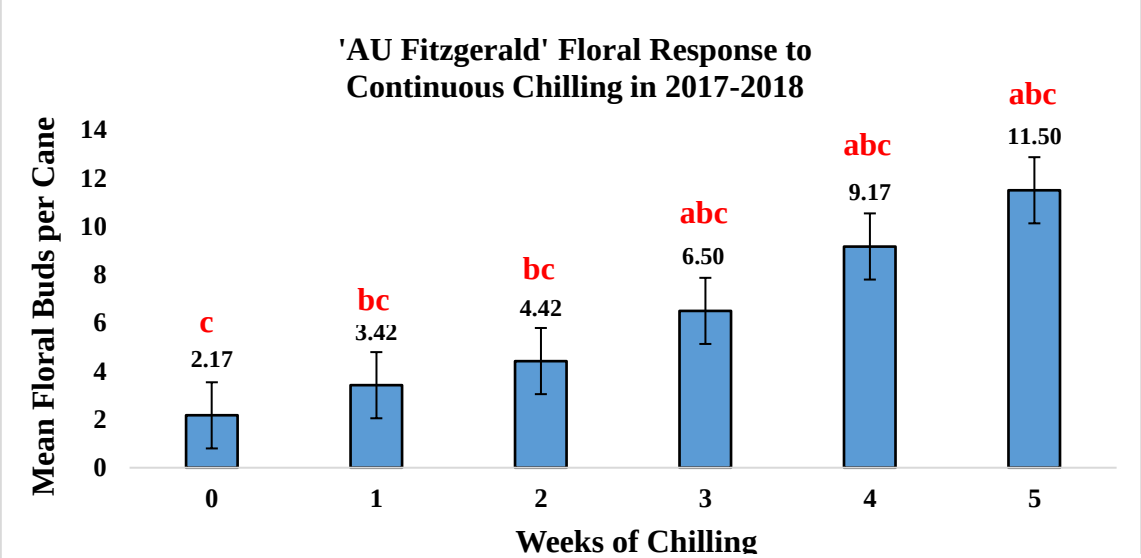
Average floral bud number per cane for ‘AU Fitzgerald’ under W.T. decreased from 5.04 at base chilling 2.75 one week chilling, before beginning a sustained climb from two weeks through five weeks chilling, ultimately reaching a maximum of 9.83. Floral response was not significantly affected by chilling level for this cultivar W.T. in 2018 (Figure 57).

Floral response for ‘AU Fitzgerald in 2018 under C.C. resulted in three different statistical groups: 1.) base chilling (2.17); 2.) one week (3.42) and two weeks (4.42) chilling; 3.) three weeks (6.50), four weeks (9.17), and five weeks (11.50) chilling. While average floral bud number continued to increase throughout all levels of chilling, three weeks chilling, was identified as the upper threshold, suggesting a maximum floral chilling requirement of approximately 838 Richardson units for ‘AU Fitzgerald’ (Figure 58).



Means separtion by Tukey's HSD P<0.05; treatment means not connected by same letter are significantly different.

Figure 57 . Histogram of 'AU Fitzgerald' kiwifruit floral (mean floral buds per cane) response to warm temperature interruption (W.T.) across six chilling levels in 2017-2018.

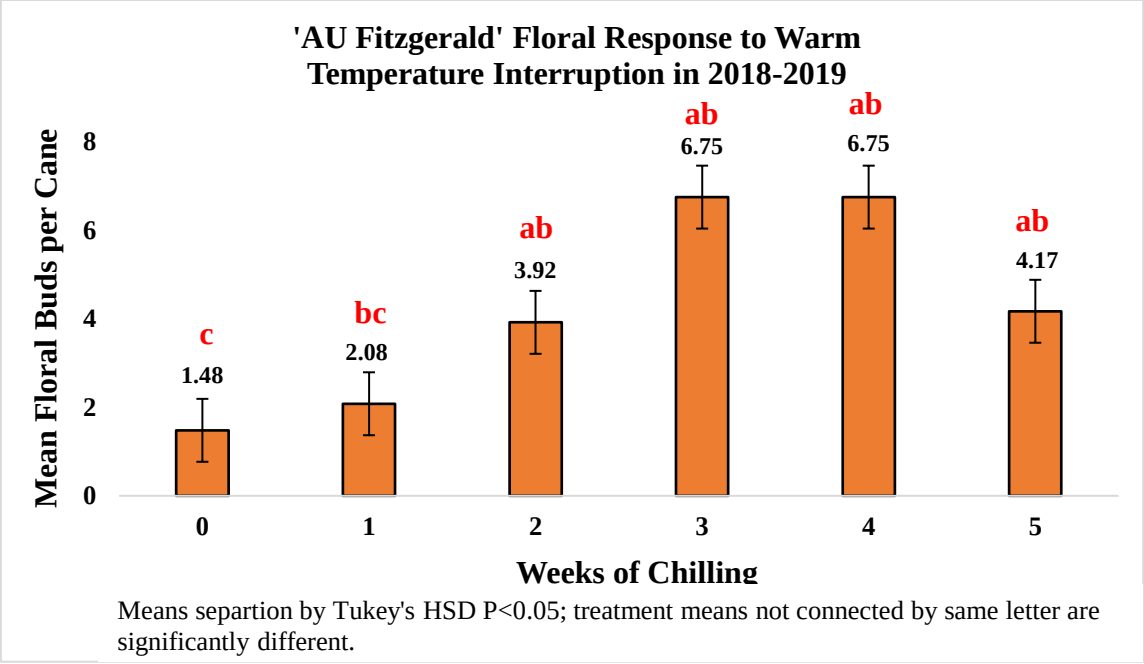


Means separtion by Tukey's HSD P<0.05; treatment means not connected by same letter are significantly different.

Figure 58 Histogram of 'AU Fitzgerald' kiwifruit floral (mean floral buds per cane) response to continuous chilling (C.C.) across six chilling levels in 2017-2018.

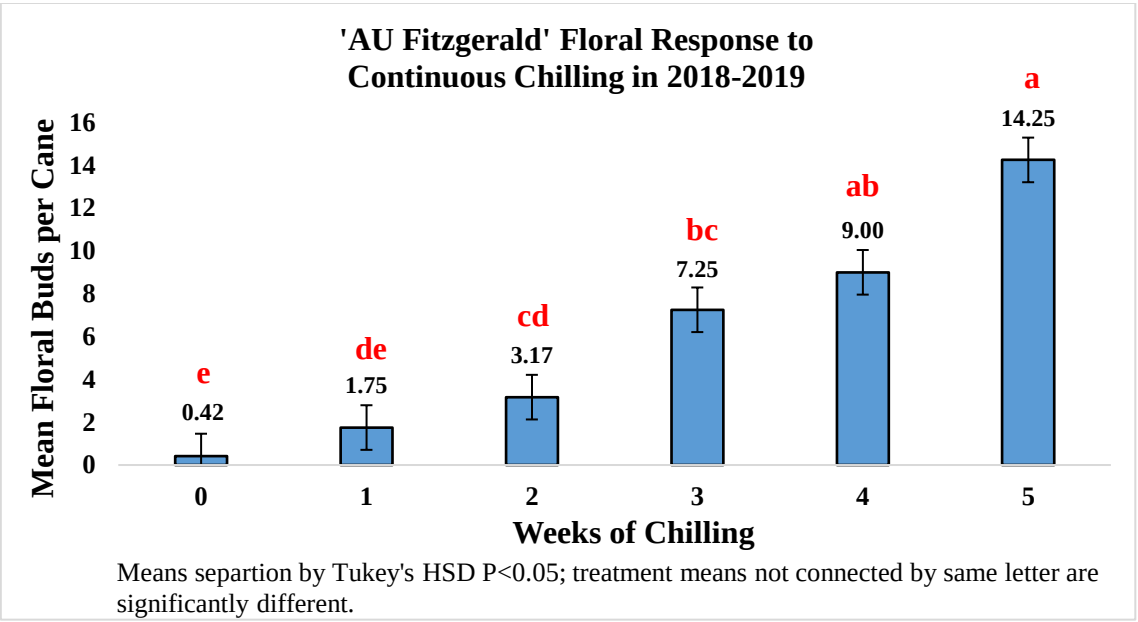
Analysis of floral response for ‘AU Fitzgerald’ under W.T. conditions in 2019 identified three statistically distinct groups, based on treatment means: 1.) base chilling (1.48); 2.) one week chilling (2.08); 3.) two weeks (3.92), three weeks (6.75), four weeks (6.75), and five weeks chilling (4.17). An upper threshold was identified at only two weeks of chilling, suggesting a chilling requirement of 696 Richardson units. Based on the experimental model for chilling negation (Table 8), this suggested requirement could be as low as 576 units, if chilling negation was present (Figure 59).

Finally, floral response to chilling under C.C. conditions for ‘AU Fitzgerald’ in 2019 revealed a continuous climb throughout all chilling levels. Furthermore, analysis indicated that treatment means for all six levels of chilling were statistically different: 1.) base chilling (0.42); 2.) one week chilling (1.75); 3.) two weeks chilling (3.17); 4.) three weeks chilling (7.25); 5.) four weeks chilling (9.00); 6.) five weeks chilling (14.25). It is suggested that the maximum chilling requirement for floral production ‘AU Fitzgerald’ was not met until five weeks or approximately 1,200 units (Table 8). However, considering the analysis’ inability to identify an upper limit, it is also possible that the maximum chilling requirement exceeded the limits of this experiment, (Figure 60).



Means separation by Tukey's HSD P<0.05; treatment means not connected by same letter are significantly different.

Figure 59 Histogram of 'AU Fitzgerald' kiwifruit floral (mean floral buds per cane) response to warm temperature interruption (W.T.) across six chilling levels in 2018-2019.



Means separation by Tukey's HSD P<0.05; treatment means not connected by same letter are significantly different.

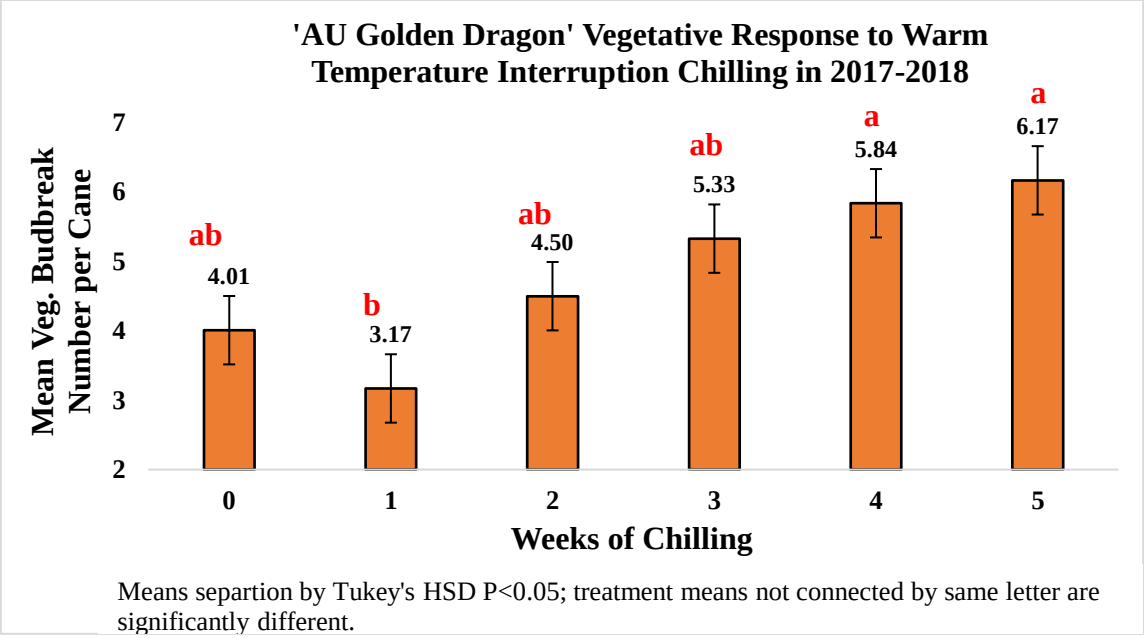
Figure 60 Histogram of 'AU Fitzgerald' kiwifruit floral (mean floral buds per cane) response to continuous chilling (C.C.) across six chilling levels in 2018-2019.

Estimation of Vegetative Chilling Requirements

Chilling generally had less of an impact on vegetative response in both cultivars, particularly ‘AU Fitzgerald. Vegetative budbreak was only significantly affected by chilling in the golden kiwifruit cultivar and only under W.T. conditions, whereas the green cultivar AU Fitzgerald did not show significant response to chilling level under either type of chilling for either year.

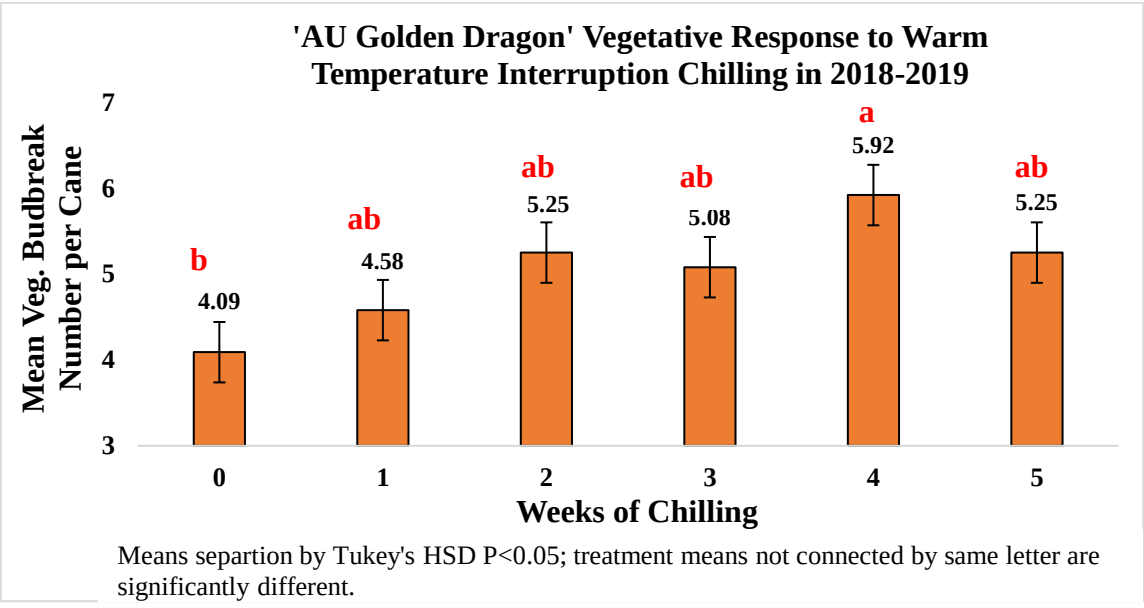
For ‘AU Golden Dragon’, chilling had a significant (P = 0.0052) effect on average vegetative budbreak per cane under W.T. conditions in 2018. Three distinct statistical groups were identified: 1.) one week chilling (3.17); 2.) base-chilling (4.01), two weeks (4.50), and three weeks chilling (5.33); 3.) four (5.84) and five weeks (6.17) chilling, suggesting a maximum threshold for vegetative budbreak at four weeks of chilling or 1,006 Richardson units (Figure 61).

Average vegetative budbreak showed a significant response to chilling (level) (P = 0.0368) for ‘AU Golden Dragon’ in 2019 under W.T. conditions. Analysis of treatment means identified three statistical groups: 1.) base-chilling (4.09); 2.) one week (4.58), two weeks (5.25), three weeks (5.08), and five weeks chilling (5.25); 3.) four weeks chilling (5.92). The upper threshold was estimated again at to be at four weeks chilling with an estimated amount of 1,032 Richardson units (Figure 62).



Means separation by Tukey's HSD P<0.05; treatment means not connected by same letter are significantly different.

Figure 61 Histogram of 'AU Golden Dragon' kiwifruit vegetative (mean vegetative budbreak per cane) response to warm temperature interruption (W.T.) across six chilling levels in 2017-2018.



Means separation by Tukey's HSD P<0.05; treatment means not connected by same letter are significantly different.

Figure 62 Histogram of 'AU Golden Dragon' kiwifruit vegetative (mean vegetative budbreak per cane) response to warm temperature interruption (W.T.) across six chilling levels in 2018-2019.

Floral and Vegetative Response to High Temperature Interruption

Assessment of floral vegetative and floral response under H.T. conditions (unreplicated), relative to W.T. and C.C. was only conducted the second year (2019). When compared to C.C. and W.T. in the cultivar AU Golden Dragon, base-chilling for H.T. resulted in a generally similar average floral bud per cane number (0.33) at base- (three days high-temperature), one- (2.67), and two weeks chilling (3.67). However, average floral bud number trended considerably lower than that of C.C. and H.T. for the remaining higher levels of chilling (1.00, 2.33, and 1.00, respectively). Across all chilling levels, H.T. resulted in an average of 1.83 floral buds per cane (Figure 63).

For vegetative response, H.T. chilling resulted in an average value of 4.33 vegetative budbreak per cane at base-level chilling, which was comparable to the other two chilling types. However, budbreak dropped to 3.33 at one week chilling, increased to 4.33 at two weeks, before dropping to 4.00 at three weeks chilling, whereas C.C. and W.T. maintained a relatively gradual but steady climb over these same chilling levels. H.T. trended with W.T., rapidly climbing to a value of 5.00 at four weeks, but continued to increase to a value of 6.67 at the final chilling level, whereas C.C. and W.T. appeared to begin a downward trend. Whereas C.C. and W.T. reached their maximum budbreak values at three and four weeks chilling (respectively), H.T. continued to increase sharply, suggesting that this trend exceeded the limits of this experiment. However, across all chilling levels, H.T. produced only slightly higher budbreak (4.64) than C.C. (4.54), and less than W.T. (5.03), on average (Figure 64 and Table 18).

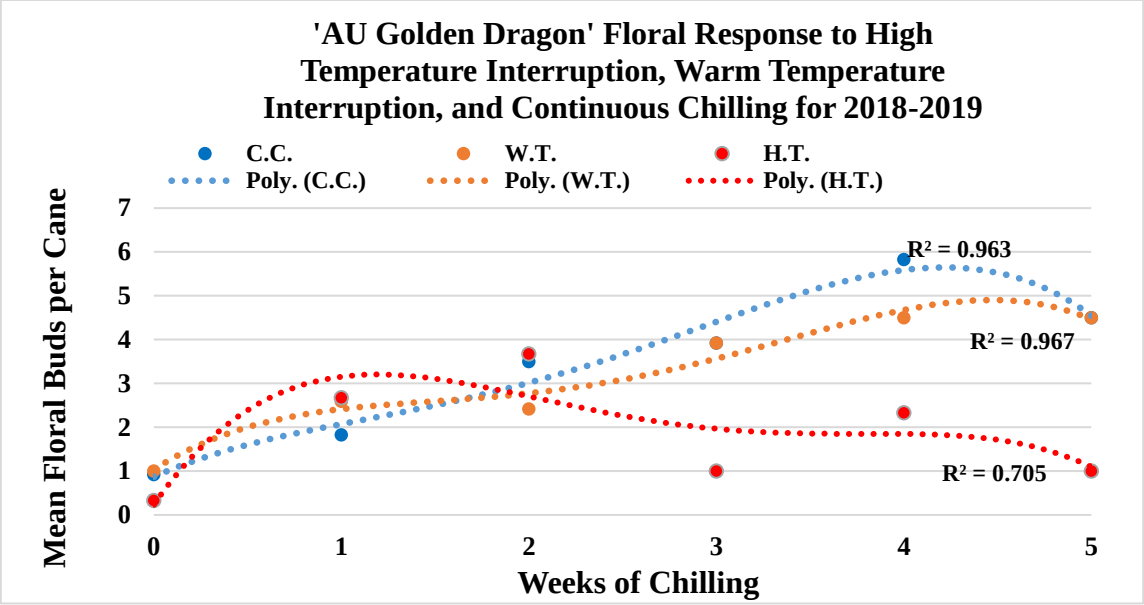


Figure 63 Scatterplot comparing the effects of high temperature interruption (H.T.) warm temperature interruption (W.T.), and continuous chilling (C.C.) on mean floral buds per cane in 'AU Golden Dragon' kiwifruit across six levels of chilling in 2018-2019.

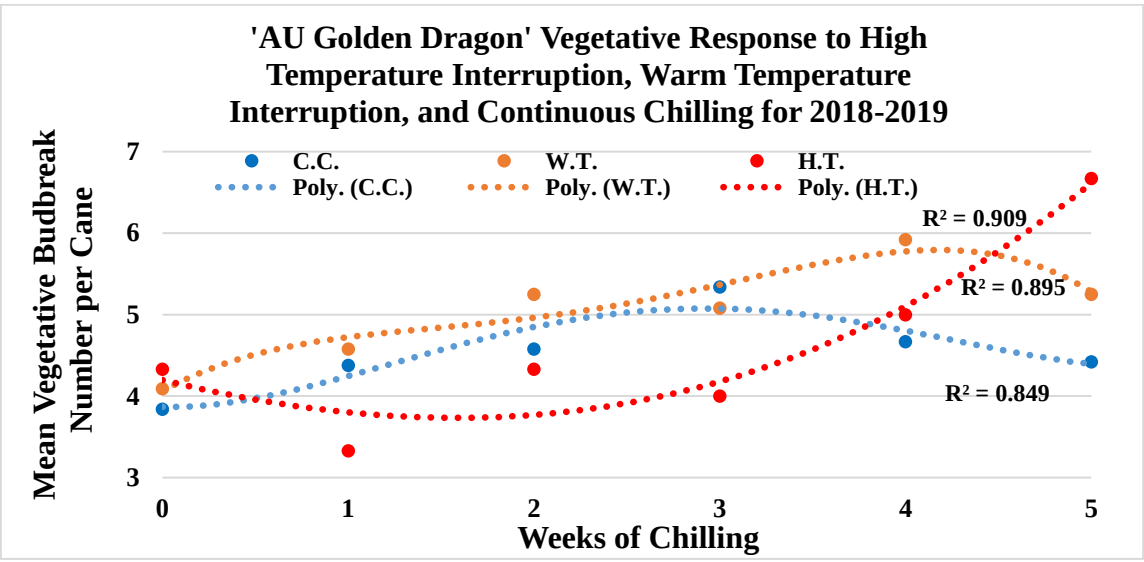


Figure 64 Scatterplot comparing the effects of high temperature interruption (H.T.) warm temperature interruption (W.T.), and continuous chilling (C.C.) on mean vegetative budbreak per cane in 'AU Golden Dragon' kiwifruit across six levels of chilling in 2018-2019.

Table 18 'AU Golden Dragon' kiwifruit floral and vegetative response to high temperature interruption chilling (H.T.) across six levels of chilling for one year (2018-2019).

Chilling Level	Days Warm Temp.	Floral Buds	Vegetative Buds	Vegetative Budbreak	Shoot Number	Percent Shoot Development	Vegetative Bud to Shoot
Base	3	0.33	4.33	54.2%	4.00	50.0%	92.3%
1 week	6	2.67	3.33	41.7%	2.67	33.3%	80.0%
2 weeks	9	3.67	4.33	54.2%	3.67	45.8%	84.6%
3 weeks	12	1.00	4.00	50.0%	3.00	37.5%	75.0%
4 weeks	15	2.33	5.00	62.5%	3.33	41.7%	66.7%
5 weeks	18	1.00	6.67	83.3%	6.00	75.0%	90.0%
Average		1.83	4.61	57.6%	3.78	47.2%	81.4%

All values on a per-cane basis
All data based on mean of single (one-jar, three-cane sample, un-replicated) observation.
All analysis of floral bud number based square root transformation
All analysis of shoot number based on non-transformed data
All analysis of percent shoot development based on non-transformed data
Non-transformed data presented for floral bud number, shoot number, and percent shoot development

In the case of ‘AU Fitzgerald’, average floral bud number per cane was very comparable to the other two chilling types at base-chilling (1.00) and one week chilling (0.67), but continued to trend well below that of W.T. and C.C. over two-, three-, and four weeks chilling (2.00, 1.67, and 2.67, respectively). H.T. resulted in a higher value (5.33) than W.T. (4.17) at five weeks chilling, although this level was much lower than that for C.C. (14.25). Overall, H.T. produced an average of 2.22 floral buds per cane across all levels of chilling (Figure 65).

For average vegetative budbreak number in ‘AU Fitzgerald’, H.T. resulted in a much lower value at the base-chilling level (1.67), as compared to C.C. and especially W.T. However, H.T. increased to a value of 2.00 at one week and 3.00 at two weeks, before dropping to 2.33 at three weeks, then increasing again to 2.67 at four weeks and ending with a value of 3.00 at five weeks chilling. In general, H.T. appeared to follow a trend that was more similar to that of W.T. H.T. reached maximum budbreak at two weeks chilling (3.00), while peaking again at five weeks (3.00). On average and across all treatments, H.T. produced less average vegetative budbreak per cane (2.45) compared to W.T. (2.70) and C.C. (2.84) (Figure 66 and Table 19).

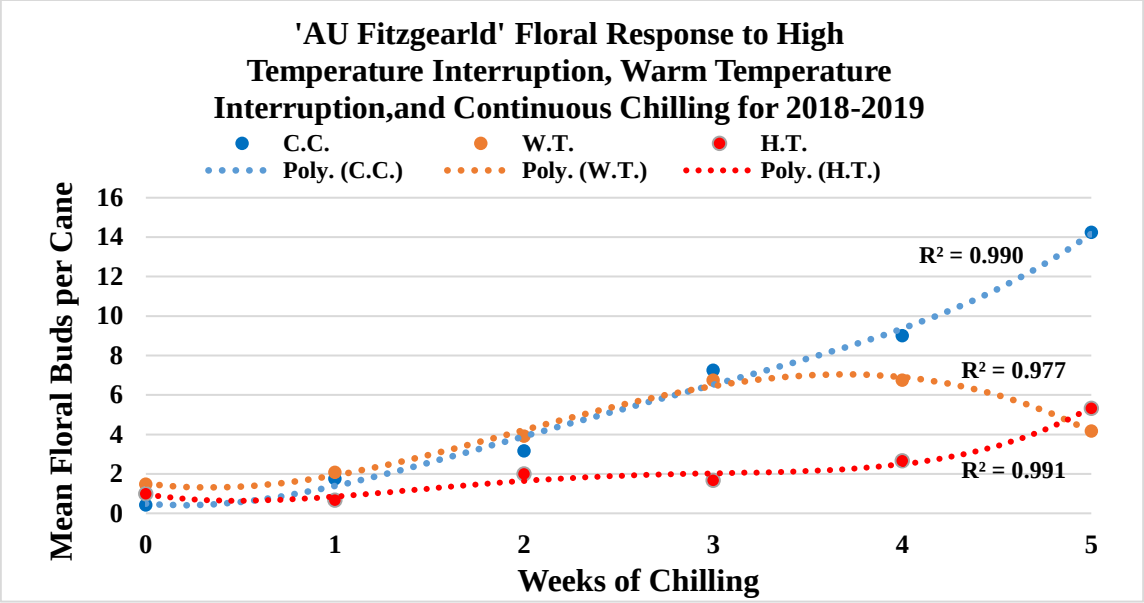


Figure 65 Scatterplot comparing the effects of high temperature interruption (H.T.), warm temperature interruption (W.T.), and continuous chilling (C.C.) on mean floral buds per cane in 'AU Fitzgerald' kiwifruit across six levels of chilling in 2018-2019.

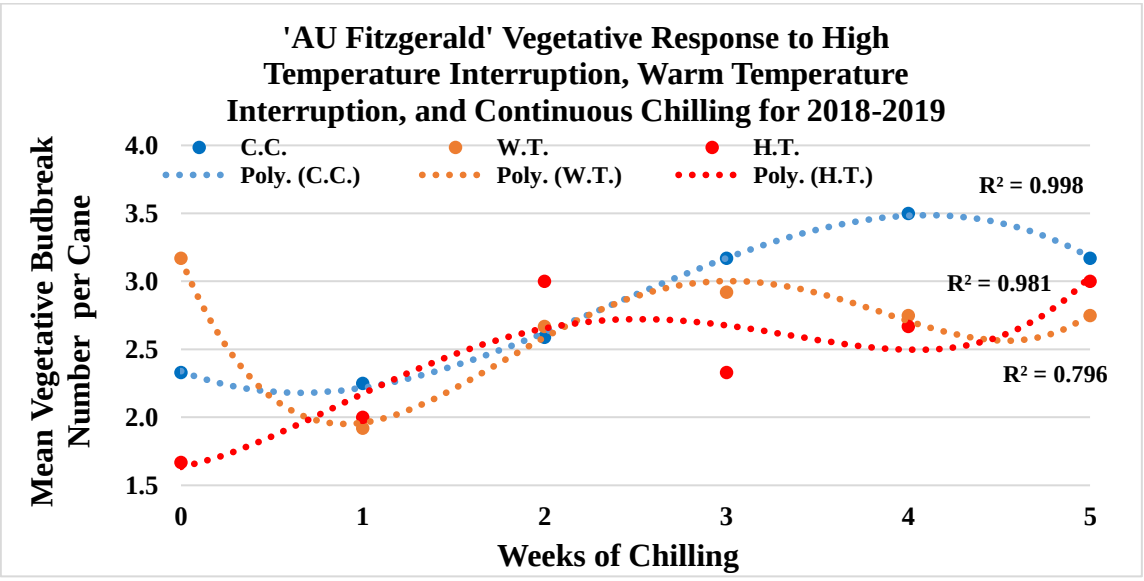


Figure 66 Scatterplot comparing the effects of high temperature interruption (H.T.), warm temperature interruption (W.T.), and continuous chilling (C.C.) on mean vegetative budbreak per cane in 'AU Fitzgerald' kiwifruit across six levels of chilling in 2018-2019.

Table 19 'AU Fitzgerald' kiwifruit floral and vegetative response to high temperature interruption chilling (H.T.) across six levels of chilling for one year (2018-2019).

Chilling Level	Days Warm Temp.	Floral Buds	Vegetative Budbreak Number	Percent Vegetative Budbreak	Shoot Number	Percent Shoot Development	Percent Vegetative Bud to Shoot
Base	3	1.00	1.67	20.8%	1.67	20.8%	100.0%
1 week	6	0.67	2.00	25.0%	2.00	25.0%	100.0%
2 weeks	9	2.00	3.00	37.5%	2.33	29.2%	77.8%
3 weeks	12	1.67	2.33	29.2%	2.33	29.2%	100.0%
4 weeks	15	2.67	2.67	33.3%	2.33	29.2%	87.5%
5 weeks	18	5.33	3.00	37.5%	2.67	33.3%	88.9%
Average		2.22	2.44	30.6%	2.22	27.8%	92.4%

All values on a per-cane basis
All data based on mean of single (one-jar, three-cane sample, un-replicated) observation.
All analysis of floral bud number based square root transformation
All analysis of shoot number based on non-transformed data
All analysis of percent shoot development based on non-transformed data
Non-transformed data presented for floral bud number, shoot number, and percent shoot development

Nodal Position Response to Chilling Type

For the first year (2018) average vegetative budbreak number and average floral bud number observations were collected on a per-node as well as per-cane basis in order to study the effect of chilling type (W.T. and C.C.) on nodal position. Comparisons were made for W.T and C.C. at each level of chilling and at each node position for both cultivars. For 'AU Golden Dragon', there were several instances in which chilling-type had a significant effect on vegetative budbreak at a specific node position. At one week chilling, C.C. resulted in significantly (P = 0.0146) greater average vegetative budbreak number (0.67) than W.T. (0.17) at node position eight. For two weeks chilling, W.T. produced a greater number (0.84) of average vegetative budbreak (P = 0.139) compared to C.C. (0.33) at node position four. A significantly higher (P = 0.0139) average vegetative budbreak number was observed with W.T. (0.67) compared to C.C. (0.17) at node position seven at the four week chilling level. At five weeks chilling, node position three exhibited a significant budbreak difference (P = 0.0338), in favor of W.T. (0.75, compared to 0.17 for CC). Significant difference in vegetative response was only observed at the three chilling level for 'AU Fitzgerald' at which C.C. resulted in a greater value (0.83) at node position nine, as compared to W.T. (0.42) (P = 0.0151) (data not shown).

Significant differences in floral response were also observed between chilling-type at specific nodal positions for the cultivar AU Golden Dragon. Average floral bud number was significantly different (P = 0.0097; 0.0217) at node positions ten and eight at four weeks chilling. W.T. resulted in greater average floral buds (3.17 and 1.25) as

compared to C.C. (0.33 and 0.00) at both of these node positions. At five weeks chilling a greater average number of floral buds with W.T. (1.33) than C.C. (0.00) was observed at node position three (P = 0.011). There were no cases in which chilling type had a significant effect on floral response at a specific node position in 'AU Fitzgerald' ((data not shown).

Comparison of chilling type influence on vegetative and floral response was also made based on nodal position, across all chilling levels. Values were expressed in terms of average number per-node as well average number per-node, relative to the total average value for the entire cane. Vegetative budbreak number was higher at node position ten, with an average value of 0.96 for all treatments (both cultivars) (Figure 67 and Table 20). However, when considered as a percentage of total budbreak relative to the total for the entire cane, 'AU Fitzgerald' exhibited greater percentage of vegetative budbreak with W.T. (29.4%) and particularly with C.C. (35.1%) compared to 'AU Golden Dragon' (20.6% and 21.9%). At node position nine, 'AU Golden Dragon' produced only a slightly lower number of average vegetative budbreak for both W.T. and C.C. (0.88 and 0.92) as compared to node position ten (1.00 and 0.97), whereas budbreak number was only approximately half as high for 'AU Fitzgerald' at node position nine (0.51 and 0.56 for W.T. and C.C.). However, average budbreak per node was more similar between cultivars and comparable between chilling type, when expressed as a percentage of total budbreak per cane (Figures 67 & 68).

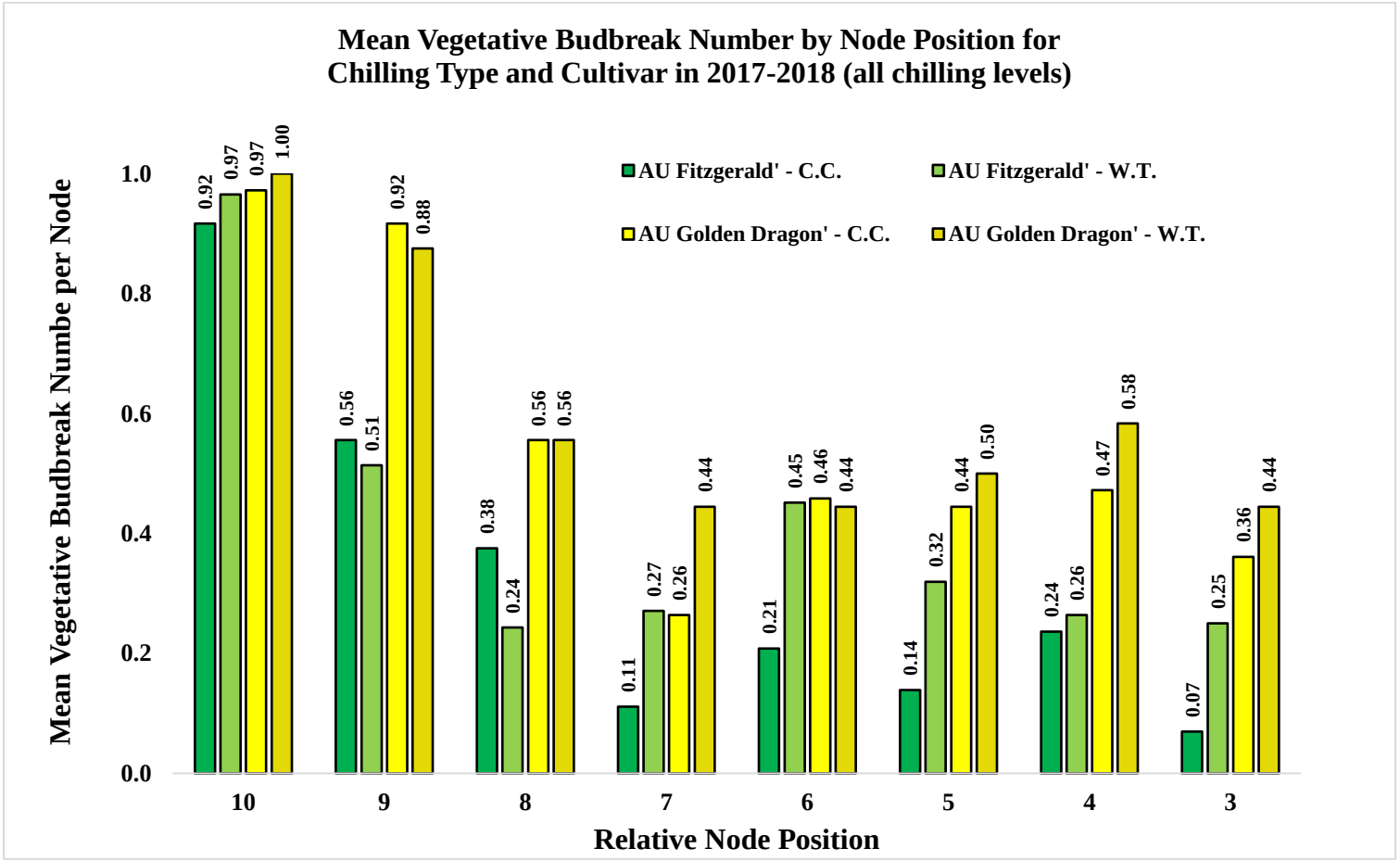


Figure 67 Mean vegetative budbreak number by nodal position for chilling type (C.C. and W.T.) and kiwifruit cultivar in 2017-2018 (all chilling levels).

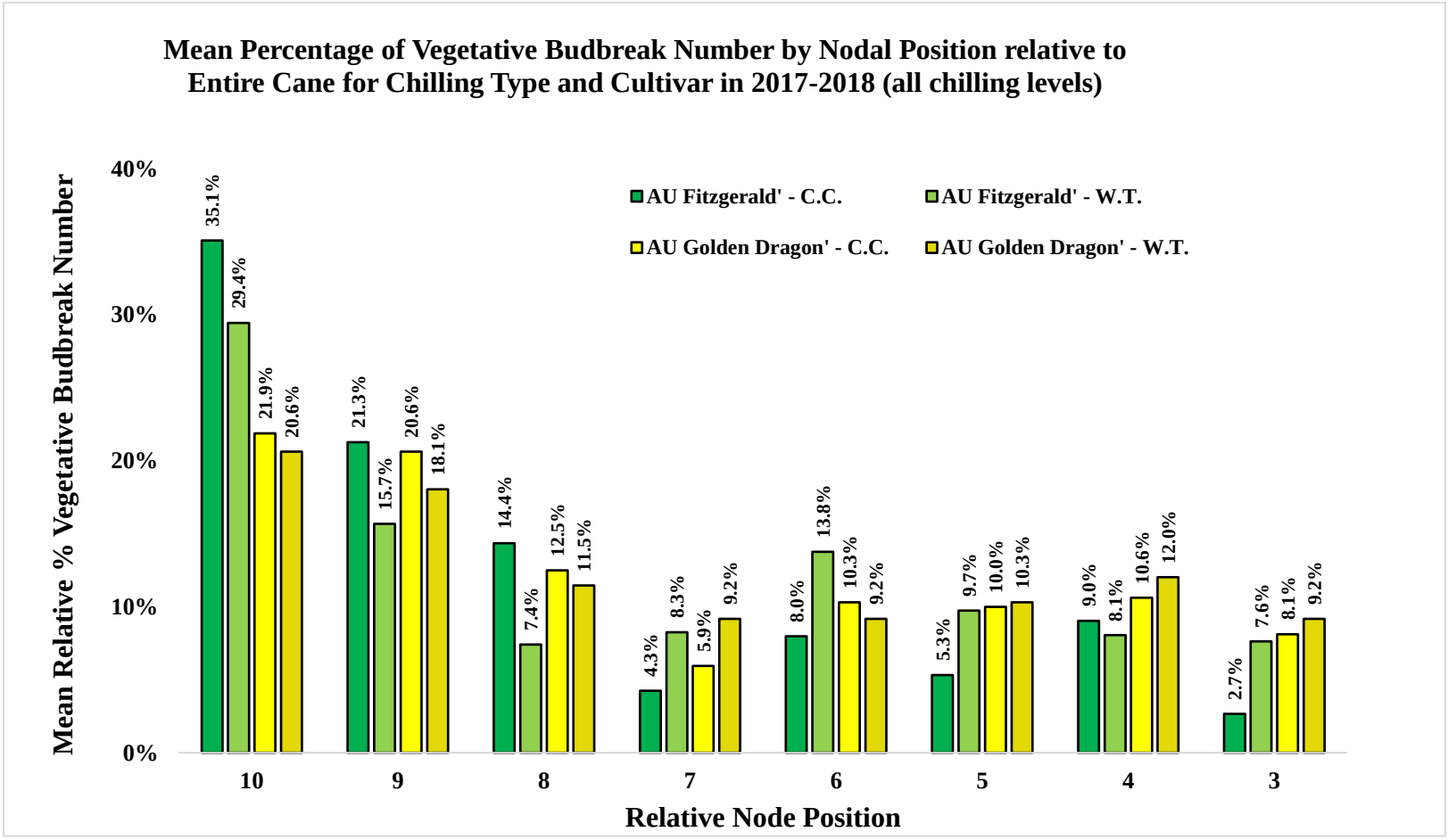


Figure 68 Mean percentage of vegetative budbreak number by nodal position relative to entire cane for chilling type (C.C. and W.T.) and kiwifruit cultivar in 2017-2018 (all chilling levels).

Average vegetative budbreak number (but not necessarily percentage relative to entire cane total) was generally higher in ‘AU Golden Dragon’ as compared to ‘AU Fitzgerald’ at the more proximal nodes (positions three through seven). A strong basipetal trend was observed for vegetative budbreak (both number and percentage relative to total for entire cane) in both cultivars. However, W.T., in comparison to C.C., tended to result in greater budbreak at the lower node (positions three through seven) for both cultivars. On average and across all chilling levels, W.T. produced a higher total vegetative budbreak number (entire cane) for both ‘AU Golden Dragon’ (4.85) and ‘AU Fitzgerald (3.27), as compared to C.C. (4.45 and 2.61, respectively) for these two cultivars (Figures 67 & 68). Mean values for each vegetative response to chilling type and cultivar with respect to specific node position (all chilling levels averaged) can be found in Tables 20 and 21.

Table 20 Mean vegetative budbreak number by node position for chilling type (C.C. & W.T) and kiwifruit cultivar in 2017-2018 (all chilling levels).

Cultivar / Chilling Type	Node 10	Node 9	Node 8	Node 7	Node 6	Node 5	Node 4	Node 3	Average	Total
'AU Fitzgerald' / C.C.	0.92	0.56	0.38	0.11	0.21	0.14	0.24	0.07	0.33	2.61
'AU Fitzgerald' / W.T.	0.97	0.51	0.24	0.27	0.45	0.32	0.26	0.25	0.41	3.27
'AU Golden Dragon' / C.C.	0.97	0.92	0.56	0.26	0.46	0.45	0.47	0.36	0.56	4.45
'AU Golden Dragon' / W.T.	1.00	0.88	0.56	0.44	0.44	0.50	0.58	0.44	0.61	4.85
Average- All Cultivars / Treatments	0.97	0.72	0.44	0.27	0.39	0.35	0.39	0.28	0.49	3.80
Values based on the average of all chilling levels for each cultivar and chilling type-combination (warm temperature and continuous chilling).										

Table 21 Mean percentage of vegetative budbreak number by node position relative to total for entire cane for chilling type (C.C. & W.T) and kiwifruit cultivar in 2017-2018 (all chilling levels).

Cultivar / Chilling Type	Node 10	Node 9	Node 8	Node 7	Node 6	Node 5	Node 4	Node 3	Average	Total
'AU Fitzgerald' / C.C.	35.1%	21.3%	14.4%	4.3%	8.0%	5.3%	9.0%	2.7%	12.5%	100.0%
'AU Fitzgerald' / W.T.	29.4%	15.7%	7.4%	8.3%	13.8%	9.7%	8.1%	7.6%	12.5%	100.0%
'AU Golden Dragon' / C.C.	21.9%	20.6%	12.5%	5.9%	10.3%	10.0%	10.6%	8.1%	12.5%	100.0%
'AU Golden Dragon' / W.T.	20.6%	18.1%	11.5%	9.2%	9.2%	10.3%	12.0%	9.2%	12.5%	100.0%
Average- All Cultivars / Treatments	20.6%	18.1%	11.5%	9.2%	9.2%	10.3%	12.0%	9.2%	12.5%	100.0%
Calculated as percentage of average vegetative budbreak per node relative to average total vegetative budbreak per cane. Values based on the average of all chilling levels for each cultivar and chilling type-combination (warm temperature and continuous chilling).										

Floral Response to Chilling Type as a Function of Nodal Position

Floral response, both in terms of number and the percentage relative to the whole cane, was by far the highest at node position ten, with an average of 48.6% of the total floral buds for both cultivars and chilling types observed at the apical node. This was particularly true in the case of 'AU Fitzgerald' for both W.T. (3.22) and C.C. (3.81) as compared to 'AU Golden Dragon (1.51 and 0.69, respectively). However, it should also be noted that 'AU Fitzgerald' produced more floral buds (average of 6.41 for both chilling types) than 'AU Golden Dragon' (4.39) per cane. As discussed earlier, 'AU Golden Dragon' produced more floral buds per cane with W.T. in the 2018. This was certainly evident at the tenth and ninth nodes, where an average of 1.51 and 0.81 (respectively) buds with W.T. were observed as compared to 0.69 and 0.26 (respectively) with C.C. Average floral number at node position nine was approximately only 39% that of the tenth node (both cultivars and chilling types. (Figures 69 & 70).

Average floral bud number and percentage relative to the per-cane total in 'AU Fitzgerald' decreased sharply and more drastically beyond the ninth node as compared to that of vegetative budbreak. Floral activity at nodes three through eight accounted for an average of only 5.4% each (collectively 32.2%) of the total floral buds per entire cane at these nodes, for both cultivars and all treatments. However, as with vegetative budbreak, total percentage of floral buds relative to entire cane were noticeably higher at these nodes with W.T for both 'AU Fitzgerald' and 'AU Golden Dragon' (33.5% and 44.1%) as compared to C.C. (16.6% and 34.5%, respectively) (Figure 70 and Tables 22 & 23).

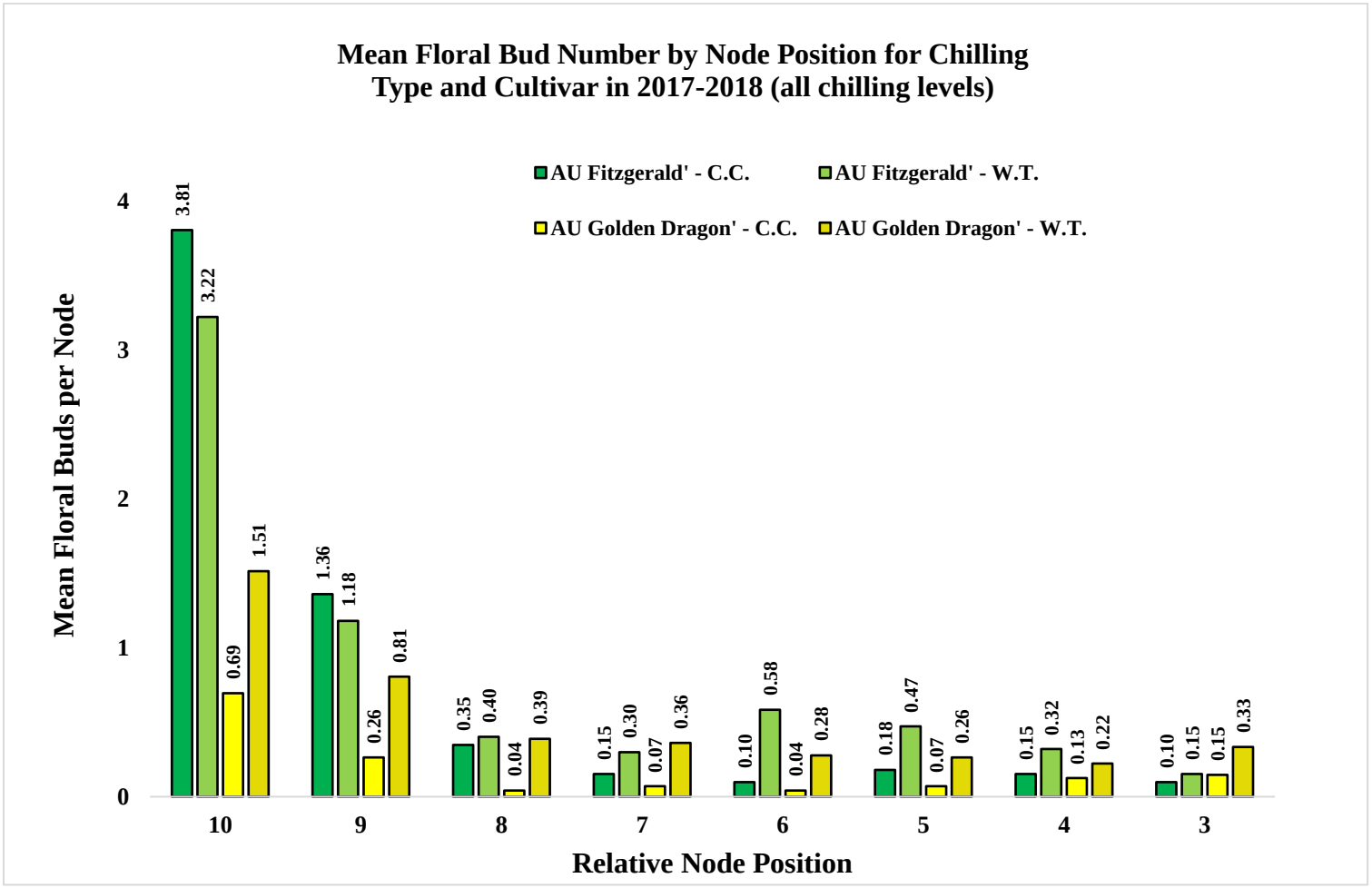


Figure 69 Mean floral bud number by nodal position for chilling type (C.C. and W.T.) and kiwifruit cultivar in 2017-2018 (all chilling levels).

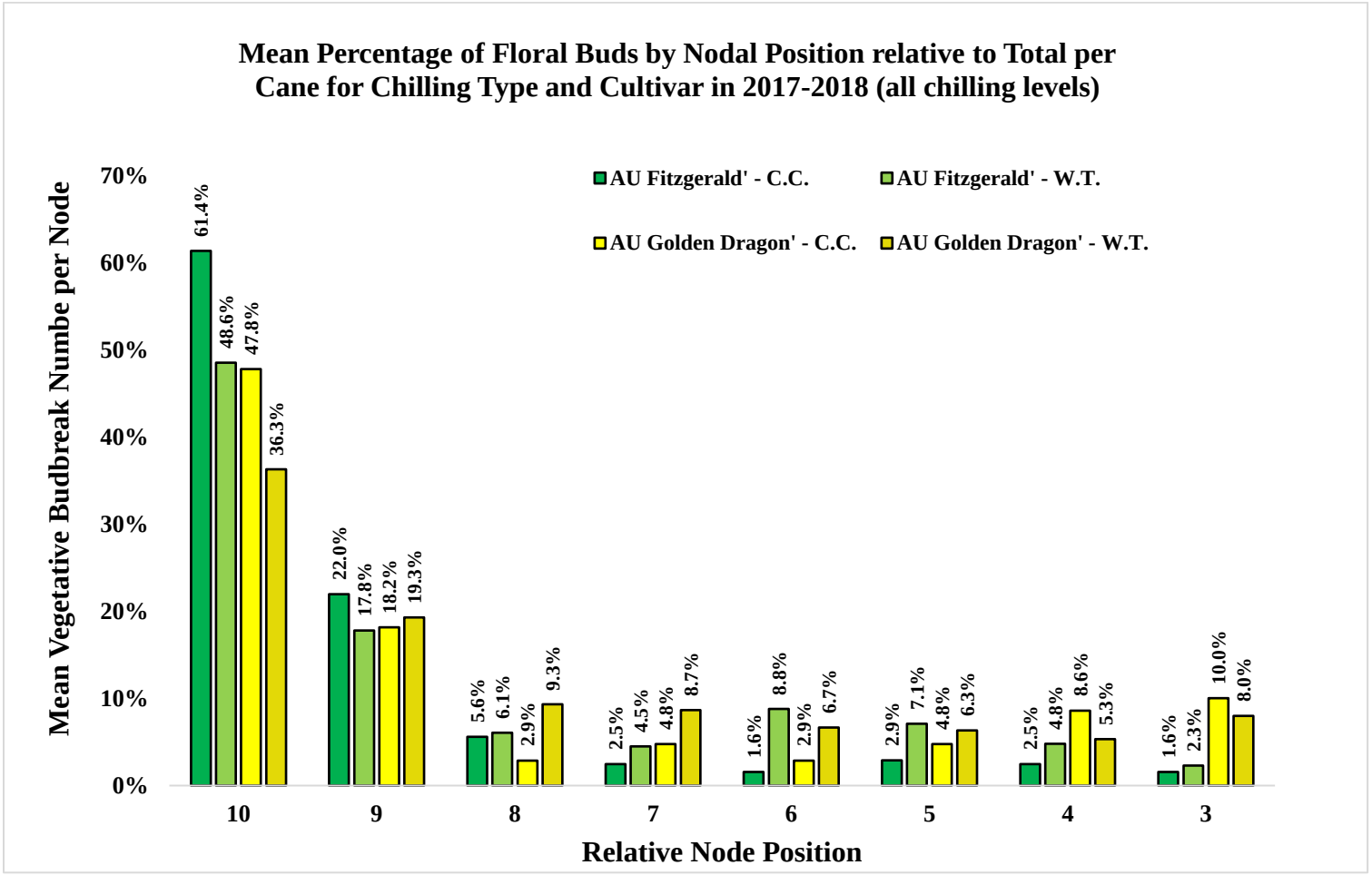


Figure 70 Mean percentage of floral bud number by nodal position relative to entire cane for chilling type (C.C. and W.T.) and kiwifruit cultivar in 2017-2018 (all chilling levels).

Table 22 . Mean floral buds by node position for chilling type (C.C. & W.T) and kiwifruit cultivar in 2017-2018 (all chilling levels).

Cultivar / Chilling Type	Node 10	Node 9	Node 8	Node 7	Node 6	Node 5	Node 4	Node 3	Average	Total
'AU Fitzgerald' / C.C.	3.81	1.36	0.35	0.15	0.10	0.18	0.15	0.10	0.77	6.19
'AU Fitzgerald' / W.T.	3.22	1.18	0.40	0.30	0.58	0.47	0.32	0.15	0.83	6.63
'AU Golden Dragon' / C.C.	0.69	0.26	0.04	0.07	0.04	0.07	0.13	0.15	0.18	1.45
'AU Golden Dragon' / W.T.	1.51	0.81	0.39	0.36	0.28	0.26	0.22	0.33	0.52	4.17
Average- All Cultivars / Treatments	2.31	0.90	0.30	0.22	0.25	0.25	0.20	0.18	0.58	4.61

Values based on the average of all chilling levels for each cultivar and chilling type-combination (warm temperature and continuous chilling).
Analysis based on non-transformed data for floral bud number.

Table 23 Mean percentage of floral buds by node position relative to total for entire cane for chilling type (C.C. & W.T) and kiwifruit cultivar in 2017-2018 (all chilling levels).

Cultivar / Chilling Type	Node 10	Node 9	Node 8	Node 7	Node 6	Node 5	Node 4	Node 3	Average	Total
'AU Fitzgerald' / C.C.	61.4%	22.0%	5.6%	2.5%	1.6%	2.9%	2.5%	1.6%	12.5%	100.0%
'AU Fitzgerald' / W.T.	48.6%	17.8%	6.1%	4.5%	8.8%	7.1%	4.8%	2.3%	12.5%	100.0%
'AU Golden Dragon' / C.C.	47.8%	18.2%	2.9%	4.8%	2.9%	4.8%	8.6%	10.0%	12.5%	100.0%
'AU Golden Dragon' / W.T.	36.3%	19.3%	9.3%	8.7%	6.7%	6.3%	5.3%	8.0%	12.5%	100.0%
Average- All Cultivars / Treatments	48.6%	19.3%	6.0%	5.1%	5.0%	5.3%	5.3%	5.5%	12.5%	100.0%

Calculated as percentage of average floral buds per node relative to average total floral buds per cane.
Values based on the average of all chilling levels for each cultivar and chilling type-combination (warm temperature and continuous chilling).
Analysis based on non-transformed data for floral bud number.

Discussion

Floral Response to Chilling Type

'AU Golden Dragon'

The difference in average floral buds per cane between 2018 and 2019 was not significant for either cultivar in this experiment. Significant differences in floral response between continuous and warm temperature-interrupted chilling were not observed in 'AU Golden Dragon' until the two highest levels of chilling. Across both years, 'AU Golden Dragon' produced substantially (approximately 84% and 110%) more flowers under W.T. as compared to C.C. at four and five weeks chilling. However, strong year x treatment interactions (P = 0.00014 and P=0.0019) present at these levels identified the need for comparison individually for each year. Treatment differences were even more dramatic when only data form the first year (2018) was analyzed. At these chilling levels, W.T. out-produced C.C. by approximately 534% and 349% (P = 0.0181 and P=0.0084).

While it is possible that higher metabolic rates associated with intermittent exposure to warm temperature might have provided for greater degradation of dormancy-promoting factors such as ABA, it is difficult to speculate the physiological mechanism responsible for these surprising results. Simulated chilling with temperatures as high as 20°C for two to four hours (in conjunction with 4°C chilling) can reportedly enhance the effectiveness of chilling exposure, while longer exposure (>6 hours) or to warmer temperatures (24°C) has resulted in negation of chilling in peach (Couvillon and Erez, 1985). Considering that temperatures simulated by W.T. conditions (17.2°C /

25.0°C) were similar to the 20°C reported previously, it is not implausible that these conditions might have actually accentuated the supplied chilling. Porlingis and Therios (1997) discovered that high (30°C) temperatures were nearly as effective as chilling in promoting budbreak, albeit vegetative, in defoliated two-year-old *A. deliciosa* plants. The apparent positive floral response to warm temperature observed in this study as compared to C.C. might be more of a reflection on the comparative lack of response to chilling exposure observed with C.C. across all chilling levels in 2018.

Whether this unusual difference in behavior observed between W.T. and C.C. is due to a lack of response to chilling under C.C. conditions or a promotive effect of chilling with W.T. is not known. Crude climatological study of *A. chinensis'* natural geographic range would suggest that this species is at least occasionally exposed to dynamic winter temperatures, similar to what was simulated in this experiment. One aspect that was not extensively surveyed was the amount or frequency of aborted floral buds, as only the number of total flowers and buds were considered in quantifying floral response. However, there was not an apparent difference in the incidence of floral bud abortion with W.T. treatments for 'AU Golden Dragon' as compared to 'AU Fitzgerald' during either year of this study. Analysis of per-node floral bud data indicated that node positions eight and ten were most influential at four weeks chilling, whereas the third node was responsible for much of the treatment difference at five weeks chilling (data not shown).

A comparison of the W.T. and C.C. effect on floral response in 'AU Golden Dragon' during the second year (2019) yielded different, but not conflicting, results.

Chilling type did not result in a significantly different number of average floral buds per cane at any chilling level and the positive response to chilling (level) observed with C.C. in addition to W.T. in 2018 suggest that data from this second year of the experiment might be more reliable. Nevertheless, there was no indication of chilling negation present for this cultivar. The absence of a treatment effect on floral response in the second year, along with the results from the first year, when in context, suggest that 'AU Golden Dragon' is, at the very least, not negatively impacted by intermittent warm temperature exposure (as simulated in this experiment). However, the resulting implication that *A. chinensis* does not respond to chilling negation is limited to the scope of a single cultivar in this study and further research on additional cultivars of this species is needed.

'AU Fitzgerald'

Across both years, 'AU Fitzgerald' followed a similar pattern to 'AU Golden Dragon', with no significant treatment effect for floral response through the first three weeks of chilling. However, average floral bud number per cane was significantly ($P = 0.0476$) lower (approximately 18%) at four weeks chilling in the W.T. treatment as compared to C.C. This difference was even greater at five weeks chilling, where W.T. resulted in considerably (46%) less floral buds than with C.C. However, as with 'AU Golden Dragon', a significant ($P = 0.0089$) year x treatment interaction observed at the highest chilling level also necessitated single-year comparisons, particularly at this level of chilling.

Analysis of floral response in 2018 did not reveal significant differences between treatments at any chilling level, except at base chilling (P = 0.0172). At the estimated 334 Richardson units, W.T. resulted in approximately 132% greater average floral buds per cane as compared to C.C. at this level. This discrepancy might be due to the fact that the canes might not have entered endodormancy at this amount of chilling. The additional exposure (three days) to warm temperature (17.2°C to 25.0°C as compared to 22.8°C to 26.0°C for forcing conditions) might have resulted in accentuation of the previously accumulated chilling, as discussed earlier and as described by Couvillon (1995).

No significant treatment differences were observed during the second year (2019) until five weeks chilling (P = 0.0016). At this highest amount of simulated chilling, W.T. produced approximately 71% fewer average floral buds per cane than C.C. While treatment differences at four weeks chilling were not significant for individual years, floral activity was significantly lower across years, in addition to the difference observed at the five week level in 2019. These results suggest that, unlike *A. chinensis,* floral response of *A. deliciosa* appears to be susceptible to negation of chilling by warm temperatures. At first glance, this response may seem minor or inconsistent, considering that its effects were limited to the highest two chilling levels. However, when one takes into account that the extent of negation might be limited to previous 20 to 40 of the previously units supplied chilling units (as described by Erez et al., 1979 in peach), it is not surprising that such response would only become evident at the culmination of several chilling – warm temperature cycles. Based on these assumptions,

effective chilling accumulation between treatments would have differed by as few as 80 and 100 units.

Based on crude assessment of regional winter temperature data from the native distribution of *A. deliciosa* in comparison to that of *A. chinensis*, it would seem *that A. chinensis* is not necessarily more likely to be exposed to W.T. (Table 4). However, fuzzy kiwifruit is typically found at higher elevations (Hongwen, 2016), where short-term temperature fluctuations might not be as pronounced. Additionally, one would presume that *A. chinensis'* more coastal origin would result in more frequent exposure incursions of warm maritime air masses. It is important to note that winter temperature patterns in the native distribution for both species are not expected to be nearly as dynamic as those in southeastern United States. Nevertheless, W.T., as imposed in this study, are reflective of such dynamic conditions along the Gulf of Mexico and such temperatures have proven adequately warm to result in chilling negation in other temperate fruit species.

Vegetative Budbreak Response to Chilling Type

As discussed earlier, vegetative budbreak was expressed as a number and percentage. Given the congruent nature of these two variables and lack of associated significant results, only average vegetative budbreak number will be included in this discussion. Average budbreak was not significantly different between years for either cultivar in this study, although budbreak was considerably higher for the golden kiwifruit cultivar as compared to the fuzzy one. Comparison of chilling type (W.T. and

C.C.) for each level of chilling did not reveal significant differences in treatment at any chilling level for either cultivar across both years of observation nor for individual years (Appendices B. 1–4).

In ‘AU Golden Dragon’, W.T. generally resulted in greater (but not significant) budbreak number as compared to C.C. across all chilling levels, with an average difference of 0.57 buds (7.1% vegetative budbreak). For ‘AU Fitzgerald’, W.T. and C.C. trended even more closely across the six levels of chilling, resulting in an average difference of only 0.27 buds or 3.4% vegetative budbreak. The greatest difference between treatments for ‘AU Golden Dragon’ was observed at the two highest chilling levels (0.88) and at base chilling level (0.60) for ‘AU Fitzgerald—both in favor of W.T. Interestingly, average vegetative budbreak number (both W.T. and C.C.) was lower following one week of chilling than with base level chilling for both cultivars, which coincided with the increase from estimated chilling values of 334 – 360 to 502 to 528 Richardson units. This brief reduction in budbreak likely represents the transition from ecodormancy (light rest) to endodormancy (deep state of rest).

Shoot Development Response to Chilling Type

Shoot Number

As discussed earlier, shoot development was signified by the presence of one or more leaves. Fruit in both species are primarily borne on one-year-old ‘replacement canes’ that typically originate from the base of previous years’ cane “stubs” in a manner similar to ‘spur-pruning’ in grapes. Consequently, a minimum number of quality,

properly-spaced fruiting canes are required on each vine for optimal production (Beutel, 1994; Sale and Lyford, 1990). As with budbreak, shoot development was quantified both in terms of number per cane and percent (possible total of eight) per cane, with only the former discussed here. Average shoot number per cane, which was only assessed in the second year, did not exhibit significant response to chilling type for either cultivar at any of the six chilling levels observed.

In the golden cultivar AU Golden Dragon, W.T. generally resulted in slightly greater shoot number (0.49 shoots or 6% shoot development, on average) across all chilling levels, with the greatest difference (0.93) observed at four and five weeks chilling. While the transformed data did not indicate that it was significant ($P = 0.052$), the treatment difference at five weeks chilling was significant ($P = 0.0484$), based on the non-transformed dataset (data not shown). Conversely and unlike budbreak in 'AU Fitzgerald', average shoot number in this fuzzy kiwifruit cultivar was generally, but not consistently higher for C.C. than W.T. by an average value of 0.22 (3% shoot development) across all levels of chilling. Treatment differences gradually increased with amount of chilling supplied, with the largest difference of 0.45 occurring at three and five weeks chilling. The short decline in budbreak from base to one week chilling (both chilling types) was also observed in shoot number response.

Shoot Development Relative to Budbreak

As discussed earlier, not all of the nodes exhibiting lateral budbreak produced shoots. Given the differential response of shoot number to chilling type (although not

significant) ‘percent vegetative budbreak to shoot’ incidence was assessed (second year only) to quantify the frequency of shoot development, given budbreak. Despite appreciable differences at individual levels of chilling, percent vegetative bud to shoot (per cane) did not differ significantly between treatments (W.T. and C.C.) at any chilling level for either cultivar. For the ‘AU Golden Dragon’, there was no clear pattern to treatment response across chilling levels. Moreover, W.T. the average margin in ‘budbreak to shoot value’ (across all chilling levels) was only different by 0.7%, with the greatest difference observed at one week chilling (7%), in favor of W.T.

Treatment response showed an even less clear pattern for ‘AU Fitzgerald’. Ultimately, C.C. resulted in only a 0.9% greater value on average (across all chilling levels) as compared to W.T., with the largest difference seen at base level chilling, where W.T. produced a 9% higher value. As previously discussed, average shoot number per cane was higher overall with C.C. than W.T. in ‘AU Fitzgerald’, despite the fact that vegetative budbreak per cane was generally higher with intermittent exposure to warm temperature. Of the four levels in which C.C. resulted in higher ‘percent budbreak to shoot’ values (base, two, three, and five weeks chilling), C.C. resulted in an average of 0.72 more shoots per cane. This apparent propensity for lower shoot development resulting from budbreak suggests that, while W.T. had a promotive effect on vegetative budbreak in ‘AU Fitzgerald’, it also might have inhibited shoot growth from these buds. Across all treatments and chilling levels, 86% and 88% of nodes showing budbreak resulted in shoot development in ‘AU Golden Dragon’ and ‘AU Fitzgerald’. Frequency of vegetative budbreak and shoot development (per cane) might have been

underestimated for ‘AU Golden Dragon’ due to occurrence of generally shorter internodes, resulting in potentially greater proportion of nodes submerged and therefore not capable of budbreak and subsequent shoot development.

Effect of Cane Diameter

The significantly ($P<0.0001$) smaller (24%) average cane diameter in the second year (2019) for both cultivars (all treatments and chilling levels) was most likely the result of a conscientious effort to avoid collecting and using excessively large canes. This lower average for the second year may have also been related to the fact that the vines from which they were collected had produced a heavier crop in 2018, likely resulting in less vigorous vegetative growth. Regardless, diameter (8.1 mm to 15.0 mm) and weight were of acceptable range (for both cultivars and all treatments) according to Snowball (1996) for such studies.

The negative relationship between average cane diameter and floral bud number in ‘AU Fitzgerald’ ($P<0.0001$) (across all treatments) in 2019 was corroborated by observations that particularly large, vigorous canes (as noted by long internodes) proved less prolific with regard to floral response. This pattern contradicted reports by Snowball (1996), suggesting that smaller canes lacked sufficient carbohydrate reserves for normal flowering. While the negative relationship between cane diameter and floral response was only significant ($P = 0.0216$) for W.T. treatments (all chilling levels) for ‘AU Golden Dragon’ in 2018, average cane diameter was not significantly different between the two chilling type.

Significant negative relationships were also identified between cane diameter and vegetative budbreak number for both cultivars in 2018 (all treatments) and for C.C. treatments (all chilling levels) in 'AU Fitzgerald' in 2019. Again, average cane diameter was not significantly different between chilling types. Excessively large shoots ("bull canes" or "water sprouts") are generally considered to be highly vegetative in many fruit crops. This appeared to be the case in this experiment as, while larger-diameter canes exhibited a lower incidence of vegetative budbreak, resulting shoots were particularly vigorous and generally lacking in floral buds. As described earlier, canes were selected systematically based on size for each jar (experimental unit), such that diameter-variability was largely accounted for within jars, rather than among treatments. While this practice attempted to minimize experimental error, it also likely obscured the full extent of cane diameter effect on floral and vegetative response.

Chilling Effect and Chilling Requirement Estimation

'AU Golden Dragon' Floral Chilling Requirements

As discussed earlier, vegetative and floral chilling requirements have already been estimated for both of the cultivars used in this study. However, for comparison with previously reported estimates, estimation of chilling requirements was carried out for chilling type (across all levels) by year and cultivar using ANOVA and Tukey's HSD. Results were similar to regression analyses using the Gompertz function (Wall et al., 2008) (data not shown).

Estimation of ‘AU Golden Dragon’ under W.T. conditions identified maximum floral chilling requirements of approximately 1,006 C.U (Richardson units) in 2018 and 864 C.U. in 2019. The lack of significant chilling effect on floral response under C.C. conditions in 2018 might suggest that intermittent exposure to warmer temperatures, either in a diurnal cycle or period of several days, is important in the completion of rest for this cultivar. However, floral development responded more normally (based on expectations) for C.C. in 2019, identifying a maximum floral chilling requirement of 1,032 C.U. The average maximum floral chilling requirement of approximately 970 C.U. estimated in this experiment is comparable to the estimate of 900 C.U by Wall et al. (2008) for ‘AU Golden Dragon.

‘AU Fitzgerald’ Floral Chilling Requirements

For floral response of ‘AU Fitzgerald’ in 2018, the lack of significant response to chilling under W.T. conditions in conjunction with the previously discussed indication of chilling negation further suggest that supplied chilling was less effective in conjunction with exposure to heat. Maximum floral requirements of 838 C.U and 1,200 Richardson were identified in 2018 and 2019 under C.C. conditions. The estimated chilling requirement of 696 C.U. (576 C.U. assuming hypothetical chilling negation) under W.T. conditions in 2019 was much lower. The associated average floral bud number of 2.59 (as compared to 3.76 with C.C. in 2019), along with the observed decrease in floral activity from four to five weeks chilling further implicate warm temperature interruption as an inhibitor to flowering in this cultivar. While flower bud abortion was not

extensively surveyed in this study, a higher incidence was generally observed with W.T. in this cultivar. The maximum floral chilling requirement of 1,019 C.U. (average for C.C. only) estimated in this experiment was slightly lower than the reported estimate of 1,100 Richardson Units for ‘AU Fitzgerald’ by Wall et al. (2008). However, the author also surmised that the actual requirement was likely lower.

‘Estimation of Vegetative Chilling Requirements

The lack of significant vegetative response to chilling (level) was surprising, considering that maximum vegetative budbreak was reported at 800 C.U. for both ‘AU Golden Dragon’ and ‘AU Fitzgerald’ (Wall et al., 2008). However, vegetative chilling response and requirement appears to be much more flexible as compared to floral bud development in kiwifruit, particularly prior to entering endodormancy, as evident when correlative inhibition is removed (Guerriero, 1991). Such responses have even led some, such as Brundell (1976) to suggest a lack of necessity for chilling altogether in kiwifruit. For ‘AU Golden Dragon’, chilling only had a significant effect on vegetative budbreak number under W.T. conditions, providing further evidence supporting that warm temperature in association with chilling is beneficial or is at least not detrimental in this cultivar. Under this scenario, maximum vegetative budbreak was achieved at 1,006 C.U. in 2018 and 1,032 C.U. in 2019—both of which were higher than previously reported. However, 50% vegetative budbreak was achieved as early as approximately 685 C.U., compared to the maximum of 74%, both years considered.

Floral and Vegetative Response To High Temperature Interruption

A 46% reduction in floral response (across all chilling levels) associated with H.T. in comparison to W.T. and C.C. as well as the considerably lower floral response over three- through five weeks chilling in 'AU Golden Dragon' indicate that these temperatures were detrimental to floral development in this cultivar. Except for base level chilling, H.T. floral average floral bud number per cane was consistently lower than C.C. across all levels of chilling for 'AU Fitzgerald'. The average of 63% reduction in floral response for H.T. relative to C.C. across all chilling levels strengthens the assertion that flowering in this fuzzy kiwifruit cultivar is negatively impacted by exposure to heat during chilling accumulation. Fortunately, these simulated conditions (30.6°C / 23.9°C day / night) were much warmer than typically encountered in the southeastern U.S. However, they successfully demonstrated that floral response in 'AU Golden Dragon' can be diminished by exposure to heat (at least at some intensity) during chilling accumulation. However, 'AU Fitzgerald' exhibited even greater susceptibility, given that its poorer performance at nearly every chilling level and greater reduction in average floral buds (across all chilling levels), as compared to 'AU Golden Dragon'. While the implication here is limited by the small sample size, it supports the earlier assertion of this fuzzy kiwifruit cultivar being susceptible to chilling negation by warmer temperature.

This difference in cultivar response was also evident in the case of vegetative budbreak. 'AU Golden Dragon consistently exhibited lower average vegetative budbreak number per cane relative to W.T. and C.C. until the two highest chilling levels. Across

all chilling levels, H.T. resulted in an amount of budbreak that was comparable to C.C., but slightly lower than W.T., with approximately 50% higher average vegetative budbreak than C.C. at five weeks chilling. Conversely ‘AU Fitzgerald’ exhibited a lower rate of vegetative budbreak with H.T. than both W.T. across all chilling levels.

Nodal Position Response to Chilling Type

Vegetative budbreak and floral bud number were both assessed on a per-node basis during the first year, with the results reflecting data from across all chilling levels. This was done both on a per-node position basis as well as per-node position relative to the total budbreak or floral bud number per-cane—the latter providing a more accurate representation of vegetative or floral productivity based on node position, relative to that of the entire cane.

The strong apical dominance exhibited by both cultivars across all treatments, evident by the high vegetative budbreak and, to a greater degree, high floral number at node position ten, was not surprising. While this was likely a function of limited available carbohydrates or hormone levels (particularly auxin, cytokinins, and gibberellins) in the tissue, cane angle or position may have also played a major role in the prevalence of apical dominance in this experiment, as reported earlier by Snelgar et al. (1997). As opposed to in field conditions in which fruiting canes are generally tied down in a horizontal (0°) position, canes in this experiment were positioned at a diagonal (60°) or vertical (90°) orientation, depending on arrangement in the jar. The length of the cane was likely also influential. Cane lengths containing ten nodes were chosen to better

simulate the behavior of whole canes (Dennis, 2003; Snowball, 1991) and to avoid underestimation of chilling requirements through removal of correlative inhibition (Guerriero, 1991). However, buds on these longer canes may have also suffered from greater inter-node competition for resources (primarily stored carbohydrates), resulting in the negative skewing of both vegetative budbreak, shoot growth, and especially floral production in non-apical nodes.

Apical dominance with arrested budbreak in the middle portion of the cane is commonly associated with inadequate chilling exposure in kiwifruit (Austin et al., 2002). Indeed, treatments receiving less chilling, regardless of chilling type, exhibited nearly 100% budbreak and strong shoot growth from the apical bud (node position ten) with noticeable suppression of lateral budbreak in the lower (especially middle portion of the cane) nodes (data not shown).

Previously discussed treatment differences (for entire cane cane) between W.T. and C.C. at specific chilling levels appeared to have been strongly affected by significant differences that were observed between treatments at specific node positions. For ‘AU Golden Dragon, significantly higher frequency of vegetative budbreak was observed with W.T. for several different levels of chilling at several different node positions—all of which were located in the middle (non-apical) portion of the cane. The same pattern was observed with floral response, where significantly greater number of floral buds were observed with W.T. for four and five weeks chilling at several node positions.

Chilling type did not appear to have any significant effect on either vegetative or floral response in the cultivar AU Fitzgerald, except for one chilling level-node position

combination, which resulted in lower vegetative response with C.C. (data not shown). In general, greater vegetative budbreak over the non-apical nodes (three through eight) was observed in ‘AU Fitzgerald’ with W.T. treatments, while floral bud production appeared to be generally higher with W.T. for both cultivars at these positions.

Conclusion

The objective of this study was to determine if exposure to warm temperature interruption, as encountered during winter in the southeastern United States, could result in negation of chilling, as evident in floral and vegetative response. Floral activity was significantly lower at the second-highest chilling level across both years and significantly lower at the highest level of chilling during the second year for ‘AU Fitzgerald’. Conversely, not only was floral response not significantly reduced by W.T. in ‘AU Golden Dragon’, but exposure actually resulted in significantly greater floral activity at the two highest chilling levels of chilling during the first year. However, exposure to even higher temperatures during chilling interruption proved capable of floral inhibition in this cultivar as well as ‘AU Fitzgerald’.

Vegetative activity showed no significant response to W.T., although vegetative budbreak was generally greater for both cultivars, while shoot development was largely greater in ‘AU Golden Dragon’ and lower in ‘AU Fitzgerald’ with W.T. Maximum floral chilling requirements for both cultivars in this study were similar to previously reported estimates. Cane diameter was generally negatively associated with vegetative budbreak

and floral bud number. Vegetative budbreak and especially floral response exhibited strong apical dominance, although W.T. exposure tended to lessen this effect.

The fuzzy cultivar AU Fitzgerald tended to be more prolific in flowering than its gold counterpart, but produced fewer vegetative budbreak and shoots. The earlier reports underscoring the need for winter chilling in *A. deliciosa,* particularly for floral production, were corroborated for both species in this study. Based on the results of this experiment, it is concluded that *A. deliciosa,* as represented by the cultivar AU Fitzgerald, is strongly responsive to negation of winter chilling by intermittent exposure to warm temperature, whereas the *A. chinensis* cultivar AU Golden Dragon is not.

Based on these findings, it is suggested that the 'Positive Utah Model' serves as a reliable predictor of chilling accumulation of golden kiwifruit, whereas the Dynamic Model or Richardson (Utah) Model is better suited for the green kiwifruit. It is cautioned that these implications are limited by the single cultivar representing each species in this study and further research on additional cultivars is needed.

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CHAPTER IV

EXPLORING THE RESPONSE OF FIELD-GROWN KIWIFRUIT (ACTINIDIA CHINENSIS AND A. DELICIOSA) PLANTS TO SOIL ALKALINITY

Introduction

Kiwifruit (*Actinidia deliciosa* A. Chev.) is a subtropical or warm-temperate fruit native to China. Recently, the commercialization of the golden kiwifruit (*A. chinensis* Planch.) along with availability of new green kiwifruit cultivars has led to new interest in this crop. Successful trialing of two golden kiwifruit ('AU' Golden Dragon' and 'AU Golden Sunshine') and one green cultivar ('AU Fitzgerald') by Auburn University researchers has led to the establishment of a small commercial industry in central Alabama. Recent success from trials in eastern Texas has led expanded research efforts as well as interest in developing a new industry in Texas. Kiwifruit are known to perform best in a well-drained soil with a pH of 5.5 to 7.0 (Norton, 1994). As the range of adaptation for kiwifruit in Texas is determined, high soil pH is expected to be one of the more limiting factors, given the fact that many of Texas soils have a pH well above this optimal range.

Soil Alkalinity

Alkaline soils are defined as those that have a greater than pH 7. Such soils are common in arid environments and where the parent material is alkaline. Alkaline soils are typically grouped into two classes: calcareous soils dominated by $CaCO_3$ and

typically have a pH range of 7.5 to 8.3 and tend to be highly-buffered; sodic soils, which are dominated by Na and are typically associated with saline conditions, typically with a pH of 8.5 to 10.0, with a transitionary zone between pH values of 8.3 and 8.5 (George et al., 2012). Approximately 30% of soils globally are alkaline (Chen and Barak, 1982), including many Texas soils.

While one of the most commonly observed limitations associated with alkaline soils is reduced availability of micronutrients (George et al., 2012), high soil pH may also have direct effects on plant growth. Soils with high pH can limit the roots' ability to maintain an electrochemical gradient and interfere with co-transport of anions and protons across the plasma membrane from the soil solution (White, 2012). Another direct effect of high soil pH is the potential for ammonia toxicity, which can inhibit root elongation, although this is only expected to be a problem when soil pH exceeds 10.0. (Schenk and Wehrmann, 1979). Additionally, the association between species such as kiwifruit, originating in arboreal environments with vesicular-arbuscular mycorrhizal fungi may be negatively impacted by soil pH outside their native range (Porter et al., 1987).

Soil Alkalinity and Nutrient Availability

Soil pH is one of the most critical determinants of nutrient availability and ultimately, plant growth (Comerford, 2005). Acidic soil-adapted species such as blueberry (*Vaccinium spp.),* raspberry (*Rubus*), muscadine (*Vistis rotundifolia*), and kiwifruit are known to exhibit chlorosis symptoms associated with deficiency when

planted in high pH soils (Tagliavini and Rombolà, 2001). Soil alkalinity is most influential on plant growth through reduced solubility of micronutrients, particularly iron, manganese, and zinc (George et al., 2012.). While attempts have been made to correct deficiencies for these nutrients using both soil- and foliar-applied products (Loupassaki et al, 1997; Rombola et al., 2003; Tagliavini and Rombola, 2001; Tagliavini et al., 1995), the vast majority of global kiwifruit production continues to be limited to sites with low soil pH.

Iron

Iron is the 9th most abundant element found in plants (Brown et al., 1987b), and primarily exists in soil as two forms: ferric iron (Fe^{3+}), which is typically most abundant, insoluble and thus unavailable to plants; and ferrous iron (Fe^{2+}), which is the plant-available form (Broadley et al., 2012). In plants, iron is almost exclusively located in the chloroplasts where it an important constituent of proteins such as Fe-S proteins used for redox reactions crucial to photosynthesis (Miller et al., 1995). Iron is required for other enzymes such as methoionine and lypoxygenases and critical for photosynthesis as a constituent of aminolevulinic acid (precursor for chlorophyll synthesis) and chloroplast formation and function, especially in the thylakoid membranes (Broadley et al., 2012). Iron supply also has a major influence on xanthophyll concentrations, and thus plays an important secondary role in photosynthesis (Timperio et al., 2007). Iron is important in detoxification of reactive oxygen species in chloroplasts and other parts of plants (Ravet et al., 2009; Briat et al., 2010). Iron has important roles in roots, where lack of iron can

result in morphological abnormalities including root elongation inhibition and proliferation of root hairs (Broadley et al., 2012).

Iron deficiency is reported to have the most limiting effects on kiwifruit growth in high pH soils (Tagliavini et al., 1995), and is the primary cause of lime-induced chlorosis (Broadley et al., 2012). Iron solubility decreases relative to pH and is heavily influenced by the dominance of bicarbonates (George et al., 2012). While graminaceous plants such as cereal crops employ strategy II uptake mechanisms for uptake including release of phytosiderphores and a high affinity Fe^{3+} transport system, most horticultural crops, including kiwifruit (Vizzotto et al., 1997), rely on the strategy I method of iron uptake involving the ferric-chelate reductase and excretion of protons and reductants (White, 2012). The latter method is strongly inhibited by high levels of bicarbonates (George et al., 2012). As with many species, iron deficiency symptoms appear primarily on younger leaves first as interveinal chlorosis, with chlorosis progressing from leaf margin toward the midrib and base as the deficiency becomes more severe. Symptoms of chlorosis may not be consistent from year to year due to factors such as crop load, temperature, rain, and may also vary from plant to plant. Iron deficiency very rarely results from inadequate levels in the soil, but are most commonly caused by factors affecting availability to the plant, notably soil pH (Tagliavini and Rombola, 2001). The most marked effect of iron deficiency is decreased photosynthesis (Broadley et al, 2012), and severe deficiency can result in leaf necrosis due to absence of chlorophyll and other pigments. Soil organic matter has also been known to influence the availability of iron through chelation by humic and fulvic acids (Tagliavini and Rombola, 2001), increased

microbial activity and resulting production of siderophores (Chen et al., 2000), as well as general improvement in root health and soil exploration.

Iron may also be inactivated in leaves due to high apoplastic pH, believed to result from the elevated bicarbonate levels associated with calcareous soils, leading to the experimental use of foliar acid sprays to combat this phenomenon (Tagliavini and Rombola, 2001; Tagliavini et al., 1995). Common agronomic practices employed in correcting iron deficiency include the lowering of soil pH, application of iron sulfates (in acid soils), and application of synthetic chelates of iron (such as EDTA and EDDHA) (Tagliavini and Rombola, 2001).

Chlorosis associated with iron deficiency in kiwifruit has been widely observed in California in soils with pH above 7.2 (Norton, 1994) and are well documented in other production regions such as Italy (Pelliconi, and Spada, 1992; Smith et al., 1987; Tagliavini and Rombola, 2001; Tagliavini et al., 1995; Viti et al., 1990; Vizzotto et al., 1999; Vizzotto et al., 1997). Appearance of these symptoms typically do not appear until concentrations fall below 60 µg/g dry weight in recently expanded leaves. However, chlorosis may persist in older leaves even after tissue concentrations have been restored to adequate levels (Smith et al, 1987). Iron deficiency typically results from insolubility in soils having a natural pH of less than 7.0 (Smith et al., 1987). Nevertheless, tissue concentrations for iron should range from 65 to 150 µg/g dry weight (Smith et al., 1987).

Manganese

Manganese is a transition metal and essential plant micronutrient, ranking 10th in tissue concentration (dry weight) among plant nutrients (Campbell and Nable, 1988). Like Iron, manganese is taken up as a divalent cation by active transport via H+ pump/antiporters, and is phloem-mobile (White, 2012). However, manganese is most prevalent as Mn^{4+}, thus must be reduced prior to uptake. Manganese is an important constituent of manganese-containing enzymes and serves as a cofactor for many enzymes. Manganese is also involved in the formation of proteins, carbohydrates, lipids, and is also required for cell division and extension (Broadley et al., 2012). Manganese is required for photosynthesis, particularly PS II, where it affects thylakoid membrane formation (Broadley et al.,), and concentration of chlorophyll and photosynthesis rate drop quickly when plants become deficient (Shenker et al., 2004). Manganese-deficient plants may exhibit inhibition of root elongation (Campbell and Nable, 1988), and fail to develop lateral roots (Abbot, 1967). Manganese availability is reduced at higher soil pH (especially in the presence of carbonates and high organic matter content) and is also dictated by soil moisture and temperature, organic matter content, and soil minerology (Broadley et al., 2012; Farley and Draycott, 1973). Availability can also increase when soils become saturated or during hypoxic conditions through the reduction of Mn^{4+} to Mn^{2+}, to the point of becoming toxic in some situations (Broadley et al, 2012).

While much less prevalent than that of iron, manganese deficiency has also been documented as nutritional problem for kiwifruit production, resulting in reduced fruit size and yield, and unique symptoms on leaves. Plant-availability was reported to be

strongly dependent on soil pH, with leaf tissue concentration decreasingly sharply from a pH range of 6.8 to 7.3. Symptoms appear on recently matured leaves first, exhibiting chlorosis similar to that of iron deficiency, although a wider border of dark green color along the veins is retained. Symptoms are more prevalent with increased soil pH (Asher et al., 1984), specifically above 6.8. Acceptable manganese tissue concentrations range from 50 to 150 µg/g dry weight (Smith et al., 1987).

Magnesium

Magnesium deficiency has proven to be a problem in some kiwifruit producing regions such as New Zealand, but is reported to be the result of naturally low levels in the soil, rather than caused by high concentrations of competing cations such as potassium and calcium (Smith et al., 1987). Given the fact that magnesium availability generally increases with pH (Broadley et al., 2012), magnesium deficiency is not expected to be a major nutritional limitation associated with high soil pH in kiwifruit.

Zinc

Zinc deficiency has also been reported as a constraint to production. Unlike for most crops, rosetting or reduction of leaf size is not observed, but rather gold color interveinal chlorosis, with inhibition of lateral root development appearing in severely deficient plants. As with other crops, excess phosphorous levels in the soil can result in deficiency of zinc. Acceptable tissue concentrations for zinc range from 15 to 28 µg/g dry weight (Smith et al., 1987).

The effects of soil alkalinity on plant nutrition is further complicated by altered relationships among nutrients with respect to availability. Iron and particularly zinc tend to become less available in soils with even adequate levels of phosphorous due to the formation of insoluble iron and zinc phosphates (Smith et al., 1987), particularly in alkaline soils.

Genotype Response to Soil Alkalinity

Development of rootstocks conferring tolerance to high soil pH are documented for several perennial crops including apricot, citrus, grape, peach, pear, and plum (Tagliavini and Rombola, 2001). While there are no known rootstocks for kiwifruit available with dependable tolerance to high soil pH (Song et al., 2003), differences in susceptibility have been observed (Pelliconi and Spada, 1992; Vizzotto et al., 1997; Viti et al., 1990). Viti et al. (1990) reported that D1, a male seedling selection resulting from open-pollination of *A. deliciosa* 'Bruno', exhibited greater vigor, higher leaf tissue micronutrient levels, and higher chlorophyll concentrations as compared to *A. deliciosa* 'Hayward' in a containerized study. Vizzotto et al. (1999) indicated that differences in tolerance and susceptibility may be associated with greater ability to lower rhizosphere pH (by as many as two units in a nutrient solution study) through the excretion of protons, although this mechanism may have very limited effectiveness (Hauter and Mengel, 1988), especially in highly-buffered calcareous soils.

The development of alkaline-tolerant rootstocks for kiwifruit would offer a tremendous advantage by eliminating or substantially reducing the need for

supplemental micronutrient applications (especially iron) and could greatly expand the geographic range of adaption for this crop (Tagliavini et al., 2001). Various methods of propagation have also resulted in differential growth responses in shoot (Clearwater et al., 2004; Diaz Hernandez et al., 1997; Loreti and Piccotino, 1991) and root architecture (Clearwater et al., 2004), suggesting that this variable might also have an effect on response to soil pH. Pelliconi and Spada (1992) reported differences in response to high soil pH associated with propagation method (micropropagation and cutting) for the same clone. Plant material used in this study represents a diverse group of *A. chinensis* and *A. deliciosa* seedling- and cutting-propagated cultivars. To date, very few studies on the effects of high soil pH on growth of kiwifruit have been conducted, none involving *A. chinensis*.

Objective

The objective of this experiment was to evaluate several *Actinidia chinensis* and *A. deliciosa* cultivars' response to contrasting soil pH and identify putative physiological and nutritional responses to soil alkalinity.

Materials and Methods

Plant Material

Plant material used in this study included a diverse collection of clonally-propagated (male and female) selections and seed-propagated material from both *A. chinensis* and *A. deliciosa* species. A total of five clonally-propagated cultivars were

used in this study. ‘AU Authur’ is a clonally-propagated *A. deliciosa* selection that was found as a chance seedling near Mobile, AL and is used as a pollinizer for ‘AU Fitzgerald’. ‘CK-3’ (CK 03 or ‘Meteor’) is a clonally-propagated *A. chinensis* selection that has been widely used as a pollinizer for *A. chinensis* ‘Hort16A’. ‘AU Golden Dragon’ and ‘AU Golden Sunshine’ are clonally propagated pistillate selections of *A. chinensis.* Both were developed at the Institute of Fruit and Tea, Hubei Academy of Agricultural Sciences of P.R. China and released and patented by Auburn University, following successful trailing in central Alabama (Spiers, unpublished). ‘AU Fitzgerald’ was patented by Auburn University as a clonally-propagated *A. deliciosa*. This female cultivar originated as a chance seedling of ‘Hayward’ near Mobile, AL and has also performed well in central Alabama (Table 24).

Three seed-propagated cultivar groups were also included in the study. These include open-pollinated seedlings of *A. chinensis* Zepsri Gold™ (‘ZEZY002’), *A. deliciosa* cultivars Bruno and Hayward. Given the dioecious flowering habit of kiwifruit, it is expected that seedlings of both groups would include plants of both male and female sex.

Table 24 List of plant material and characteristics included in assessment of kiwifruit response to soil pH.

Cultivar	Cultivar Abbreviation	Species	Propagation Method	Sex	Remarks
'AU Authur'	AUTHUR	*Actinidia deliciosa*	Clonal	Male	Pollinizer for 'AU Fitzgerald'
'AU Golden Dragon'	DRAGON	*Actinidia chinensis*	Clonal	Female	
'AU Fitzgerald'	FITZ	*Actinidia deliciosa*	Clonal	Female	
'AU Golden Sunshine'	SUN	*Actinidia chinensis*	Clonal	Female	
'Bruno' Seedling	BRUNO	*Actinidia deliciosa*	Sexual	[a]Mixed	Commercially used rootstock
'CK-3' / 'Meteor'	CK-3	*Actinidia chinensis*	Clonal	Male	Pollinizer for 'AU Golden Dragon'
'Hayward' Seedling	HAYWARD	*Actinidia deliciosa*	Sexual	[a]Mixed	Commercially used rootstock
Zespri Gold™ Seedling	GOLD	*Actinidia chinensis*	Sexual	[a]Mixed	

[a]Open-pollinated seedlings expected to segregate in a 1:1 female to male ratio.

Seed collected from ripe store-bought Zespri Gold 3™ golden kiwifruit and ‘Hayward’ and ‘Bruno’ green kiwifruit were mechanically separated from flesh. Cleaned seed were stratified for 6 weeks at approximately 2°C prior to planting November 2017 in 38-cell seedling plug trays. The resulting seedlings were transplanted into 2.84 L nursery containers approximately eight weeks later. Clonal material was propagated by softwood cuttings from vigorous new growth during the summer of 2017 with 3,000 mg/kg IBA “quick-dip” under intermittent mist. After approximately eight weeks, rooted cuttings were also transplanted into 2.84 L nursery containers containing a pine bark-based soil-less medium.

Seedling- and clonally-propagated material was grown in greenhouse conditions (22°C/32°C night / day temperatures) through the remainder of the fall and winter under long day (12-hour photoperiod) lighting. Plants received approximately 10 grams of 15-9-12 Scotts Osmocote® Plus (3-4 month) slow-release fertilizer during the nursery phase of establishment. Material was transitioned to an outdoor nursery in March 2018 to acclimatize for one to two months prior to field planting.

Description of Field Sites

College Station

Two field sites with contrasting soil pH conditions were selected for this study. The TAMU HORT-TREC field lab is located approximately 16 km southwest of College Station, TX. (30°36’N 96°18’W). The site is situated in the Brazos River alluvial floodplain at an elevation of approximately 71.02 meters above sea level.

Climate is considered sub-humid warm-temperate with nearby College Station, TX ranging from 5.1°C (ave. January min. temp.) to 35.7°C (ave. August max. temp.), with 1,017.5 mm of average annual precipitation. College Station historically receives an average of 274 frost-free days, with the average first and last day of frost occurring on November 30 and March 1 (Brazos County AgriLife). The lowest and highest temperatures ever recorded for College Station are -19.4°C and 44.4°C, respectively (National Weather Service). Winter chilling accumulation generally ranges from 600 to 700 units (0°C to 7°C).

Soil within the experimental plot is classified by the United States Department of Agriculture-Natural Resources Conservation Service (USDA NRCS) as a Westwood silt loam, with preliminary soil tests revealing an average soil pH values of 7.6. The same analysis revealed a calcium concentration of 6,288 mg/kg (Table 25), based on a sampling depth of approximately 30 cm (after field prep). Irrigation water was sourced from a reservoir pumped from the Brazos River. Water quality was analyzed several times during the experiment, for sodium, boron, chloride, pH, conductivity, and alkalinity (Appendix C. 1). During the first several months of the growing season in 2019 (March through May), well water was used as an alternative source for irrigation water while the river water was unavailable. This source had greater concentrations of Ca, Na, B, bicarbonates, conductivity, alkalinity, and total dissolved solids (TDS) (Appendix C. 2).

Table 25 Chemical and fertility results of preliminary soil analysis of College Station, TX site used in the assessment of kiwifruit response to soil pH.

Parameter	Result	Unit	[1]Critical Level
pH	7.6	-	≥5.8
Conductivity	0.281	dS/m	None
Nitrate-N	7	mg/kg	-
Phosphorus	21	mg/kg	-
Potassium	306	mg/kg	-
Calcium	6,288	mg/kg	180
Magnesium	287	mg/kg	50
Sulfur	22	mg/kg	13
Sodium	82	mg/kg	-
Iron	7.34	mg/kg	4.25
Zinc	0.66	mg/kg	0.81
Manganese	6.49	mg/kg	1.00
Copper	0.48	mg/kg	0.16
Boron	1.19	mg/kg	0.60
Organic Matter	4.76	%	-

[1]Critical level determination based on estimated soil chemical and fertility requirements for fruit production, as applicable
Analyses based on sampling to depth of 15 cm and 30 sample cores
Samples collected April 2018
Results generated by Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory, 2478 TAMU College Station, TX 77843

Nacogdoches

Field site two is located on the Stephen F. Austin State University campus at Nacogdoches, TX (31°36′32″N 94°39′3″W) at an elevation of approximately 92 meters above sea level. Climate is considered humid warm-temperate ranging from 2.2°C (ave. January min. temp.) to 34.4°C (ave. August max. temp.), with 1,251 mm of average annual precipitation. Winter chilling accumulation is estimated between 700 to 800 units (0°C to 7°C). Soil is classified by the USDA NRCS as a Tuscosso-Hanahatchee loamy soil. Preliminary soil analysis (30 cm depth) indicated that the soil was strongly acid with an average pH value of 5.2 and calcium concentration of 828 mg/kg (after field prep) (Table 26). Irrigation water at this site was from the Nacogdoches municipal water supply. Water quality from this source was not of concern and therefore not extensively monitored.

Table 26 Chemical and fertility results of preliminary soil analysis of College Station, TX site used in the assessment of kiwifruit response to soil pH.

Parameter	Result	Unit	Critical Level
pH	5.2	-	≥5.8
Conductivity	0.105	dS/m	None
Nitrate-N	7	mg/kg	-
Phosphorus	5	mg/kg	-
Potassium	63	mg/kg	-
Calcium	828	mg/kg	180
Magnesium	240	mg/kg	50
Sulfur	18	mg/kg	13
Sodium	16	mg/kg	-
Iron	49.47	mg/kg	4.25
Zinc	1.46	mg/kg	0.81
Manganese	31.34	mg/kg	1.00
Copper	0.40	mg/kg	0.16
Boron	0.14	mg/kg	0.60
Organic Matter	2.36	%	-

[1]Critical level determination based on estimated soil chemical and fertility requirements for fruit production, as applicable
Analyses based on sampling to depth of 15 cm and 30 sample cores
Samples collected April 2018
Results generated by Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory, 2478 TAMU College Station, TX 77843

Field Preparation, Plant Establishment, and Plot Maintenance

Field preparation was conducted in spring 2017 at the College Station site, whereas the Nacogdoches field site was not prepared until spring 2018. At both sites, existing vegetation was destroyed by glyphosate application and mowing. The soil was

then loosened using a disc harrow. Approximately 8 cm of composted pine bark material was thoroughly incorporated into the planting beds to an estimated depth of 25 to 35 cm using a tractor-mounted rotary tiller. This was done in order to improve the overall physical and chemical soil properties such as in the field production of other high-value nursery crops (Fitzpatrick, 2001). In total, an estimated 5.25 and 4.25 cubic meters of the composted bark material was added at the first and second sites (respectively), with differences due to plot configuration. Raised (30 to 38 cm tall by 45 to 60 cm wide) planting beds were erected using a disc-type bed-maker.

Plants were installed in May 2018. Root circling in the containerized plants was corrected by careful hand removal of an approximately 1.0 cm thick layer of media from the root ball. Plants were also pruned at the time of planting. This was done by removing approximately one half of the total shoot system, while retaining the most dominant shoot on all plants. Resulting plants consisted of a single shoot approximately 10 cm to 15 cm in height (from soil line).

Beds were also top-dressed with 2.5 cm to 5.0 cm of fine-texture (<1.25 cm) pine bark mulch for weed suppression and erosion control. Irrigation was supplied using a single line of drip irrigation (plastic tee tape) along each row with emitter spacing of 0.33 meters (1.02 liters per hour). Deficiencies of nitrogen, phosphorus, and potassium were corrected, based on preliminary soil analyses, via water-soluble fertilizers (ammonium sulfate, mono-ammonium phosphate, mono-potassium phosphate, and potassium nitrate) delivered via the drip irrigation shortly after planting. Irrigation applications were generally made twice per week during the growing season

(approximately 254 mm per hectare equivalent), depending on weather conditions. Continuous nitrogen application (every irrigation) through the drip irrigation (200 mg/L N) began approximately one month after planting and continued until September 1st. Nitrogen was applied using liquid urea / ammonium nitrate (UAN) solution (32% N). Soil fertility was managed the same in 2018 and 2019, including correction of nitrogen, phosphorus, and potassium.

Plants were allowed to grow naturally (without sucker removal), with several shoots trained to grow up a single 2.0 meter bamboo stake during both years. New plants were installed during March 2019 at both sites to replace failed plants (estimated 5% mortality rate during first year). These younger plants were not accounted for in analysis of leaf weight and pruning weight.

Experimental Design

Experimental design consisted of a 2 x 2 x 8 factorial with two different sites, two years of data collection, and eight cultivar types. Field layout at both two sites was configured in a randomized complete block design (RCBD), with four blocks and experimental units consisting of consecutive five-plant subsamples. Field layout at the College Station (CS) site consisted of two rows with adjacent border rows on each side, whereas the Nacogdoches (NAC) site consisted of only one row with border rows on each side. Data plants at the end of each row were bordered by five buffer plants. Plants were spaced 0.46 meter centers, with 3.31 meters between rows.

Field Data Collection

Data collection took place from the end of September through early October 2018 toward the end of the growing season and in late-June through early-July in 2019. These dates roughly correspond with mid- and late-season sampling for plant tissue analysis of nutritional status (Smith et al. 1987). All surveyed plants were in an active state of growth during both periods of data collection. All assessments were individually made for each plant, with the five-plant (subsample) average reported for each experimental unit. For most assessments, this involved two leaves per plant. In the event that fewer than five plants were available, assessments were based on more than two leaves per plant, so that the total number of observations per experimental unit was not less than ten (Table 27).

Percent canopy chlorosis was visually estimated as the proportion of foliage exhibiting chlorosis relative to normal healthy (asymptomatic) foliage. This trait was employed as an estimate of the prevalence of chlorosis over the entire plant and assessed from both sides of the plant (row). Simultaneously, RSPAD values were collected from leaves or regions of the specific leaves that represented the most severely chlorotic foliage tissue using FieldScout SPAD 502 Chlorophyll Meter (Spectrum Technologies, Inc. Plainfield, IL). Additionally, RSPAD values were collected from leaves that were typical of healthy leaf tissue for that specific plant. For both chlorotic and healthy SPAD, reported values were based on the average of two leaves with five measurement points from around each leaf (total of ten measurements per plant). Leaves from the

Table 27 Parameters assessed for eight kiwifruit cultivars in response to soil pH soil pH over two years.

Parameter	Unit	Method	Remarks
Percent Canopy Chlorosis	Percent	Visually estimated	Whole plant
Healthy SPAD	RSPAD Value	SPAD 502 Chlorophyll Meter	[1]Average of two healthy leaves per plant (five points per leaf)
Chlorotic SPAD	RSPAD Value	SPAD 502 Chlorophyll Meter	[1]Average of two chlorotic leaves (or chlorotic regions) per plant (five points per leaf)
Reference SPAD	RSPAD Value	SPAD 502 Chlorophyll Meter	Average of four healthy leaves (five points each) from non-stressed greenhouse-grown plants
Photosynthesis	µmol CO_2 m^{-2} s^{-1}	LICOR 6400XT	[1]Two youngest fully expanded leaves per plant
Stomatal Conductance	mol H_2O m^{-2} s^{-1}	LICOR 6400XT	[1]Two youngest fully expanded leaves per plant
Transpiration	mmol H_2O m^{-2} s^{-1}	LICOR 6400XT	[1]Two youngest fully expanded leaves per plant
Leaf Dry Weight	grams	Drying oven; average per leaf	[1]Two youngest fully expanded leaves from each plant
Pruning Dry Weight	grams	Drying oven; average per plant	[2]Removal of entire shoot system above 20 cm during dormant season; [3] Removal of approximately 50% of shoot system
Leaf Tissue Nutritional Analysis	mg/kg		[1]Two youngest fully expanded whole leaves from each plant
Soil Analysis	mg/kg	Core method at three, 15-cm depths	32 cores per block, 20 cm from base of vine

[1]Data collection based on three to four leaves per plant were when less than five plants per treatment were available
[2]Material for pruning dry weight collected during dormant season during 2018
[3]Material for pruning dry weight collected during growing season (June) in 2019
All data based on average of five plants per treatment

same position (but not necessarily the same age) were sampled, as possible. Digital photographs were also taken of each group of five plants to allow for visual comparison of foliar chlorosis symptoms later.

At approximately the same date, RSPAD values were surveyed from healthy containerized plants produced in a greenhouse. These plants were grown in soil-less (pine bark-based) medium and received continuous fertigation/reverse-osmosis water with water-soluble fertilizer (Peters® Professional 21-7-7 Acid Special) at a nitrogen concentration of 200 mg/L. However, unlike the field-grown plants, this material received corrective measures for micronutrient deficiencies (according to visual symptomology and leaf tissue sampling) via supplemental drench applications of chelated micronutrient products (BASF Sprint®138 6% EDDHA-chelated iron at the labeled concentration of 0.6 g / L; Growth® Products 5% Glucoheptonate-chelated manganese/2% sulfur at the labeled concentration of 1.5 mL / L). RSPAD values were collected from five points around the leaf and four leaves per plant (total of 20 measurements per plant). The 'reference SPAD' was the RSPAD value, which represented the optimal, non-stressed phenotype associated with each cultivar. Reference SPAD for each cultivar was based on the average four surveyed plants (80 total measurements per cultivar). Leaf tissue samples were collected for nutritional analysis along with substrate pH using the 1:2 extract method (Nelson, 2003).

Physiological measurements including photosynthesis, stomatal conductance, and transpiration, were surveyed for the two cultivars that represented the most and least severe visual symptoms of chlorosis (percent canopy chlorosis) at both sites and for both

years. Based on comparison of average PCC values at the College Station site in 2018, ‘AU Golden Dragon’ and ‘AU Authur’ (respectively) were selected. All physiological measurements were done using a LICOR 6400-XT portable photosynthesis system (LI COR, Inc., Lincoln, NE). The same two youngest fully-expanded leaves per plant that were surveyed, were also later collected and used for assessment of leaf dry weight and for plant tissue nutritional analysis. As earlier, leaves from the same region or position of the plant were selected. Constant variables for gas exchange were programmed as the following: photosynthetically active radiation (PAR): 1,400 µmol/m^2/sec^{-1}; sample CO_2: 400 mg/kg; leaf temperature: 20°C; fan mode setting: ‘fast’; relative humidity range of approximately 60% to 70%. Measurements were made on well-hydrated plants between the hours of 8:00 AM and 11:30 AM.

Whole-leaf (blade and petiole) tissue samples were collected shortly after completion of gas exchange measurements. Two youngest fully-expanded leaves were collected from each plant (generally from the same region), for a total of ten leaves per sample. Three to four leaves were harvested when fewer than five plants were available in order to collect a total of ten or more leaves for each experimental unit. Samples were placed in polyethylene bags and placed in refrigerated (4°C to 6°C) storage until processing (two to four days later). Leaves were hand-washed in a mild phosphate-free detergent solution, double-rinsed in reverse-osmosis water, and allowed to partially air-dry. After washing, each sample was placed in a labeled paper bag and placed in a forced-air drying oven at 80°C for at least 48 hours (Romheld, 2012). Digital photographs of each sample were also collected for latter evaluation. After drying,

samples were weighed (g) to calculate average dry weight per leaf for measure of leaf size and to facilitate the expression of nutrient status alternatively in terms of content on a per-leaf basis, as suggested by Negrao et al. (2017). Tissue samples were held in polyethylene bags until further processing.

After plant-based measurements were completed, soil samples were collected to assess nutrient availability and chemical analysis of the soil. This was accomplished using a hand-coring probe (approximately 20 cm from plant base) to a depth of approximately 46 cm. Cores were collected randomly from throughout each block for a total of approximately 30 cores. Cores were partitioned into three 15 cm subsamples based on depth via separate probe insertions into the same hole. Core samples were bulked according to soil depth and block for a total of 12 individual samples per site. Samples were allowed to air-dry completely at room temperature for several days before being pulverizing with a rubber mallet, sieving, and thorough mixing. A mass of approximately 500 g from each sample was placed in polyethylene bags for chemical and nutritional analysis.

Pruning weight data was collected as the primary measure of growth and vigor. For the first year, this was done in at the end of the season in early January (2019) during dormancy by cutting all shoot material to a standard height of 20 cm above ground level. Pruning material was bulked together with plants of the same experimental unit. Care was taken to separate material by treatment, block, and site. Pruning wood was cut into short (<30 cm) lengths to facilitate packing into large paper bags, then placed in a forced-air drying oven (80°C) for 10 to 14 days. Samples were weighed (g) to calculate

an average dry pruning weight for all treatments. For mid-season data collection in the second year (2019), all shoot growth was cut back by an estimated amount of approximately 50% (in height) shortly after collection of other field data and leaf tissue samples (late-June to early-July). The leafy material was spread out on greenhouse benches to partially dry for three to five days before being cut, bagged, dried, and weighed as previously described for the first year.

Chemical and Nutritional Analyses

Dried plant tissue and soil samples were taken to the Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory (2610 F&B Road, College Station, TX 77845) for analysis. Prior to analysis, soil samples were heated in a forced air oven at 60°C for 16 hours or until dry. Soil was then pulverized and sieved to remove >2mm particles. Mehlich III extraction in conjunction with inductively coupled plasma mass spectrometry (ICP) was used for analysis of soil P, K, Ca, Mg, Na, and S (Mehlich, 1984; Mehlich, 1978). Soil nitrate-N was determined using a 1 N KCl solution followed by cadmium reduction in conjunction with spectrophotometry (Kachurina et al., 2000; Keeney et al., 1982). Soil micronutrients (Cu, Fe, Mn, and Zn) were extracted using 0.005 M diethylenetriaminepentaacetic acid (DTPA), 0.01 M $CaCl_2$, and 0.10 M triethanolamine solution and determined by ICP (Lindsay et al., 1978). Boron was analyzed via hot-water extraction and quantified using ICP (de Abreu et al., 1994). Soil organic matter was determined by combustion procedure in conjunction with grinding to pass an 80 mesh screen (Schulte and Hopkins, 1996; Storer, 1984). Soil pH was

determined using a hydrogen-selective electrode following 1:2 soil:deionized water extraction for a minimum of 30 minutes (Schofield and Taylor, 1955). Electrical conductivity (E.C.) was determined using a conductivity probe after extraction in 1:2 soil:deionized water solution for a minimum of 30 minutes (Rhoades, 1982). Soil Texture was analyzed using the hydrometer procedure (Day, 1965; Murphy and Riley, 1962) (Table 28).

Total plant tissue nitrogen was determined by high temperature-combustion (Nelson and Sommers, 1973; McGeehan and Naylor, 1988). Plant minerals (B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn) were determined by nitric acid digestion in conjunction with ICP (Isaac and Johnson, 1975; Havlin and Soltanpour, 1989) (Table 29).

Table 28 Soil-related response variables used in the response of kiwifruit plants to soil pH over two years for eight kiwifruit cultivars in response to soil pH.

Response Variable	Abbreviation	Unit	Derivation
Topsoil pH	Soil-pH	Logarithmic	Hydrogen-selective probe (1:2 soil: water solution)
Topsoil conductivity	Soil Conductivity	dS/m	Conductivity probe (1:2 soil: water solution)
Topsoil nitrate	Soil-N	mg/kg	Cd reduction / spectrophotometry
Top soil phosphorus	Soil-P	mg/kg	Mehlich III / ICP
Topsoil potassium	Soil-K	mg/kg	Mehlich III / ICP
Topsoil calcium	Soil-Ca	mg/kg	Mehlich III / ICP
Topsoil magnesium	Soil-Mg	mg/kg	Mehlich III / ICP
Topsoil sulfur	Soil-S	mg/kg	Mehlich III / ICP
Topsoil sodium	Soil-Na	mg/kg	Mehlich III / ICP
Topsoil iron	Soil-Fe	mg/kg	DPTA / ICP
Top soil zinc	Soil-Zn	mg/kg	DPTA / ICP
Top soil manganese	Soil-Mn	mg/kg	DPTA / ICP
Topsoil copper	Soil-Cu	mg/kg	DPTA / ICP
Topsoil boron	Soil-B	mg/kg	Hot water extraction / ICP
Topsoil organic matter	Soil-OM	Percent	High temperature combustion
Topsoil texture	Soil-texture	Textural class	Hydrometer procedure

Soil samples collected from 32 cores per block, 20 cm from plant base, to depth of 30 cm.
Results generated by Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory, 2478 TAMU College Station, TX 77843

Table 29 Plant tissue-related response variables used in the response of kiwifruit plants to soil pH over two years.

Response Variable	Abbreviation	Unit	Derivation
Tissue nitrogen	Tissue-N	mg/kg	High-temperature combustion
Tissue phosphorus	Tissue-P	mg/kg	Nitric acid digestion / ICP
Tissue potassium	Tissue-K	mg/kg	Nitric acid digestion / ICP
Tissue calcium	Tissue-Ca	mg/kg	Nitric acid digestion / ICP
Tissue magnesium	Tissue-Mg	mg/kg	Nitric acid digestion / ICP
Tissue sodium	Tissue-Na	mg/kg	Nitric acid digestion / ICP
Tissue zinc	Tissue-Zn	mg/kg	Nitric acid digestion / ICP
Tissue iron	Tissue-Fe	mg/kg	Nitric acid digestion / ICP
Tissue copper	Tissue-Cu	mg/kg	Nitric acid digestion / ICP
Tissue manganese	Tissue-Mn	mg/kg	Nitric acid digestion / ICP
Tissue sulfur	Tissue-S	mg/kg	Nitric acid digestion / ICP
Tissue boron	Tissue-B	mg/kg	Nitric acid digestion / ICP

Data based on whole-leaf sampling, two leaves per plant.
Samples based on three to four leaves per plant when fewer than five plants were available (minimum total of ten leaves per sample).
Results generated by Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory, 2478 TAMU College Station, TX 77843

Response Variables

Response variables consisted of a combination of visual or symptomatic responses, physiological measurements, horticultural responses, tissue-based nutritional analyses, and soil-based analyses. As mentioned earlier, all data points used in the analysis were reported as the average of each experimental unit (generally five plants). Percent canopy chlorosis (PCC) was used to quantify the extent of chlorotic foliage over the entire plant. Healthy SPAD (SPADh) represented the average RSPAD values

exhibited in leaf tissue that was considered to be healthy or asymptomatic in regard to chlorosis. Conversely, chlorotic SPAD (SPADc) represented the average RSPAD value for tissue exhibiting the most severe visual symptoms of chlorosis. Percent SPAD (P-SPAD) was calculated as the quotient of SPADc and SPADh, expressed as a percentage: $PSPAD = (SPADc/SPADh)x100$. Expressing the chlorotic SPAD value as a proportion of the healthy SPAD value provided a relative inverse measure for intensity of chlorosis. Chlorosis index (CI) was calculated to include the effects of PCC, SPADc, SPADh, and SPADr: $I = PCC * [(1 - (SPADc/SPADh)) + (1 - (SPADh/SPADr))$]. Not only does this formula attempt to calculate the prevalence of chlorosis and intensity of chlorosis, but also takes into account healthy SPAD as a proportion of the reference SPAD. This additional provision was included in the formula in response to frequent observations indicating a noticeably lower SPADh as compared to SPADr for respective cultivars (Table 30).

Physiological response variables included photosynthesis (*PS*) (µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s) (mmol m^{-2} s^{-1}), and transpiration (*E*) (mol H_2O m^{-2} s^{-1}). Physiological responses such as photosynthesis and chlorophyll fluorescence are known to be deleteriously affected by soil alkalinity (Gonzáles-Mas et al., 2009), generally as a function of nutritional stress (Flore and Lakso, 1989).

Table 30 Visual, physiological, and Horticultural response variables used in the response of kiwifruit plants to soil pH over two years.

Response Variable	Abbreviation	Unit	Derivation
Percent Canopy Chlorosis	PCC	Percent	Visually estimated, whole plant
Healthy SPAD	SPADh	RSPAD Value	SPAD 502 Chlorophyll Meter, average of two healthy leaves per plant (five points per leaf)
Chlorotic SPAD	SPADc	RSPAD Value	SPAD 502 Chlorophyll Meter, average of two healthy leaves per plant (five points per leaf)
SPAD Percentage	P-SPAD	Percent	$PSPAD = \left(\frac{SPADc}{SPADh}\right) x100$
Chlorosis Index	CI	Numerical	$CI = PCC * [\left(1 - \left(\frac{SPADc}{SPADh}\right)\right) + \left(1 - \left(\frac{SPADh}{SPADr}\right)\right)]$
Photosynthesis	*PS*	µmol CO_2 m^{-2} s^{-1}	LICOR 6400XT, two youngest fully expanded leaves per plant
Stomatal Conductance	g_s	mmol m^{-2} s^{-1}	LICOR 6400XT, two youngest fully expanded leaves per plant
Transpiration	*E*	mol H_2O m^{-2} s^{-1}	LICOR 6400XT, two youngest fully expanded leaves per plant
Leaf Dry Weight	LW	grams	Sample Leaf Dry Weight ÷ Leaf Number
Pruning Dry Weight	PW	grams	Sample Pruning Dry Weight ÷ Plant Number

[1]Data collection based on three to four leaves per plant were when less than five plants per treatment were available
[2]Material for pruning dry weight collected during dormant season during 2018
[3]Material for pruning dry weight collected during growing season (June) in 2019
All data based on average of five plants per treatment

As discussed earlier, plant tissue sampling was conducted concurrently with collection of visual and symptomatic data. Results were conveyed both in terms of concentration (mg/kg dry weight) as well as content (mg / leaf), as a function of previously surveyed leaf dry weight (LW). Tissue analyses included all macronutrients and micronutrients: N (expressed as % and mg/kg), P, K, Ca, Mg, Na, Zn, Fe, Cu, Mn, S, and B. All nutrient concentration are referred to from here on with 'tissue' as a prefix preceding the specific element (example: 'tissue-N'). Cl was not included in tissue or soil analysis. Tissue-Na data in 2019 was reported as a minimum of 79.956 mg/kg for both sites (likely as a result of testing method).

For soil-related data, all analyses were based on an average of the top two 15-cm horizons, collectively referred to as 'top soil' (0 to 30 cm in depth). Parameters included pH, conductivity, nitrate-N, P, K, Ca, Mg, S, Na, Fe, Zn, Mn, Cu, B, and soil percent organic matter. Top soil analyses results are referred to with 'soil' as a prefix for each specific parameter (example: top soil-pH). Soil texture was analyzed for each block at both sites during 2019 for reference purposes.

Statistical Analysis

All statistical analyses were performed using JMP software, Version 14.0, SAS Institute Inc., Cary, NC. Data for all variables was checked for normality using the Shapiro-Wilcox Test at the 0.05 alpha level. The test revealed that data from the following variables was not from the normal distribution: PCC, Healthy SPAD, CI, stomatal conductance, LW, PW, tissue-N, tissue-P, tissue-Mg, tissue-Na, tissue-Zn,

tissue-Fe, tissue-Mn, tissue-S, tissue-B, soil-pH, soil-nitrate, soil-K, soil-Ca, soil-S, soil-Na, soil-Fe, soil-Mn, soil-Cu, soil-B, and soil-OM. Of these variables, stomatal conductance, tissue-N, tissue-P, tissue-Zn, tissue-Fe, tissue-S, tissue-B, soil-S, soil-Na, soil-Cu, and soil-B were successfully transformed using the natural log (Ln) method, whereas the square root transformation was used for CI. For all other variables, the non-transformed data was used for all analysis.

A multi-factor ANOVA model (0.05 alpha-level) was used to estimate effects for site, year, cultivar, block, and interactions between site and year, site and cultivar, year and cultivar, and site x year x cultivar for each dependent variable. Student's t-Test (0.05 alpha-level) was used for estimating response to site and year (as applicable). All dependent variables were assessed for cultivar response by one-way ANOVA (0.05 alpha-level). Tukey's HSD Test (0.05 alpha-level) was used to compare means among cultivar response to all response variables with the exception of *PS*, g_s, and *E*. These physiological parameters' (*PS*, g_s, and *E*) response to cultivar, along with visual parameters (PCC, SPAD-P, and CI) response to species and propagation method, were assessed using Student's t-Test (0.05 alpha-level).

Principle Component Analysis (PCA) on correlations was performed on a total of 33 response variables. Determination of variable retention from PCA was based on eigenvalue of 1.0 or greater for non-rotated factors. Eigenvalue score plots were used to estimate the effect of vectors on total variance. Rotational factor analysis (Principle Components factoring method and Principle Components prior communality) with orthogonal Varimax and oblique Promax were used to potentially reduce the number of

variables by grouping those with similar characteristics. For each rotation, three factors were used. Factors that had a significant loading factor of <0.50 were considered non-significant.

Correlations between response variables were estimated using the Row-wise method. Correlation strengths were categorized based on correlation probability as * = $P<0.05$; ** = $P<0.01$; *** = $P< 0.001$.

Results

Soil Analyses

Comparison of soil horizon pH and physical properties by site and year

Field soil at the Nacogdoches (NAC) plot was classified as a Tuscosso-Hanahatchee loam by the USDA NRCS. However, approximately 25 to 35 cm of a coarse sandy topsoil material was added to the top of each planting bed, prior to compost incorporation and planting, creating a unique and abnormal soil profile. Based on textural analysis, the upper horizon (0-15 cm depth) was as a sandy loam (77% sand; 12% silt; 12% clay) and the middle horizon (16-30 cm) also a sandy loam (68% sand; 16% silt; 17% clay). Analysis of the lower (31-45 cm) horizon indicated that this sandy clay loam (54% sand; 23% silt; 24% clay) was more representative of the native soil (data not shown).

Soil at the College Station (CS) plot, originally mapped as a Westwood silt loam was, with soil texture revealing a loam (28% sand; 49% silt; 24% clay) in the upper horizon (0-15 cm depth),) clay loam (26% sand; 46% silt; 29% clay) in the middle

horizon (16-30 cm), and silt loam (26% sand; 50% silt; 25 % clay) in the lower (31-45 cm) horizon based on textural analysis (data not shown).

Average soil-pH (top soil) was significantly higher (P<0.0001) at CS (7.60) as compared to NAC (5.63) across both years. Specifically, average pH in the upper, middle, and lower 15-cm horizons were all significantly higher (P<0.0001) at CS (7.59, 7.61, and 7.82, respectively) in comparison to those at NAC (5.64, 5.62, and 5.44, respectively) (Figure 71).

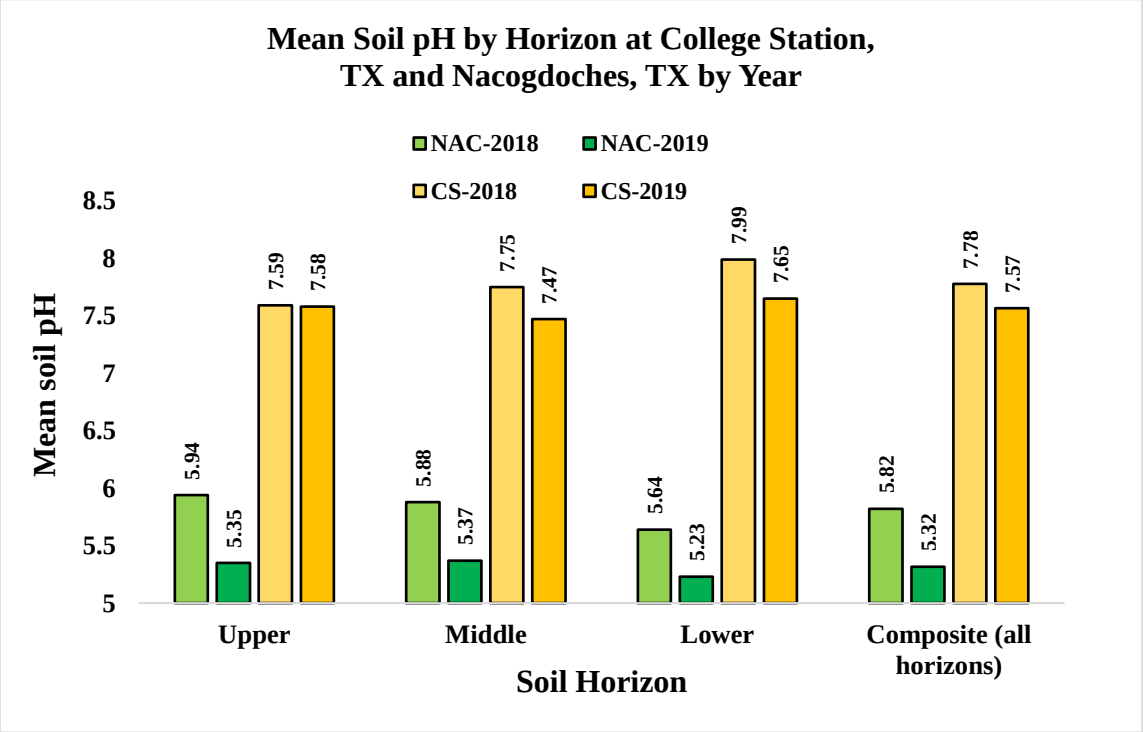


Figure 71 Mean soil pH value by soil horizon (15-cm depth) at two sites and two years used in the assessment of kiwifruit response to soil pH.

Comparison of Topsoil Parameters by Site over Years

Soil nutrient and parameter results were compared with recommended concentrations provided by the Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory for fruit crops along with B, Na, pH, and conductivity, which were specific for kiwifruit (Norton, 1994; Sale and Lyford, 1990) (Table 31). The vast majority of top soil parameters showed a significant response to site over two years. A listing of all significant effect and interactions of topsoil parameters is presented in Table 32.

Table 31 Recommended soil nutrient and chemical parameters for fruit crops used in the assessment of kiwifruit response to soil pH.

Element / Parameter	Low Threshold	High Threshold	Remarks
[1]Nitrogen	30.0 mg/kg	-	
[1]Phosphorus	50.0 mg/kg	-	
[1]Potassium	175.0 mg/kg	-	
[1]Calcium	180.0 mg/kg	-	
[1]Sulfur	13.0 mg/kg	-	
[1]Magnesium	50 mg/kg	-	
[1]Manganese	1.0 mg/kg	-	
[1]Iron	4.25 mg/kg	-	
[1]Zinc	0.27 mg/kg	-	
[1]Copper	0.16 mg/kg	-	
[2]Boron	-	0.5 mg/kg	Specific to kiwifruit
[2]Sodium	-	Minimal	Specific to kiwifruit
[1]Chloride	-	-	
[2,3]pH	6.0	7.2	Specific to kiwifruit
[1,2]Conductivity	105 µmol/cm	1,000 µmol/cm	Specific to kiwifruit (high threshold)

[1]Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory, 2478 TAMU College Station, TX 77843 (general fruit crops)
[2]Norton, 1994 (kiwifruit)
[3]Sale and Lyford, 1990 (kiwifruit)

Table 32 List of significant effects and interactions for topsoil parameters used for eight kiwifruit cultivars in the assessment of response to soil pH.

Response Variable	Site	Year	Site x Year	Block
Soil-pH	P<0.0001	P=0.0323	ns	ns
Soil-Conductivity	P<0.0001	P<0.0001	ns	P=0.0421
Soil-nitrate	ns	P<0.0001	ns	ns
Soil-P	P<0.0001	ns	P=0.0029	ns
Soil-K	P<0.0001	ns	ns	ns
Soil-Ca	P<0.0001	ns	ns	ns
Soil-Mg	P<0.0001	ns	ns	ns
Soil-S	P<0.0001	P<0.0001	ns	ns
Soil-Na	P<0.0001	P<0.0001	ns	ns
Soil-Fe	P<0.0001	P=0.0071	P=0.0093	ns
Soil-Zn	P = 0.0038	ns	ns	ns
Soil-Mn	P<0.0001	ns	ns	ns
Soil-Cu	ns	ns	ns	ns
Soil-B	P<0.0001	P<0.0001	P=0.0013	ns
Soil-OM	P<0.0001	ns	ns	ns

Soil-conductivity (P = 0.04), soil-K (P<0.0001), soil-Ca (P<0.0001), soil-Mg (P<0.0001), soil-S (P = 0.01), and soil-Na (P = 0.02) values were all higher at CS (0.271 dS/m, 250.2 mg/kg, 5,607 mg/kg, 306.8 mg/kg, 23.3 mg/kg, and 59.7 mg/kg, respectively), as compared to average values at NAC (5.63, 144.6 µmol/cm, 67.2 mg/kg, 1,029.7 mg/kg, 162.3 mg/kg, 11.8 mg/kg, and 14.0 mg/kg, respectively) across years. A significant block effect was present for soil-conductivity. Additionally, average soil-OM was significantly (P< 0.0001) higher at CS (6.07% than at NAC (2.29%) across years.

Conversely, average values for soil-Zn (P = 0.02) and soil-Mn (P< 0.0001) were significantly lower (1.42 mg/kg and 14.8 mg/kg, respectively) at CS than those at NAC (2.05 mg/kg and 38.8 mg/kg, respectively) across years. Only soil-nitrate and soil-Cu were not significantly different between sites over the two years. Significant (P<0.05) site x year interactions were observed for soil-P, soil-Fe, and soil-B, requiring that site comparisons be made for single years (Figures 72-74).

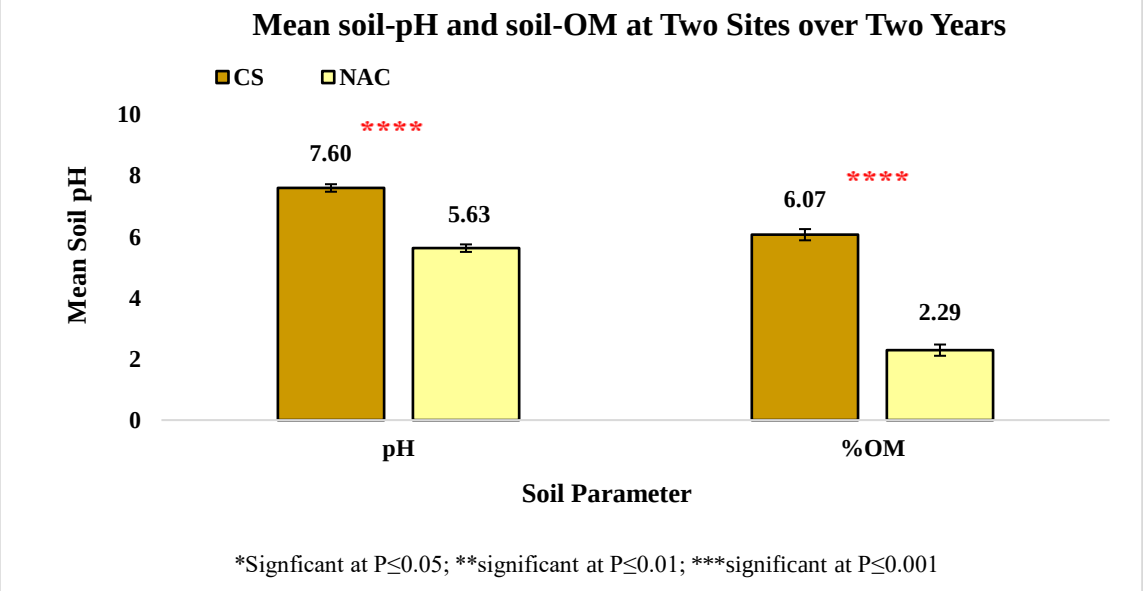


Figure 72 Comparison of mean soil-pH and soil-OM at College Station, TX and Nacogdoches, TX across two years for eight kiwifruit cultivars in the assessment of response to soil pH.

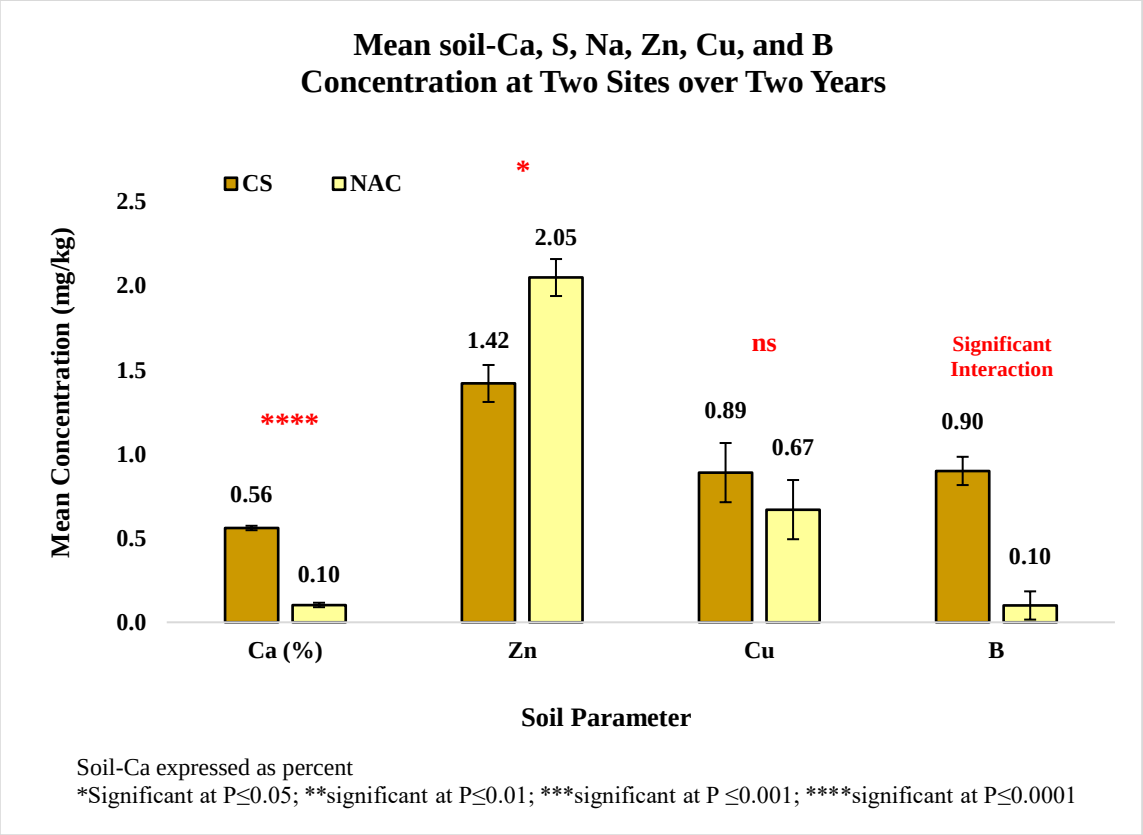
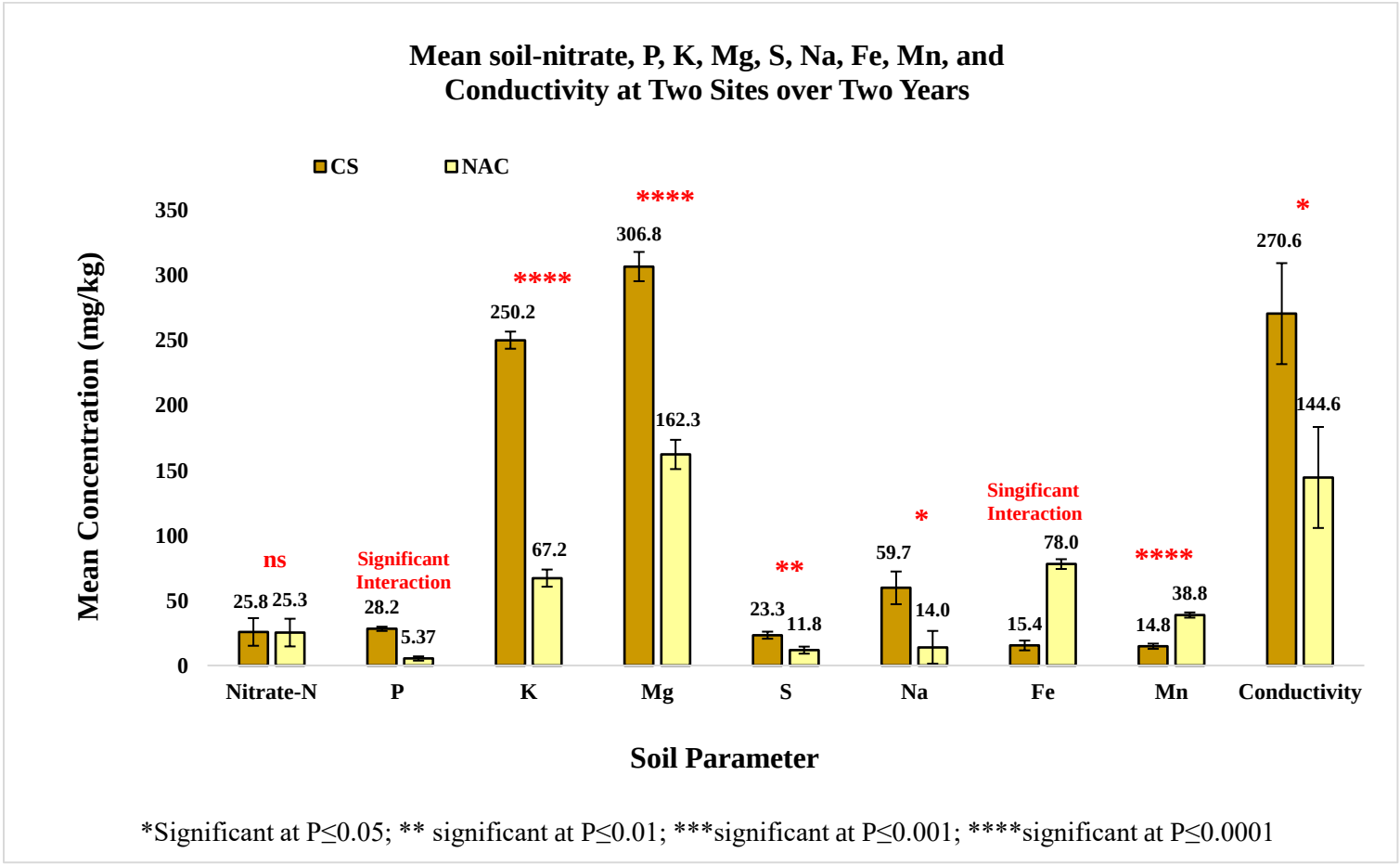


Figure 73 Comparison of mean soil-Ca, soil-Zn, soil-Cu, and soil-B at College Station, TX and Nacogdoches, TX across two years for eight kiwifruit cultivars in the assessment of response to soil pH.



*Significant at P≤0.05; ** significant at P≤0.01; ***significant at P≤0.001; ****significant at P≤0.0001

Figure 74 Comparison of mean soil-nitrate, soil-P, soil-K, soil-Mg, soil-S, soil-Na, soil-Fe, soil-Mn, and soil-conductivity at College Station, TX and Nacogdoches, TX across two years for eight kiwifruit cultivars in the assessment of response to soil pH

Comparison of Topsoil Parameters Response to Site by Individual Year

Separate analysis of top soil parameters by site for the year 2018 indicated that average soil-P (P = 0.003) and soil-B (P = 0.0002) were significantly higher at CS (32.3 mg/kg and 0.68 mg/kg, respectively) than at NAC (9.2 mg/kg and 0.03 mg/kg, respectively). The average soil-Fe value of 15.6 mg/kg at CS was significantly (P = 0.0004) lower than that of 88.6 mg/kg at NAC (Figures 75 - 77).

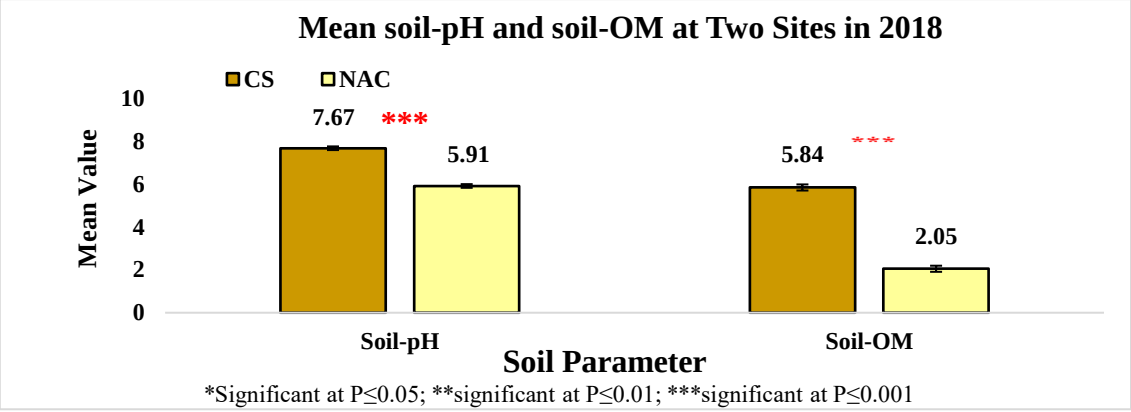


Figure 75 Comparison of mean soil-pH and soil-OM at College Station, TX and Nacogdoches, TX in 2018 for eight kiwifruit cultivars in response to soil pH.

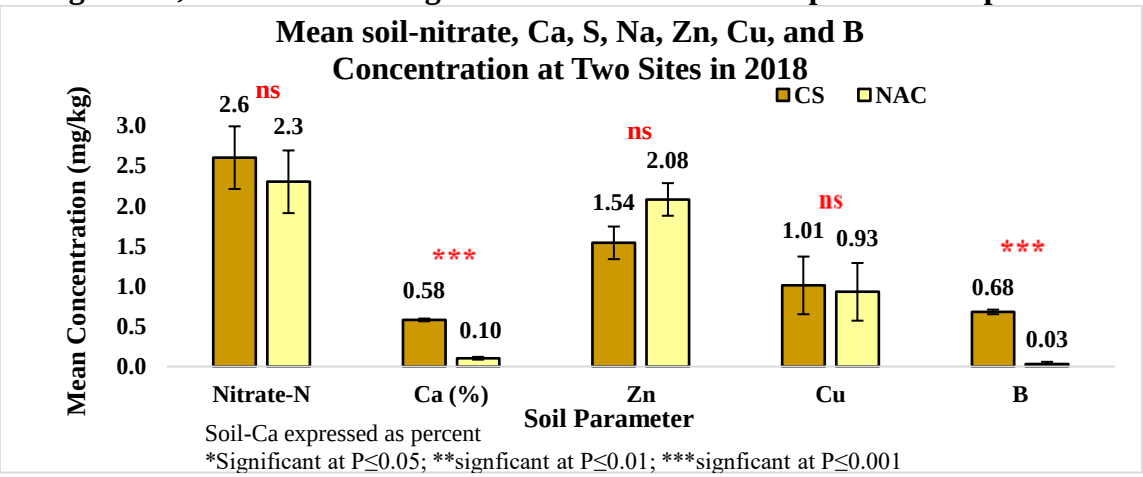


Figure 76 Comparison of mean soil-nitrate-N, soil-Ca, soil-Zn, soil-Cu, and soil-B at College Station, TX and Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

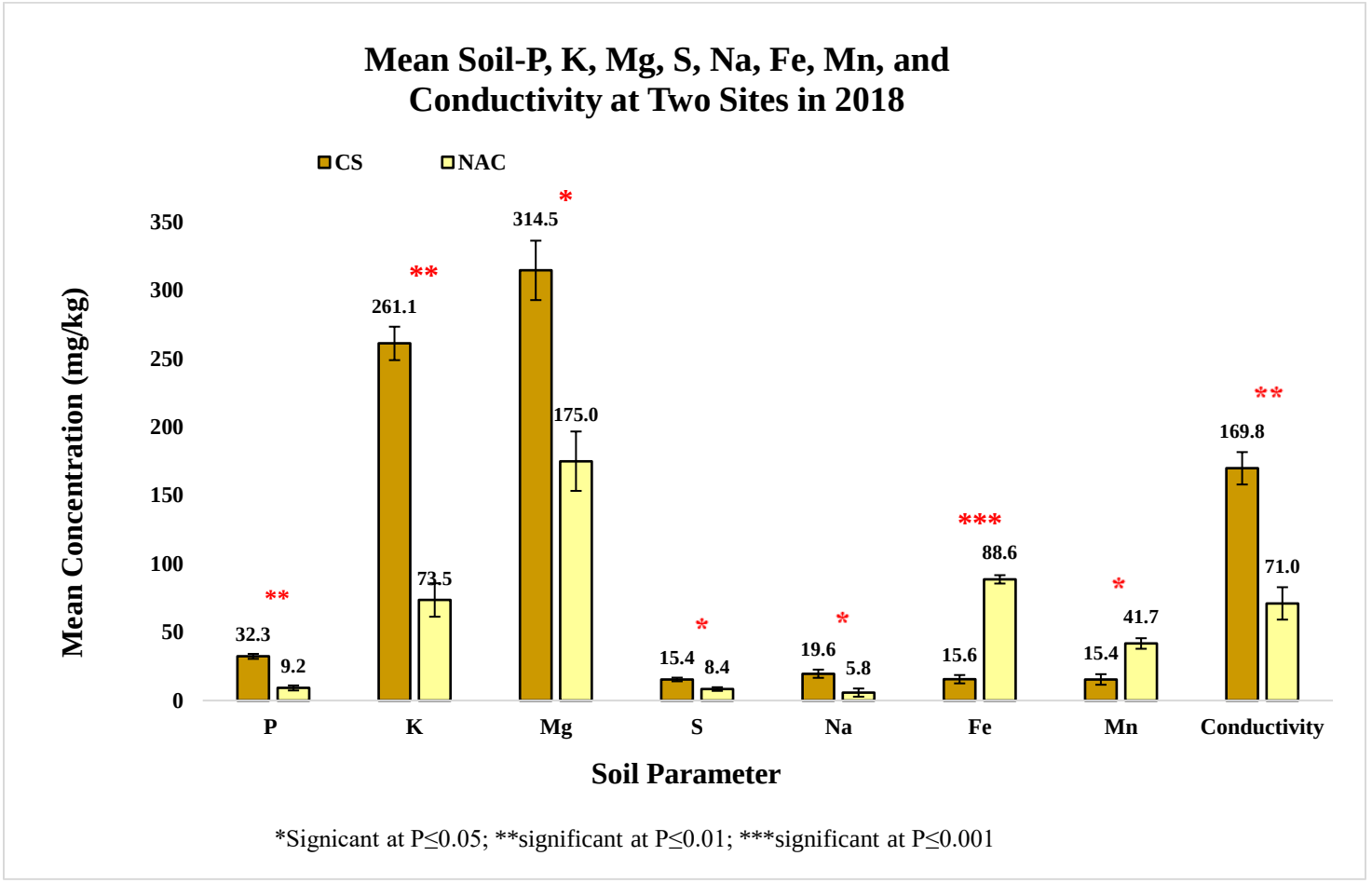


Figure 77 Comparison of mean soil-P, soil-K, soil-Mg, soil-S, soil-Na, soil-Fe, soil-Mn, and soil-conductivity at College Station, TX and Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

Site comparison for 2019 yielded similar results, with significantly higher average values for soil-P and soil-B (P = 0.0002; 0.004) at CS (24.0 mg/kg and 1.12 mg/kg, respectively) as compared to those at NAC (12.3 mg/kg and 0.16 mg/kg, respectively). As with the first year, soil-Fe was significantly lower (P = 0.0022) at CS (15.1 mg/kg) than at NAC (67.5 mg/kg). Additionally, there was a significant site response for soil-Zn (P = 0.02) and soil-Cu (P = 0.01), with CS yielding lower average soil-Zn (1.3 mg/kg) and higher average soil-Cu (0.77 mg/kg) in comparison to NAC (2.01 mg/kg and 0.41 mg/kg, respectively) (Figures 78 - 80).

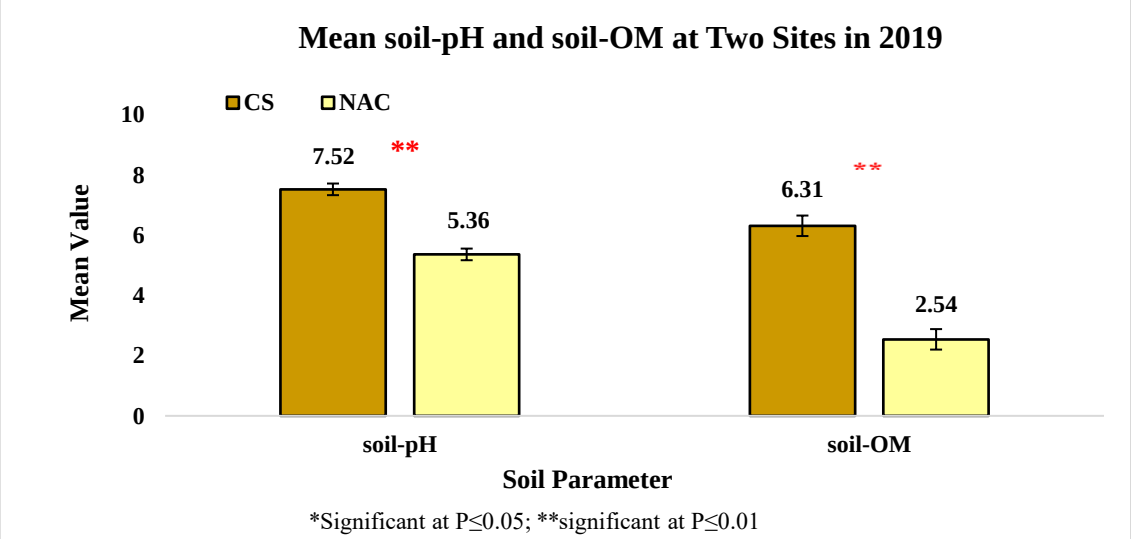


Figure 78 Comparison of mean soil-pH and soil-OM at College Station, TX and Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

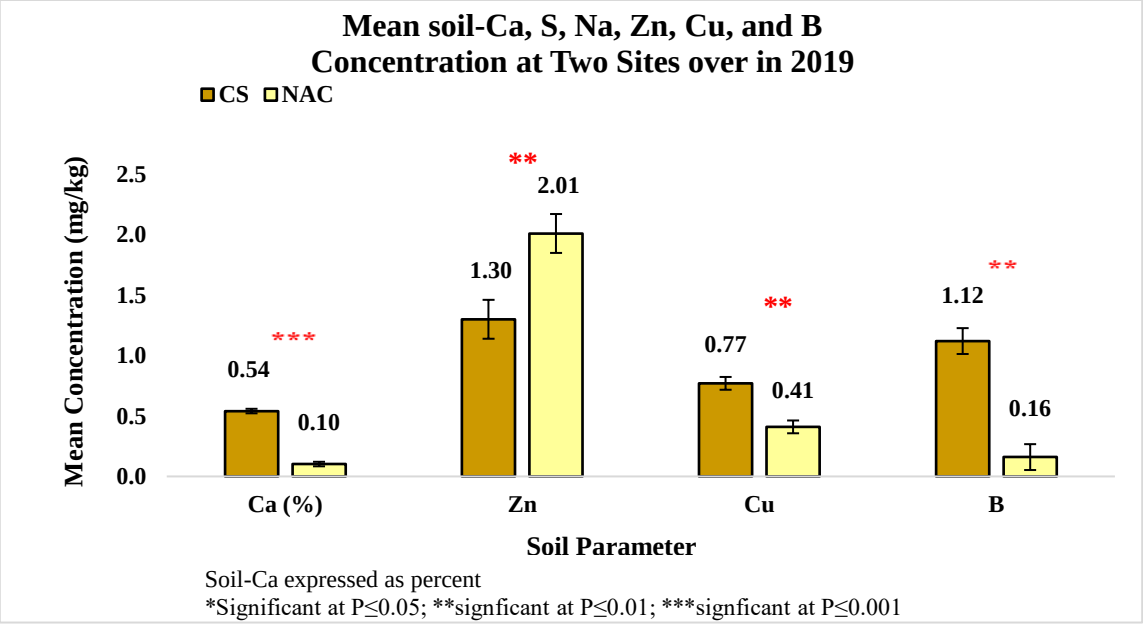


Figure 79 Comparison of mean soil-Ca, soil-Zn, soil-Cu, and soil-B at College Station, TX and Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

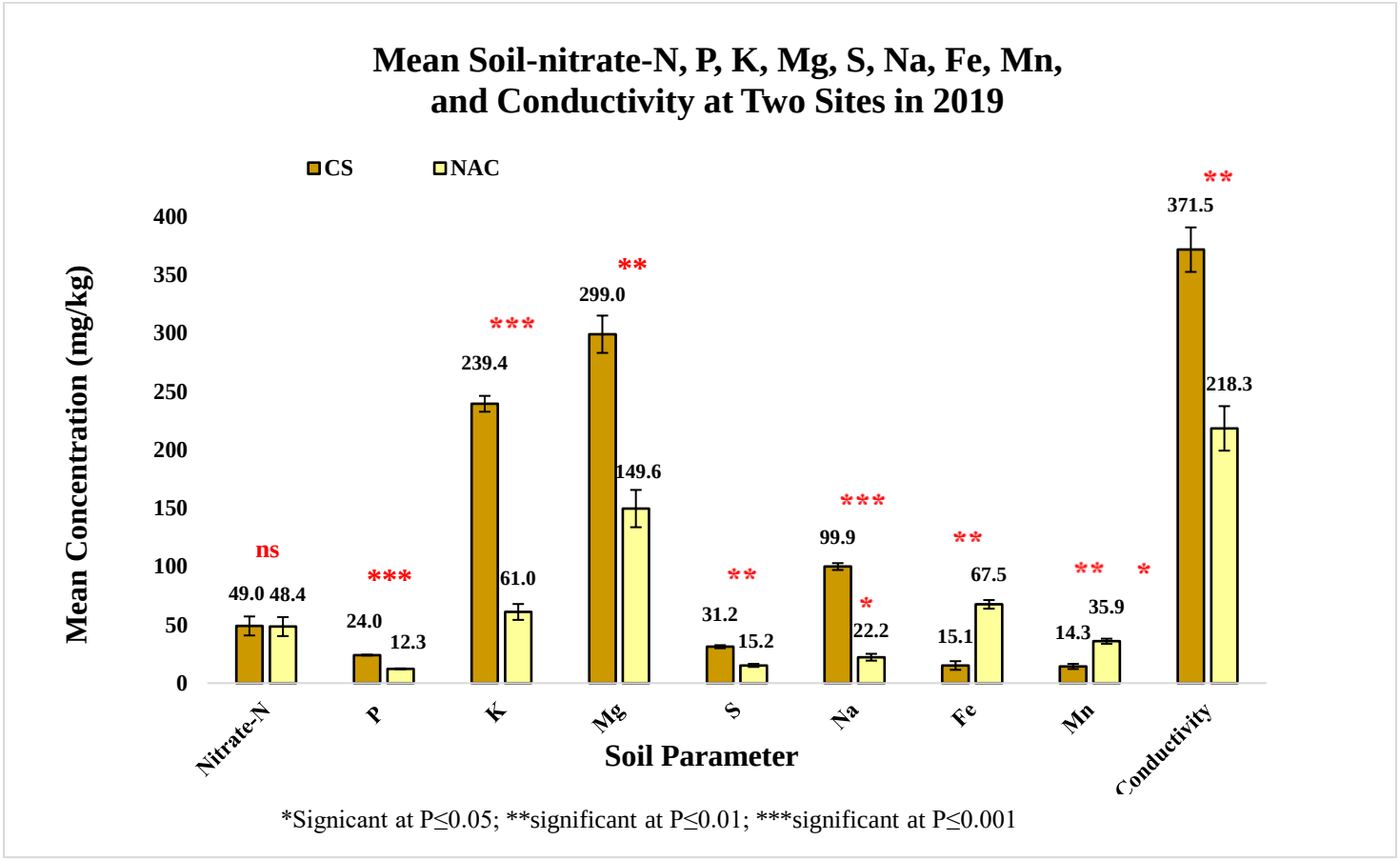


Figure 80 Comparison of mean soil-nitrate-N, soil-P, soil-K, soil-Mg, soil-S, soil-Na, soil-Fe, soil-Mn, and soil-conductivity at College Station, TX and Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Comparison of Topsoil Parameters Response by Year at Each Site

Comparison for each individual site also revealed significant response to year for several topsoil parameters. At CS, soil-conductivity (P = 0.03), soil-nitrate-N (P = 0.002), soil-S (P = 0.006), soil-Na (P = 0.002), and soil-B (P = 0.02) all showed higher average values in 2019 (371.5 µmol/cm, 49.0 mg/kg, 31.2 mg/kg, 99.9 mg/kg, and 1.12 mg/kg, respectively) than in 2018 (169.8 µmol/cm, 2.6 mg/kg, 15.4 mg/kg, 19.6 mg/kg, and 0.68 mg/kg, respectively). Conversely, average values for soil-Zn (P = 0.02) and soil-Cu (P = 0.02) were lower in the 2019 (1.30 mg/kg and 0.77 mg/kg, respectively) than those in 2018 (1.54 mg/kg and 1.01 mg/kg, respectively) at CS. The average soil-pH of 7.52 in 2019 was also significantly lower (P = 0.03) than that of 7.67 in 2018 at CS (Table 33). This was even more apparent, considering that the surrounding native (non-amended) topsoil pH averaged 8.1 (Table 33). With respect to specific 15-cm depth regions, average pH was significantly different in the middle and lower horizons (P = 0.01; P=0.04) between 2018 (7.75; 7.99) and 2019 (7.47; 7.65), but not in the upper horizon (7.59; 7.58) (data not shown).

Several topsoil parameters showed a significant response to year at NAC as well. Average values for soil-conductivity (P = 0.02), soil-nitrate (P = 0.03), soil-P (P = 0.02), soil-S (P = 0.002), soil-Na (P = 0.03), and soil-B (P = 0.002) were significantly higher in the second year (218.3 µmol/cm, 48.4 mg/kg, 12.3 mg/kg, 15.2 mg/kg, 22.2 mg/kg, and 0.16 mg/kg, respectively) than those in 2018 (71.0 µmol/cm, 2.3 mg/kg, 9.2 mg/kg, 8.4 mg/kg, 5.8 mg/kg, and 0.03 mg/kg, respectively) at NAC. Soil-K, soil-Mg, and soil-Fe exhibited an opposite trend, with these nutrients having significantly (P = 0.01; 0.02;

0.03, respectively) lower average values in 2019 (61.0 mg/kg, 149.6 mg/kg, and 67.5 mg/kg, respectively) than those in 2018 (73.5 mg/kg, 175.0 mg/kg, and 88.6 mg/kg) at NAC. Soil-pH was not significantly different between years at NAC (Table 34). However, the average pH value of 5.37 in the middle (15 to 30 cm-depth) horizon in 2019 was significantly lower (P = 0.03) than the value of 5.88 in 2018 (data not shown).

Table 33 Comparison of average topsoil parameter results by Year at College Station, TX for eight kiwifruit cultivars in the assessment of response to soil pH.

Parameter	2018			2019			Significance
	Mean	Standard Deviation	Standard Error	Mean	Standard Deviation	Standard Error	
Soil-pH	7.67	0.039	0.028	7.52	0.039	0.028	P = 0.0335
Soil-conductivity (µmol/cm)	169.8	15.06	10.65	371.5	15.06	7.53	P = 0.0009
Soil-nitrate-N (mg/kg)	2.6	4.52	3.20	49.0	4.52	3.20	P = 0.002
Soil-P (mg/kg)	32.3	2.93	2.07	24.0	2.93	2.07	ns
Soil-K (mg/kg)	261.1	8.87	6.27	239.4	8.87	6.27	ns
Soil-Ca (mg/kg)	5,806.5	262.46	185.59	5,407.6	262.46	185.59	ns
Soil-Mg (mg/kg)	314.5	11.05	7.81	299.1	11.05	7.81	ns
Soil-S (mg/kg)	15.4	1.39	0.98	31.2	1.39	0.98	P = 0.0059
Soil-Na (mg/kg)	19.6	7.89	5.58	99.9	7.89	5.58	P = 0.0022
Soil-Fe (mg/kg)	15.6	1.56	1.10	15.1	1.56	1.10	ns
Soil-Zn (mg/kg)	1.54	0.048	0.034	1.30	0.048	0.0334	P = 0.0218
Soil-Mn (mg/kg)	15.4	0.74	0.52	14.2	0.74	0.52	ns
Soil-Cu (mg/kg)	1.01	0.041	0.029	0.77	0.041	0.029	P = 0.0152
Soil-B (mg/kg)	0.68	0.129	0.091	1.12	0.129	0.091	P = 0.0195
Soil-Carbon OM (%)	5.84	0.405	0.286	6.31	0.405	0.286	ns
Soil OM (%)	5.44	0.349	0.247	5.44	0.349	0.247	ns

Student t-Test between year for each parameter (α = 0.05).
Analysis of soil-S, soil-Na, soil-Cu, and soil-B data based on natural log transformation.

Table 34 Comparison of average topsoil parameter results by Year at Nacogdoches, TX for eight kiwifruit cultivars in the assessment of response to soil pH.

Parameter	2018			2019			Significance
	Mean	Standard Deviation	Standard Error	Mean	Standard Deviation	Standard Error	
Soil-pH	5.94	0.186	0.132	5.36	0.186	0.132	ns
Soil-conductivity (µmol/cm)	71.0	30.55	21.60	218.3	30.55	21.60	P = 0.017
Soil-nitrate (mg/kg)	2.3	12.03	8.51	48.4	12.03	8.51	P = 0.0313
Soil-P (mg/kg)	9.2	0.67	0.48	12.3	0.67	0.48	P = 0.0181
Soil-K (mg/kg)	73.5	2.19	1.55	61.0	2.19	1.55	P = 0.0106
Soil-Ca (mg/kg)	1,035.6	249.85	176.67	1,023.8	249.85	176.67	ns
Soil-Mg (mg/kg)	175.0	4.76	3.37	149.6	4.76	3.37	P = 0.0175
Soil-S (mg/kg)	8.4	0.86	0.61	15.2	0.86	0.61	P = 0.0017
Soil-Na (mg/kg)	5.8	3.37	2.38	22.2	3.37	2.38	P = 0.0275
Soil-Fe (mg/kg)	88.6	5.57	3.94	67.5	5.57	3.94	P = 0.0323
Soil-Zn (mg/kg)	2.08	0.234	0.165	2.01	0.234	0.165	ns
Soil-Mn (mg/kg)	41.7	3.50	2.47	35.9	3.50	2.47	ns
Soil-Cu (mg/kg)	0.93	0.439	0.311	0.41	0.439	0.311	ns
Soil-B (mg/kg)	0.03	0.028	0.020	0.16	0.028	0.020	P = 0.0023
Soil OM (%)	2.05	0.171	0.121	2.54	0.171	0.121	ns

Student t-Test between year for each parameter (α = 0.05).
Analysis of soil-S, soil-Na, soil-Cu, and soil-B data based on natural log transformation.

Comparison of Plant Tissue Nutrition

Plant Tissue Nutrition by Site

Plant tissue nutrient concentrations were compared between sites and years in reference to recommended levels in Table 35. A list of significant responses and interactions associated with analysis of plant tissue nutritional data and comparison of plant tissue nutrients by site over years and for individual years can be found in Table 36 and Figures 81-86.

Table 35 Recommended nutrient concentrations for leaf tissue sampling in kiwifruit used in the assessment of response to soil pH.

Element	Deficiency Threshold	Low Threshold	High Threshold	Toxicity Threshold
Nitrogen	1.5 – 1.6 percent	2.2 percent	2.8 percent	5.0 - 5.5 percent
Phosphorus	0.11 - 0.12 percent	0.13 - 0.18 percent	0.22 - 0.30 percent	1.0 percent
Potassium	1.0 - 1.5 percent	1.5 - 1.8 percent	2.5 percent	-
Calcium	0.2 percent	2.0 - 3.0 percent	3.5 -3.6 percent	-
Sulfur	0.18 percent	0.25 percent	0.45 percent	-
Magnesium	0.10 percent	0.3 percent	0.4 – 0.8 percent	-
Manganese	30 µg/g	50 µg/g	100 - 200 µg/g	1,200 – 1,500 µg/g
Iron	60 µg/g	80 µg/g	100 – 200 µg/g	-
Zinc	12 µg/g	15 µg/g	25 - 30 µg/g	1,000 - 1,100 µg/g
Copper	3 µg/g	7 µg/g	15 µg/g	-
Boron	20 µg/g	25 - 40 µg/g	50 µg/g	100 µg/g
Molybdenum	0.01 µg/g	0.04 µg/g	0.2 µg/g	-
Sodium	-	100 µg/g	500 µg/g	1,200 µg/g
[1]Cloride	0.2 - 0.6 percent	0.3 – 1.0 percent	1.0 – 3.0 percent	1.1 - 0 percent

[1]Cloride requirements are higher in association with deficiency of potassium.
Low and high thresholds indicate range for adequate or normal concentrations.
All concentrations based on whole, youngest fully-expanded leaf samples collected from mid-season fruiting shoots.
Sources: Beutel et al., 1994; Clark et al., 1986; Smith et al. 1987.

Table 36 List of significant effects and interactions in the comparison of plant tissue nutrient concentrations for eight kiwifruit cultivars at two sites over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

Response Variable	Site	Year	Cultivar	Block	Site x Year	Site x Cultivar	Year x Cultivar	Site x Year x Cultivar
Tissue-N	P=0.0496	P<0.0001	P<0.0001	ns	P<0.0001	ns	ns	ns
Tissue-P	P=0.0039	P<0.0001	P<0.0001	P=0.0288	ns	P=0.0056	P=0.0009	P<0.0001
Tissue-K	P=0.0406	P<0.0001	P<0.0001	ns	P<0.0001	ns	ns	ns
Tissue-Ca	P=0.0001	P<0.0001	P<0.0001	ns	P=0.0392	ns	P=0.0003	ns
Tissue-Mg	P<0.0001	P<0.0001	P<0.0001	ns	P<0.0001	ns	P=0.0076	ns
Tissue-S	P<0.0001	P<0.0001	P<0.0001	ns	P=0.0305	ns	ns	ns
Tissue-Na	P=0.0005	ns	ns	P=0.0037	P=0.0025	ns	ns	ns
Tissue-Zn	P=0.0051	P<0.0001	P<0.0001	P=0.025	ns	P=0.0109	P=0.002	ns
Tissue-Fe	P<0.0001	ns	P<0.0001	P=0.0134	ns	ns	ns	ns
Tissue-Cu	P<0.0001	ns	P<0.0001	P<0.0001	P=0.0086	ns	ns	P=0.0141
Tissue-Mn	P<0.0001	P<0.0001	P<0.0001	P=0.0126	P<0.0001	ns	ns	ns
Tissue-B	P<0.0001	P<0.0001	P<0.0001	P=0.0051	P<0.0001	P=0.006	P=0.0228	ns

All P-values based on whole model (two sites, two years, and eight cultivars)
Analysis of tissue-N, tissue-P, tissue-S, tissue-Zn, tissue-Fe, and tissue B based on transformed data

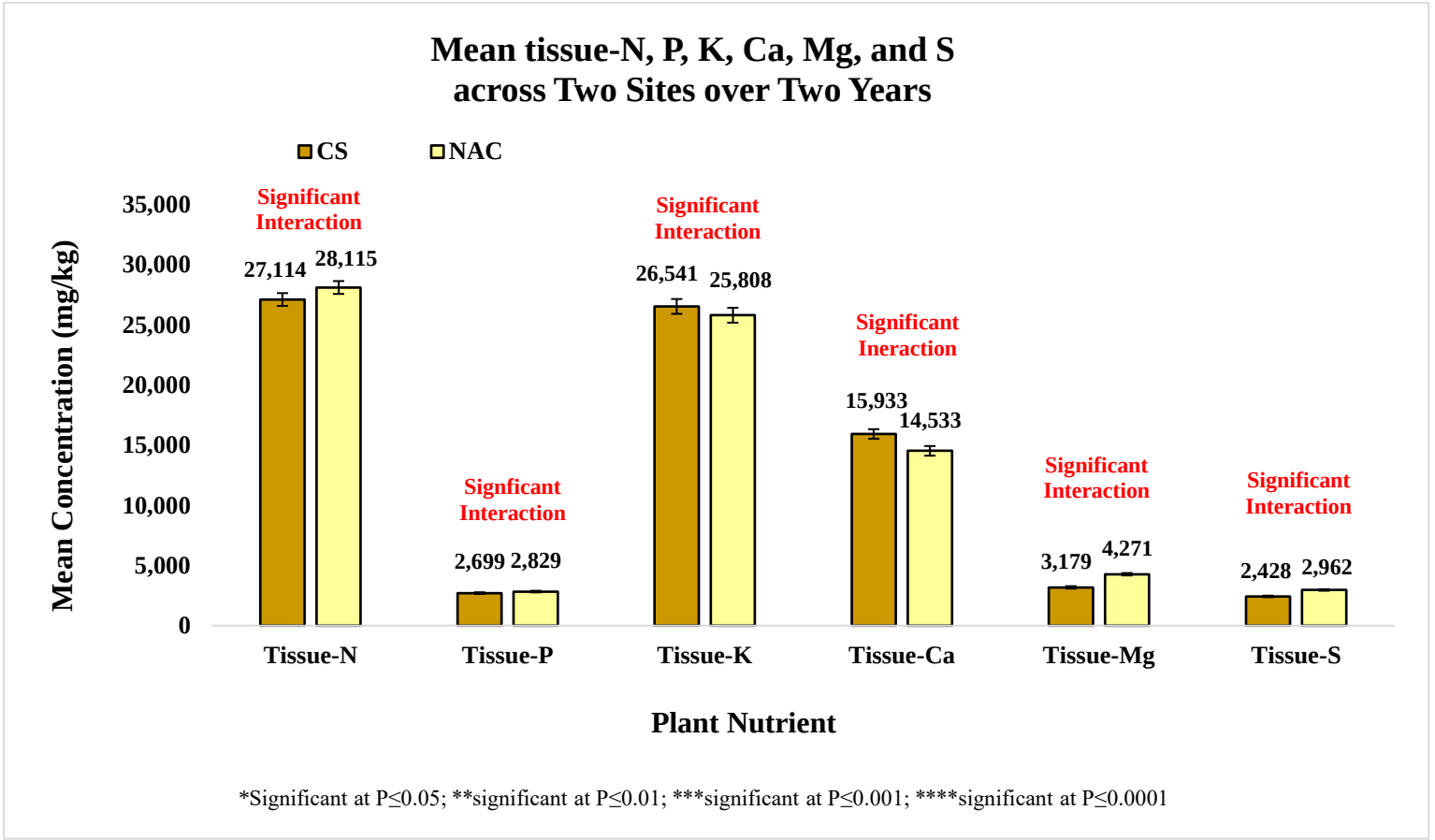


Figure 81 Comparison of mean tissue-N, tissue-P, tissue-K, tissue-Ca, tissue-Mg, and tissue, S by site over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

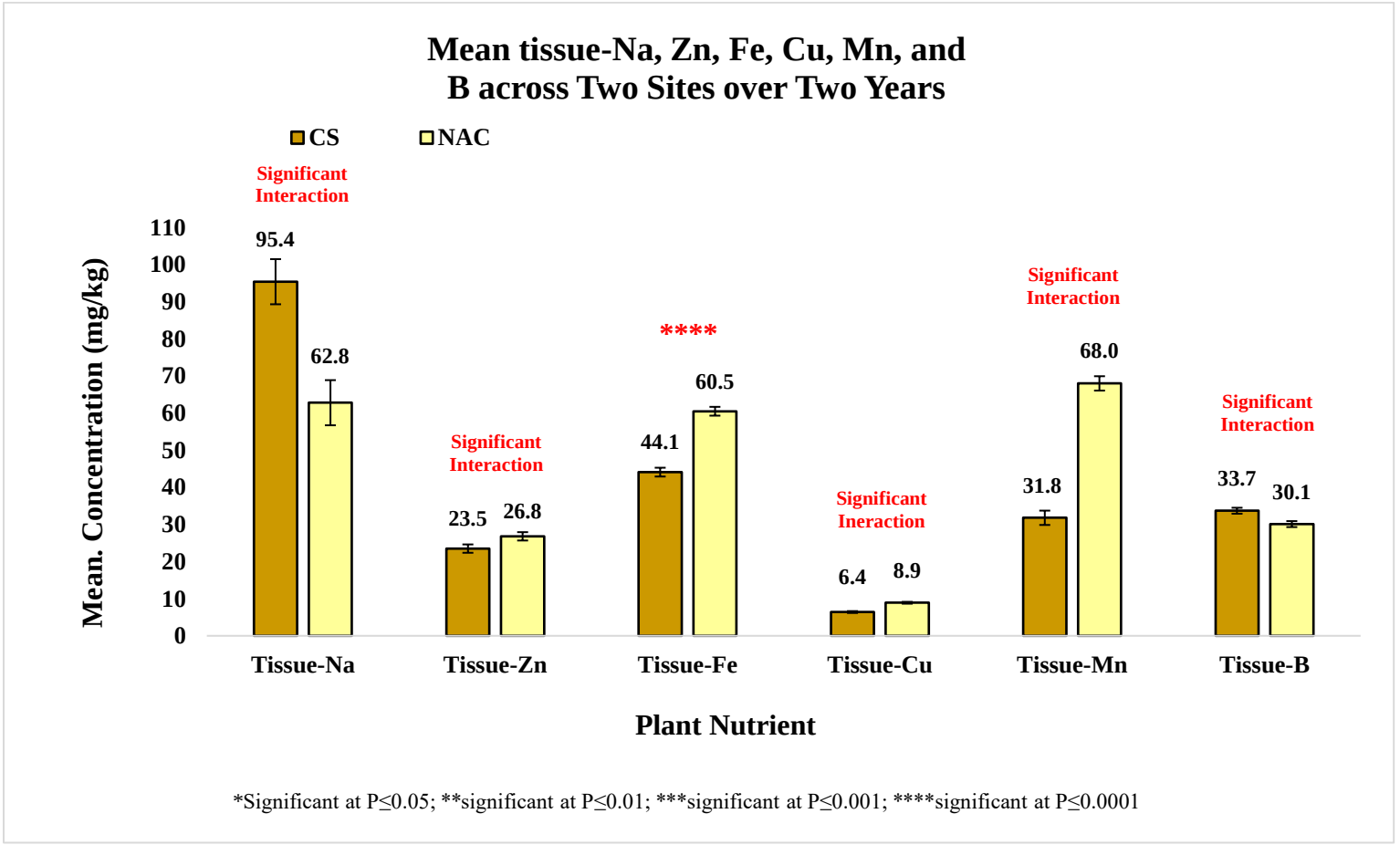
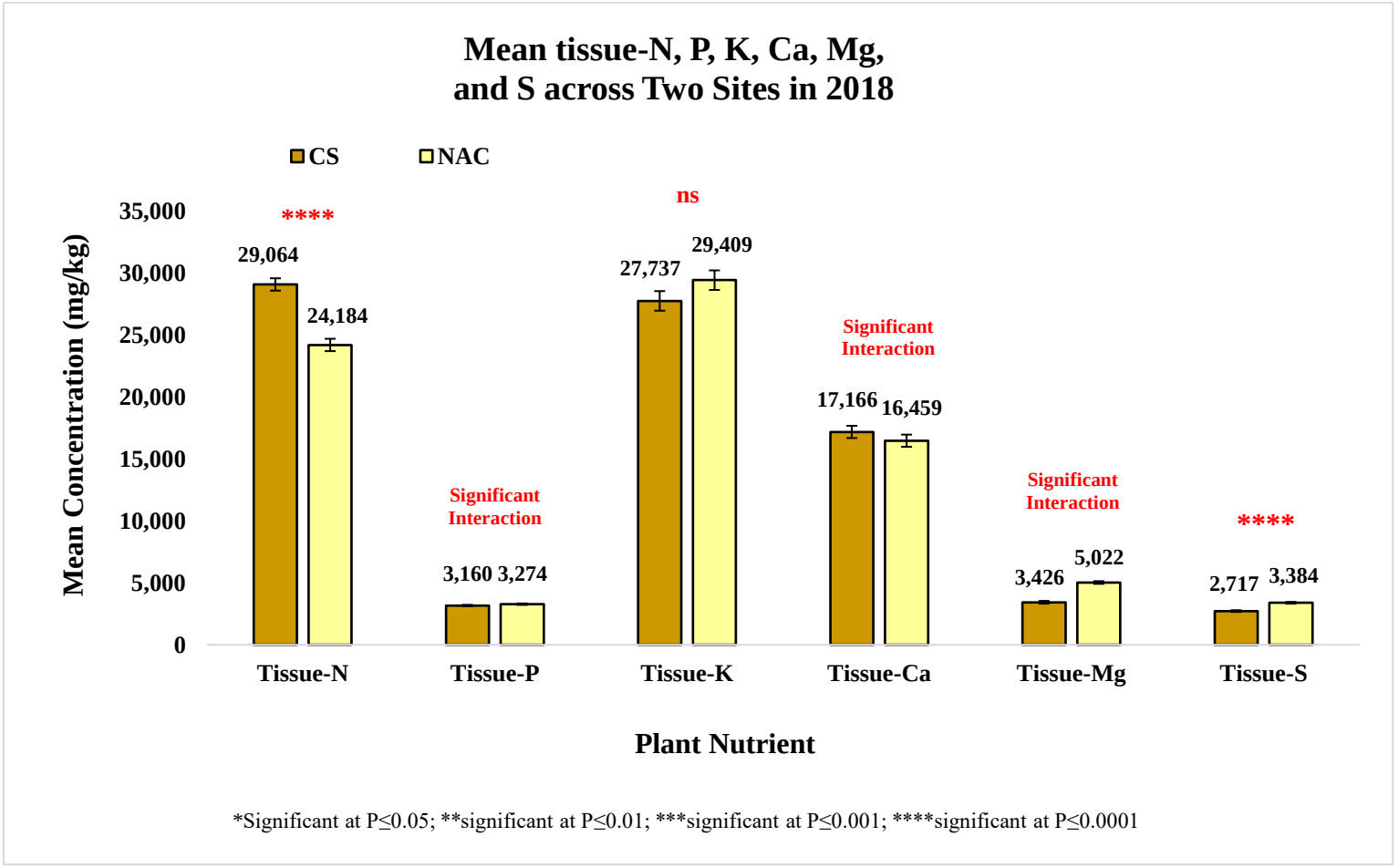


Figure 82 Comparison of mean tissue-Na, tissue-Zn, tissue-Fe, tissue-Cu, tissue-Mn, and tissue-B by site over two years for eight kiwifruit cultivars in the assessment of response to soil pH.



*Significant at P≤0.05; **significant at P≤0.01; ***significant at P≤0.001; ****significant at P≤0.0001

Figure 83 Comparison of mean tissue-N, tissue-P, tissue-K, tissue-Ca, tissue-Mg, and tissue, S by site in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

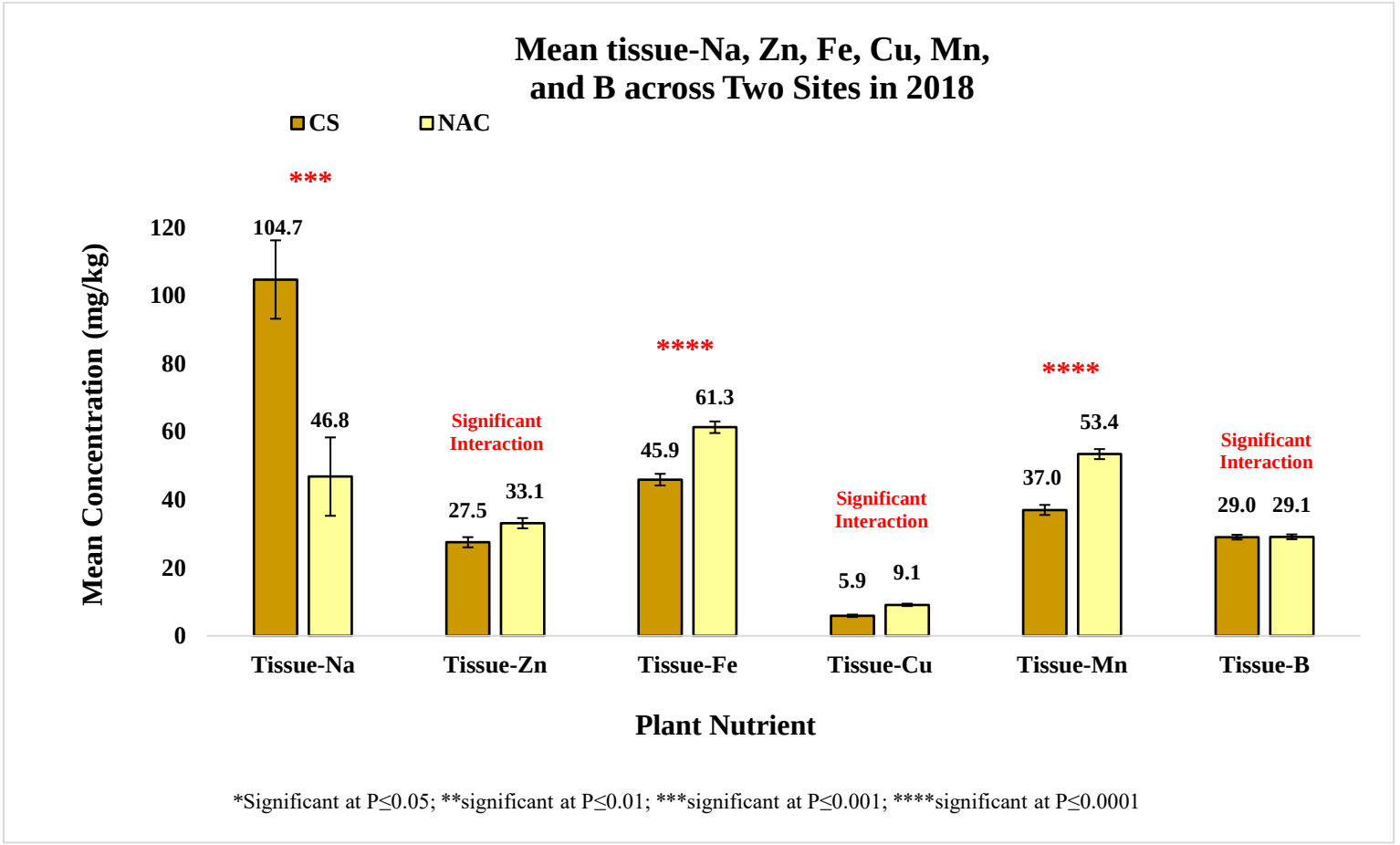


Figure 84 Comparison of mean tissue-Na, tissue-Zn, tissue-Fe, tissue-Cu, tissue-Mn, and tissue-B by site in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

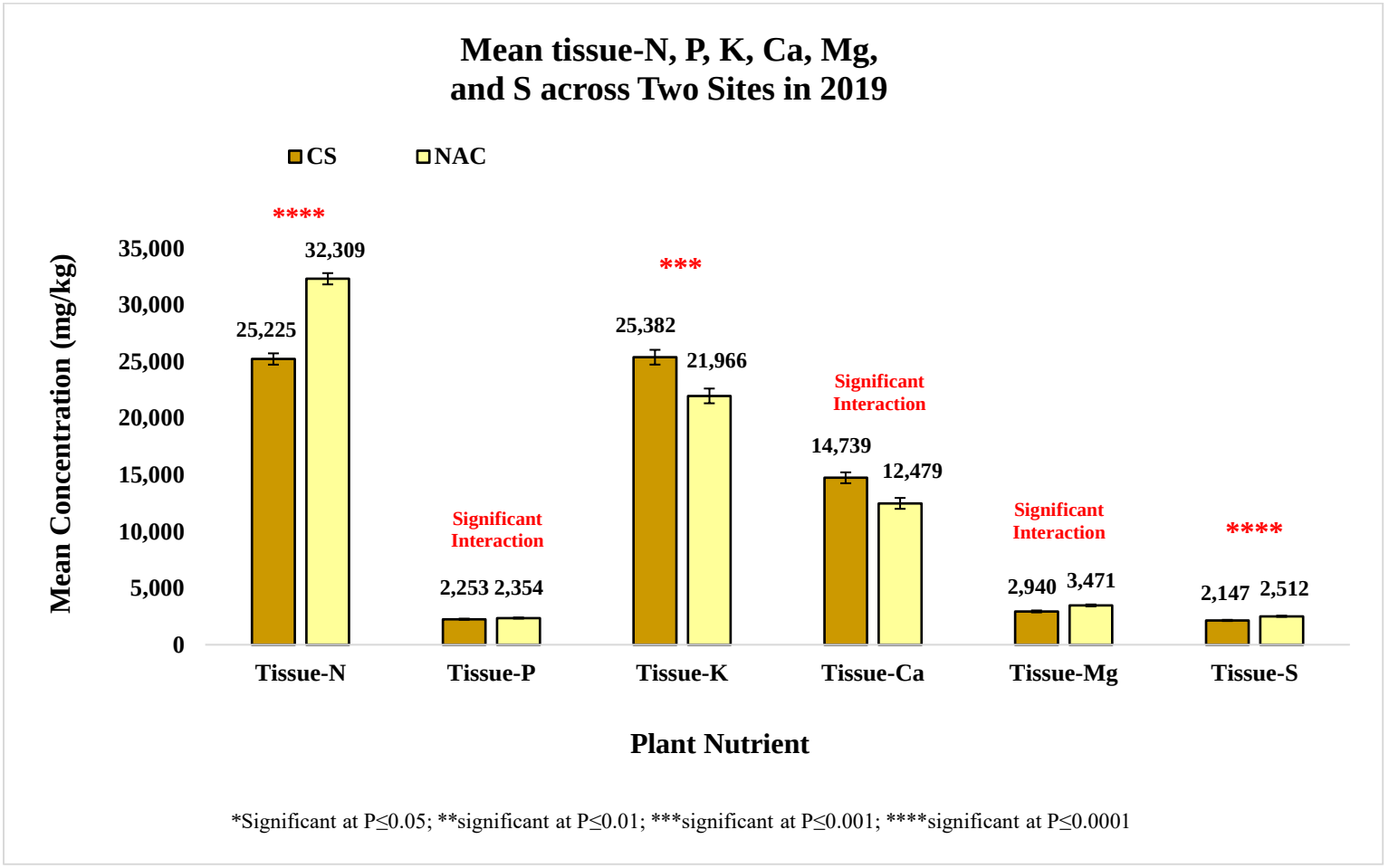


Figure 85 Comparison of mean tissue-N, tissue-P, tissue-K, tissue-Ca, tissue-Mg, and tissue, S by site in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

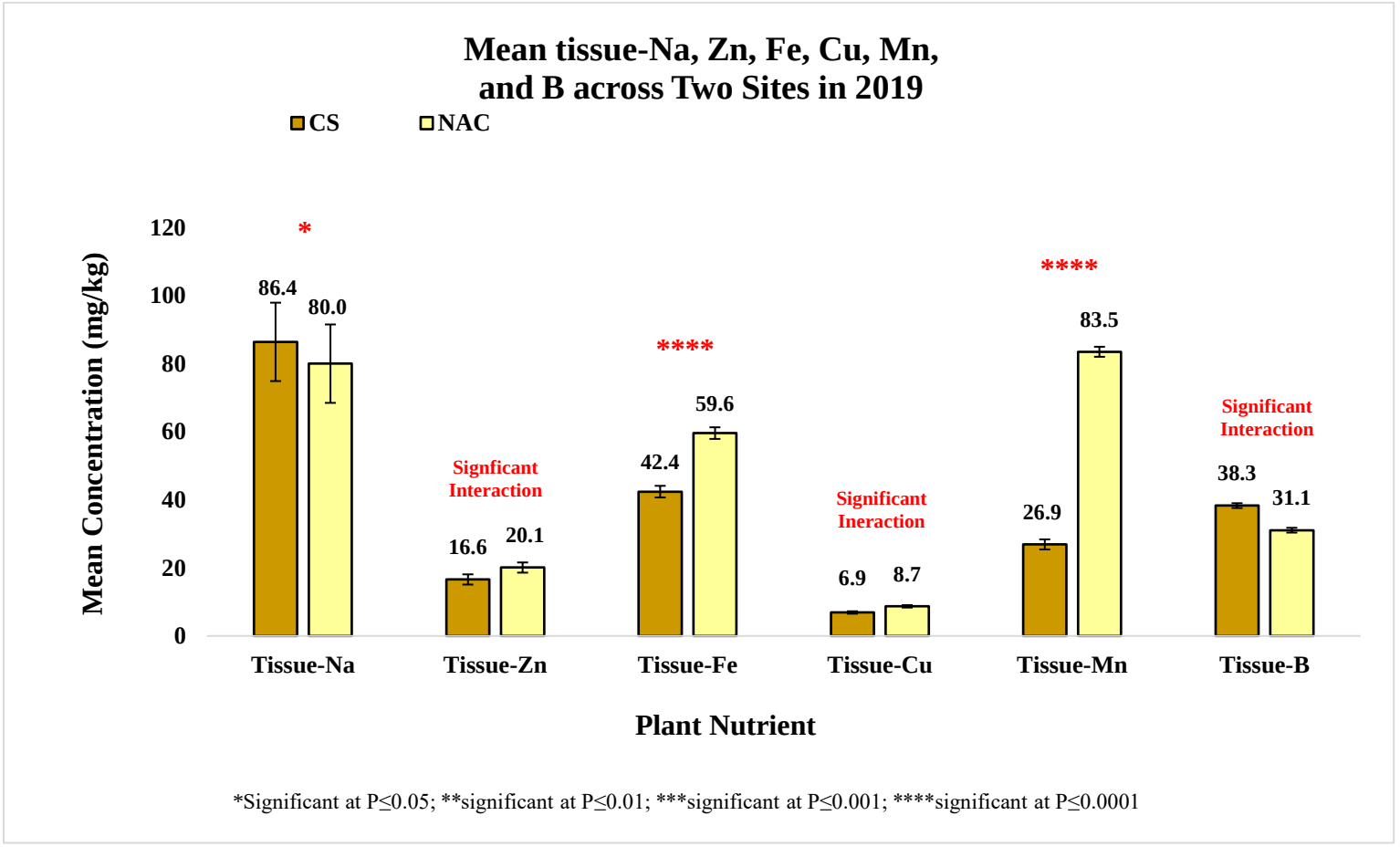


Figure 86 Comparison of mean tissue-Na, tissue-Zn, tissue-Fe, tissue-Cu, tissue-Mn, and tissue-B by site in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

There was a significant (P<0.0001) site x year interaction for tissue-N, requiring that site comparisons be made for individual years (Figure 81). Average tissue-N was significantly higher (P<0.0001) at CS (29,064 mg/kg) as compared to NAC (24,184 mg/kg) in 2018 (Figure 83). Conversely, higher (P<0.0001) average tissue-N was observed at NAC (32,309 mg/kg) than in CS (25,225 mg/kg) in 2019 (Figure 85). For tissue-P, site x cultivar, year x cultivar, and site x year x cultivar interactions were significant (P = 0.0056; 0.0009; <0.0001) as well as block effect (P = 0.0288). Site x year interaction (P<0.0001) was present for tissue-K (Figure 81). Average tissue-K was noticeably higher at NAC (29,409 mg/kg) in relation to CS (27,737 mg/kg) in 2018 (Figure 83), whereas average the average concentration was significantly (P = 0.0005) higher at CS (25,382 mg/kg) than at NAC (21,966 mg/kg) during the second year (Figure 85).

Site x year interactions were present for tissue-Ca, tissue-Mg, and tissue-S (P = 0.0392; <0.0001; 0.0305) along with year x cultivar interactions for tissue-Ca and tissue-Mg (P = 0.0003; 0.0076) (Figure 81). Average tissue-Ca was similar between CS and NAC (17,166 and 16,459 mg/kg) in 2018 (Figure 83), however, the average value at NAC (14,739 mg/kg) was considerably higher as compared to that of NAC (12,479 mg/kg) in 2019 (Figure 85). Average tissue-Mg was noticeably higher in 2018 and 2019 at NAC (5,022 and 3,471mg/kg) than at CS (3,426 and 2,940 mg/kg) (Figures 83 & 85). Average tissue-S was significantly higher (P<0.0001) at NAC in 2018 and 2019 (3,384 and 2,512 mg/kg) as compared CS (2,717 and 2,147 mg/kg) (Figures 83 & 85).

Analysis indicated a significant (P = 0.0025) site x year interaction for tissue-Na and significant site x cultivar and year x cultivar interactions for (P = 0.0109; 0.002) tissue-Zn (Figure 82). Block effect was also significant for tissue-Na, tissue-Zn, and tissue-Fe (P = 0.0037; 0.025; 0.0134). Average tissue-Na was higher in 2018 and 2019 (P = 0.0007; 0.0227) at CS (104.7 and 86.4 mg/kg) as compared to NAC (46.8 and 80.0 mg/kg). Average tissue-Zn was considerably higher at NAC in 2018 and 2019 (33.1 and 20.1 mg/kg) in comparison to CS (27.5 and 16.6 mg/kg) (Figures 84 & 86). Tissue-Fe was consistently higher (P<0.0001) across years at NAC (60.5 mg/kg) than at CS (44.1 mg/kg) (Figure 82).

Site x year interaction was present for tissue-Cu, tissue-Mn, and tissue-B (P<0.0001; 0.0126; 0.0051) along with block effect (P<0.0001; 0.0126; 0.0051), while a site x cultivar and year x cultivar interaction (P = 0.006; 0.0228) was present for tissue-B. Site x year x cultivar interaction was also significant (P = 0.0141) for tissue-Cu (Figure 82). Average tissue-Cu was noticeably higher at NAC in 2018 and, to a lesser degree, in 2019 (9.1 and 8.7 mg/kg) compared to at CS (5.9 and 6.9 mg/kg). A significantly (P<0.0001) higher average concentration for tissue-Mn was observed at NAC in 2018 and 2019 (53.4 mg/kg and 83.5 mg/kg) as compared to at CS (37.0 and 26.9 mg/kg). Finally, average tissue-B was concentration was nearly identical between CS and NAC (29.0 and 29.1 mg/kg) in 2018, whereas the average value was considerably higher at CS (38.3 mg/kg) than at NAC (31.1 mg/kg) during the second year (Figures 84 & 86).

Plant Tissue by Cultivar

Plant Tissue Nitrogen

All cultivar comparisons are made with mention of abbreviated cultivar names (Table 24).Tissue-N was consistent over year at each site for cultivar. Average tissue-N response to cultivar was not significant at CS in 2018, although concentrations (cultivar means) ranged from 26,675 mg/kg to 32,601 mg/kg (Figure 87). As discussed earlier, average tissue-N was significantly lower at NAC in 2019. However, cultivars did not vary significantly in spite of a range of 21,955 mg/kg and 25,486 mg/kg (Figure 88). During the second year at CS, average tissue-N ($P<0.0001$) ranged from 21,885 mg/kg to 28,789 mg/kg and consisted of six statistical groups, based on cultivar means (Tukey's HSD) in descending order: 1) BRUNO and SUN; 2) HAYWARD; 3) AUTH; 4) DRAGON and FITZ; 5) GOLD; 6) CK-3 (Figure 89). Average tissue-N did not vary significantly among cultivars at NAC in 2019, although a range of 29,630 mg/kg and 34,175 mg/kg was observed (Figure 90). Across years, average tissue-N ranged from 24,356 mg/kg to 30,423 mg/kg at CS and from 25,657 mg/kg to 39,392 mg/kg at NAC (Table 37).

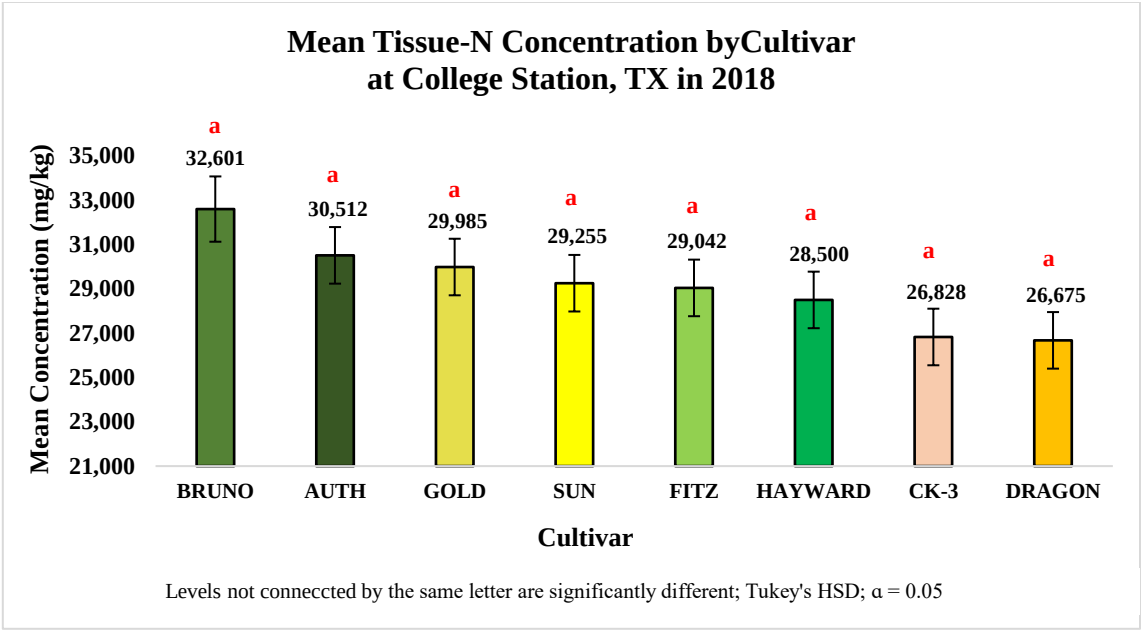


Figure 87 Comparison of mean plant tissue-N concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

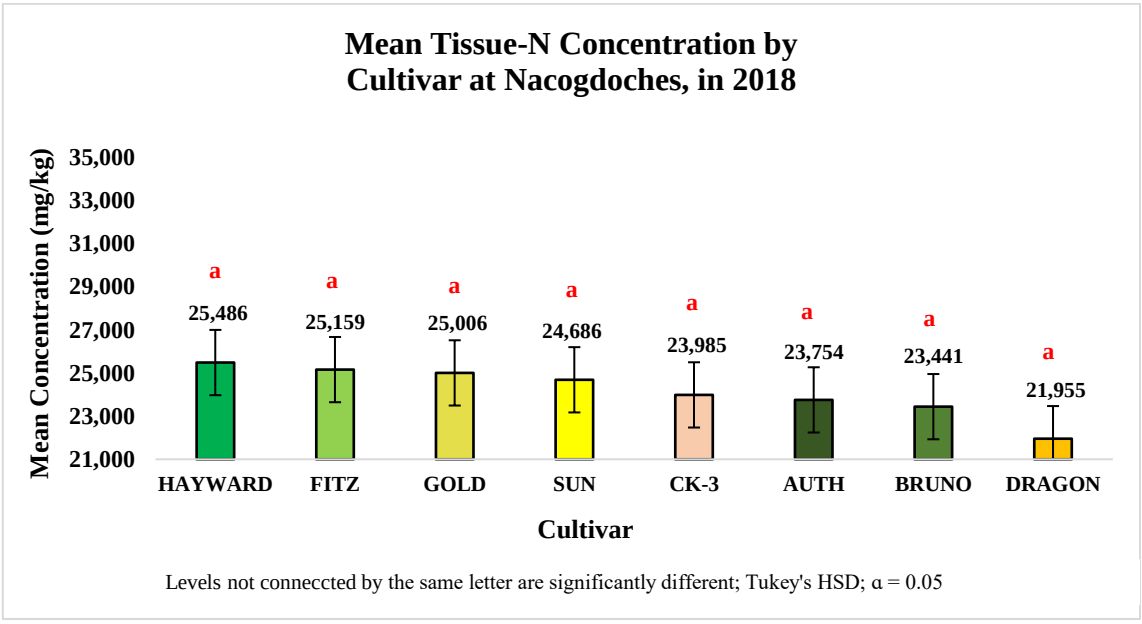


Figure 88 Comparison of mean plant tissue-N concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

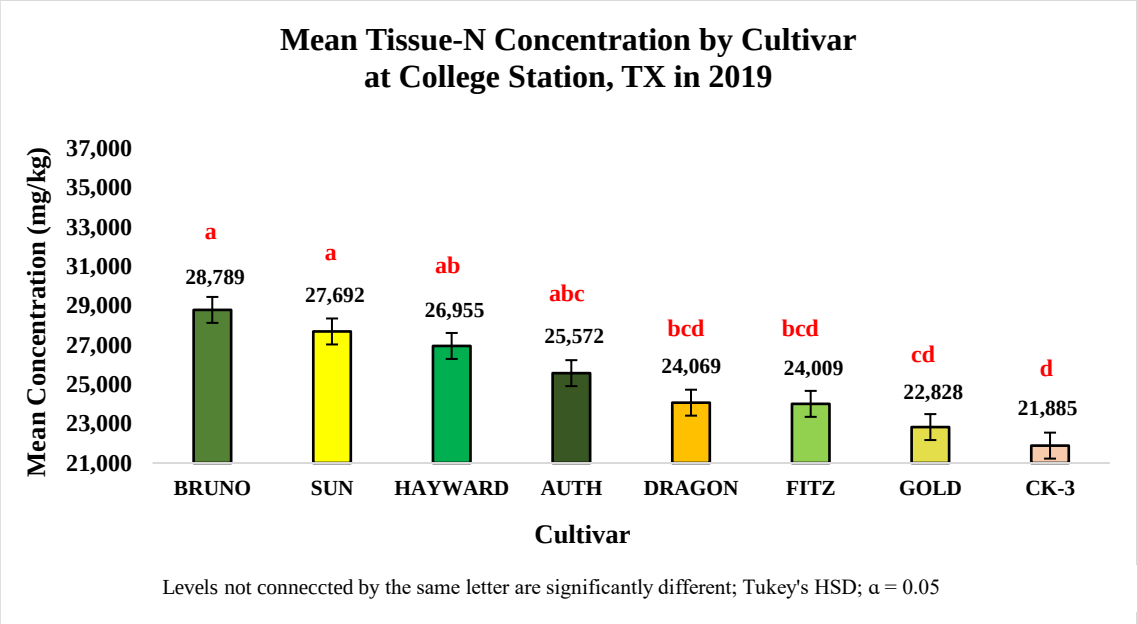


Figure 89 Comparison of mean plant tissue-N concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

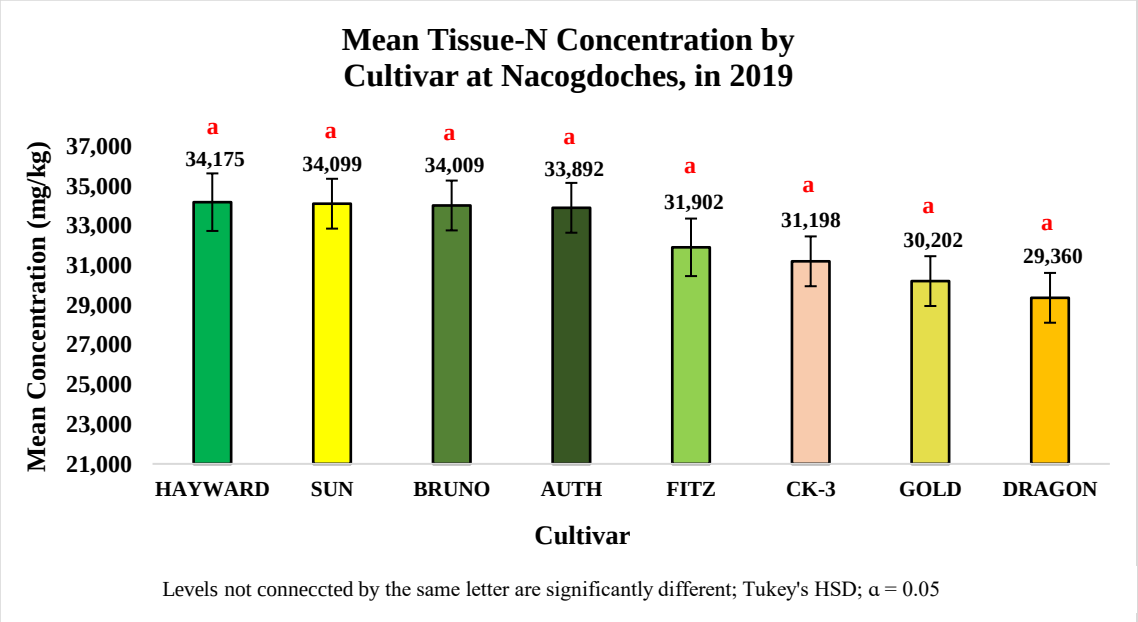


Figure 90 Comparison of mean plant tissue-N concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 37 Comparison of mean tissue-N concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
BRUNO	30,423	2,657.2	1,141.6	SUN	29,392	5,241.2	1,810.0
SUN	28,473	3,137.4	1,067.9	HAYWARD	29,210	5,143.7	1,934.9
AUTH	28,042	2,977.0	1,067.9	AUTH	28,823	6,224.7	1,810.0
HAYWARD	27,728	2,439.3	1,067.9	BRUNO	28,725	6,247.8	1,810.0
FITZ	26,526	2,788.7	1,067.9	FITZ	28,049	3,955.9	1,934.9
GOLD	26,407	4,324.6	1,067.9	GOLD	27,604	5,143.7	1,810.0
DRAGON	25,372	1,914.8	1,067.9	CK-3	27,592	5,776.6	1,810.0
CK-3	24,356	3,290.0	1,067.9	DRAGON	25,657	4,193.1	1,810.0

Tissue-N response to cultivar significant (P=0.0082) at College Station, TX only
Significant (P<0.0001) site x cultivar interaction present
Analysis based on natural log-transformed data

Plant Tissue Phosphorus

Average tissue-P (P = 0.0059) ranged from 2,785 mg/kg to 3,740 mg/kg and comprised three statistical groups in descending order: 1) GOLD; 2) AUTH, BRUNO, CK-3, FITZ, and HAYWARD; 3) SUN and DRAGON (Figure 91). Tissue-P response to cultivar was not significant at NAC in 2018, although cultivar averages ranged between 3,030 mg/kg and 3,589 mg/kg (Figure 92). Average concentrations at both sites were considerably lower during the second year. Average tissue-P (P<0.0001) exhibited a range of 1,803 mg/kg and 2,583 mg/kg with five statistical groups: 1) CK-3; 2) BRUNO, AUTH, HAYWARD, and SUN; 3) FITZ; 4) DRAGON; 5) GOLD (Figure 93). A range of 1,999 mg/kg to 2,584 mg/kg was observed at NAC in 2019 (P = 0.0001) where

cultivars consisted of three statistical groups: 1) GOLD, AUTH, HAYWARD, SUN, and BRUNO; 2) CK-3; 3) DRAGON and FITZ (Figure 94). Across years, average tissue-P by cultivar ranged from 2,492 mg/kg to 2,909 mg/kg at CS and from 2,776 mg/kg to 2,879 mg/kg at NAC (Table 38).

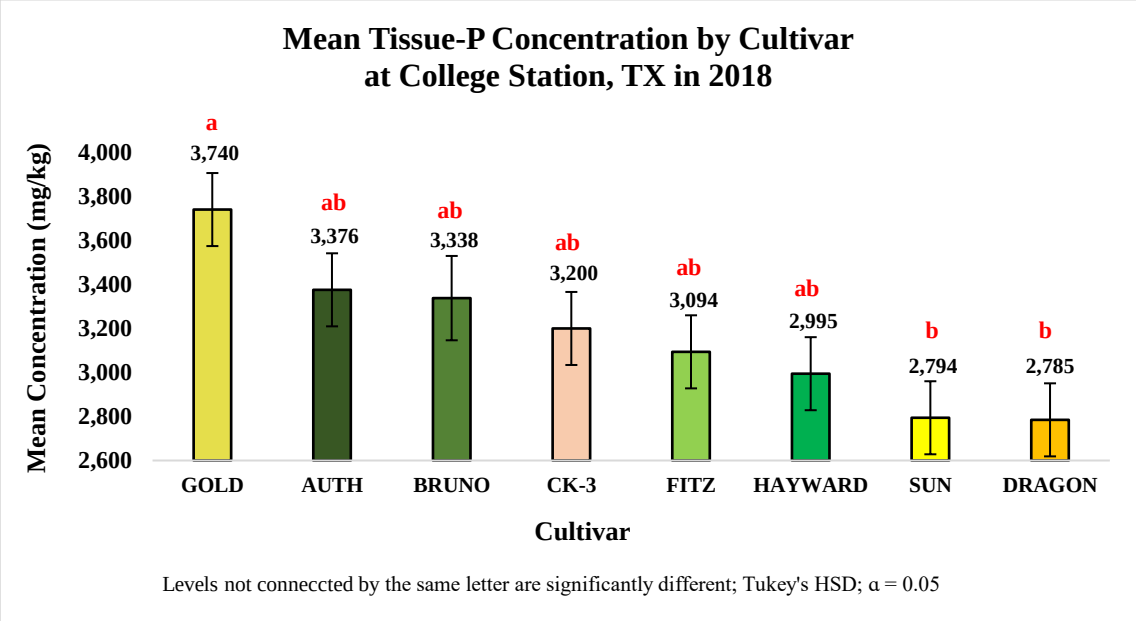


Figure 91 Comparison of mean plant tissue-P concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

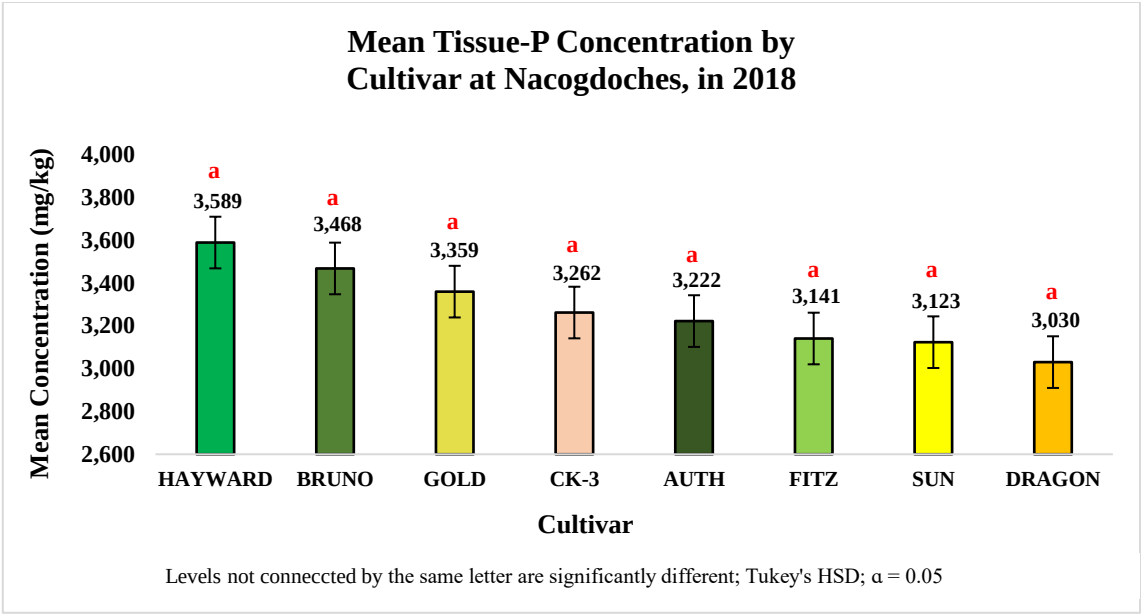


Figure 92 Comparison of mean plant tissue-P concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

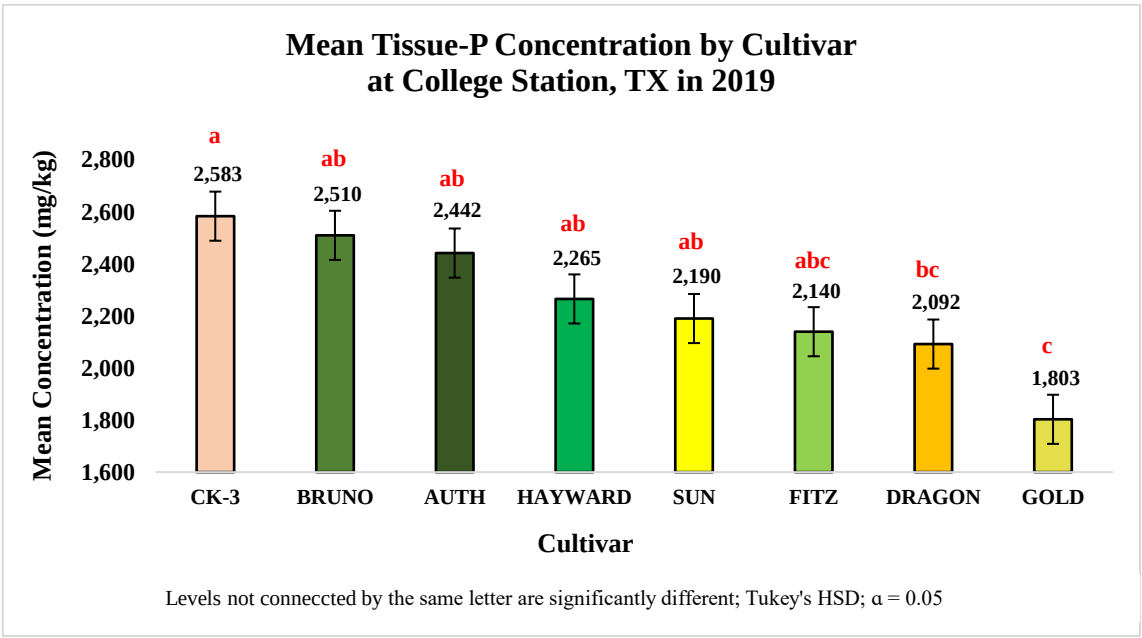


Figure 93 Comparison of mean plant tissue-P concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

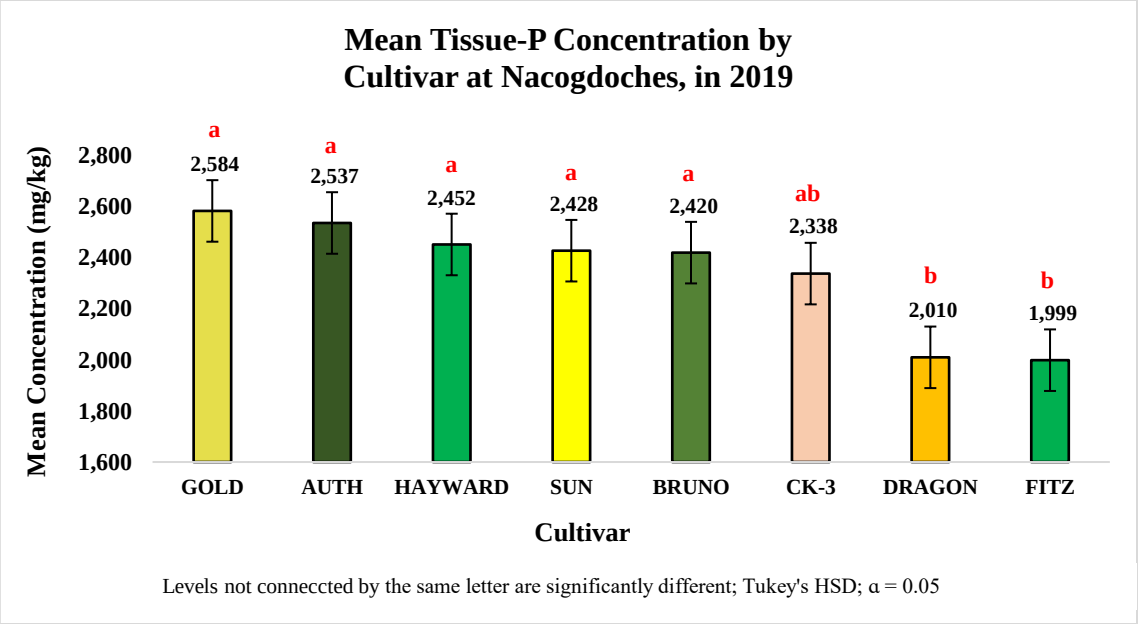


Figure 94 Comparison of mean plant tissue-P concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 38 Comparison of mean tissue-P concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
AUTH	2,909	516.2	208.2	HAYWARD	3,102	638.1	204.1
CK-3	2,892	440.4	208.2	GOLD	2,972	480.1	190.9
BRUNO	2,865	498.3	222.6	BRUNO	2,944	580.3	190.9
GOLD	2,772	1,112.7	208.2	AUTH	2,879	412.9	190.9
HAYWARD	2,630	482.0	208.2	CK-3	2,800	525.6	190.9
FITZ	2,617	553.1	208.2	SUN	2,776	430.7	190.9
SUN	2,492	350.1	208.2	FITZ	2,651	620.0	204.1
DRAGON	2,438	390.4	208.2	DRAGON	2,520	608.9	190.9

Tissue-P response to cultivar not significant at either site
Significant (P<0.0001) site x cultivar, year x cultivar (P=0.0009), and site x year x cultivar (P<0.0001) interaction present.
Analysis based on natural log-transformed data

Plant Tissue Potassium

Average tissue-K at CS in 2018 ($P<0.0001$) ranged drastically from 20,5674 mg/kg to 32,538 mg/kg and comprised four statistical groups in descending order: 1) HAYWARD and BRUNO; 2) FITZ and GOLD; 3) AUTH and SUN; 4) CK-3 and DRAGON (Figure 95). At NAC in 2018, average concentrations ranged among cultivars ranged even more drastically between 22,742 mg/kg and 34,598 mg/kg with six statistical groups: 1) HAYWARD; 2) BRUNO, FITZ, and AUTH; 3) GOLD; 4) SUN; 5) DRAGON; 6) CK-3 (Figure 96). During the second year, average tissue-K ($P<0.0001$; block: $P=0.0451$) included a range of 20,525 mg/kg and 29,383 mg/kg with six statistical groups: 1) FITZ and BRUNO; 2) HAYWARD; 3) SUN; 4) AUTH; 5) GOLD and CK-3; 6) DRAGON (Figure 97). Lastly, a range of 16,929 mg/kg to 26,401 mg/kg was observed at NAC in 2019 ($P = 0.0004$) with five statistical groups: 1) HAYWARD; 2) AUTH; 3) GOLD, FITZ, BRUNO, and SUN; 4) CK-3; 5) DRAGON (Figure 98). Average concentrations among cultivars ranged from 20,546 mg/kg to 30,609 mg/kg at CS and from 20,536 mg/kg to 31,085 mg/kg at NAC across years (Table 39).

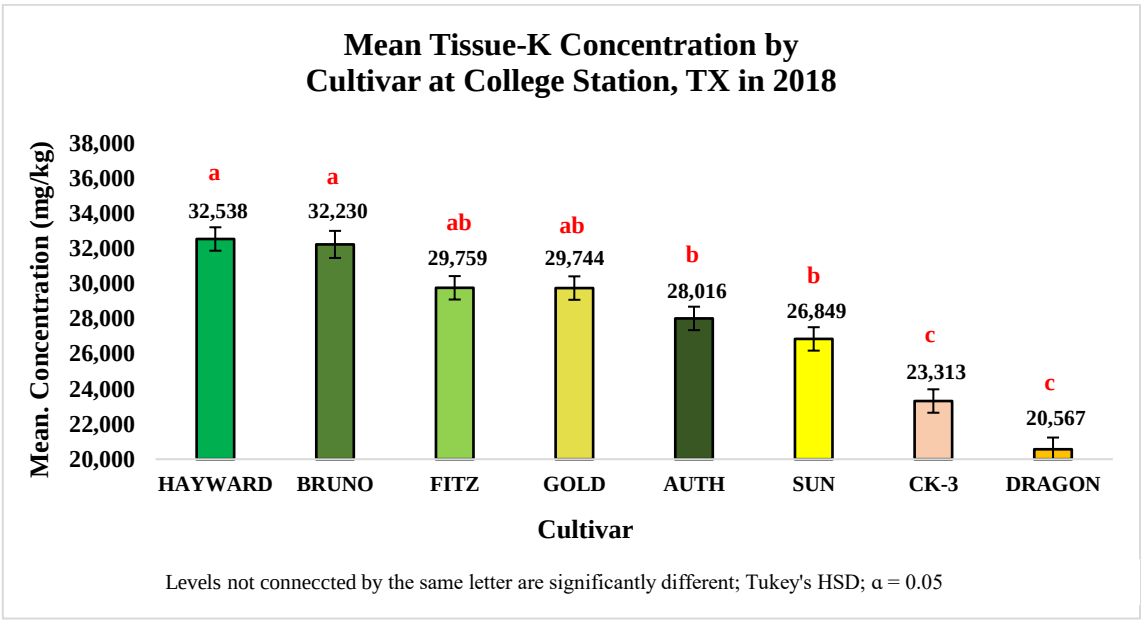


Figure 95 Comparison of mean plant tissue-K concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

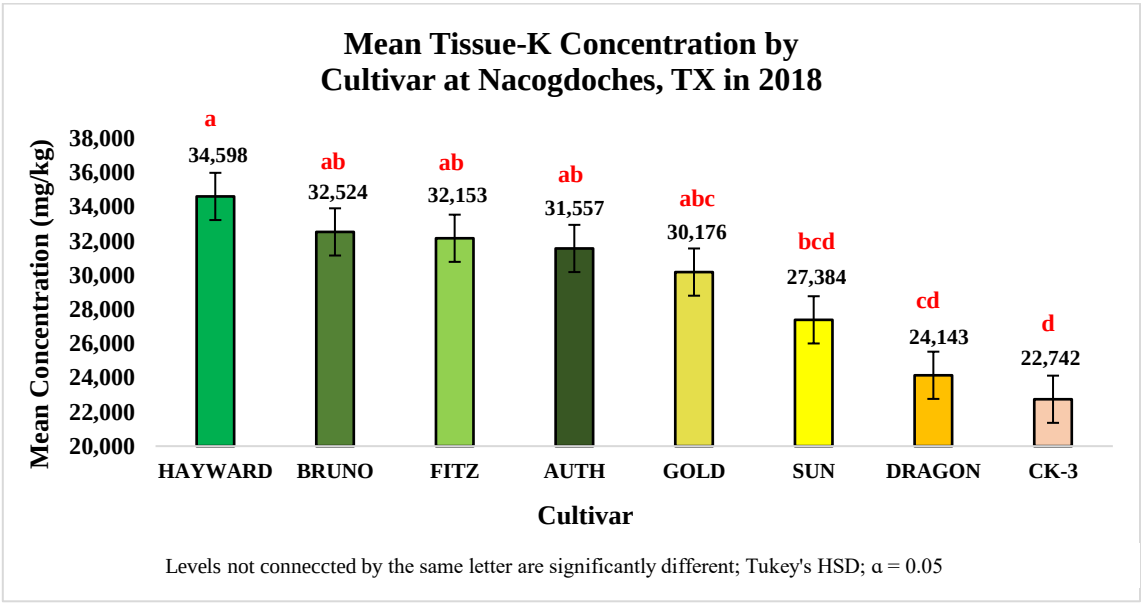


Figure 96 Comparison of mean plant tissue-K concentration by cultivar at Nacogdoches TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

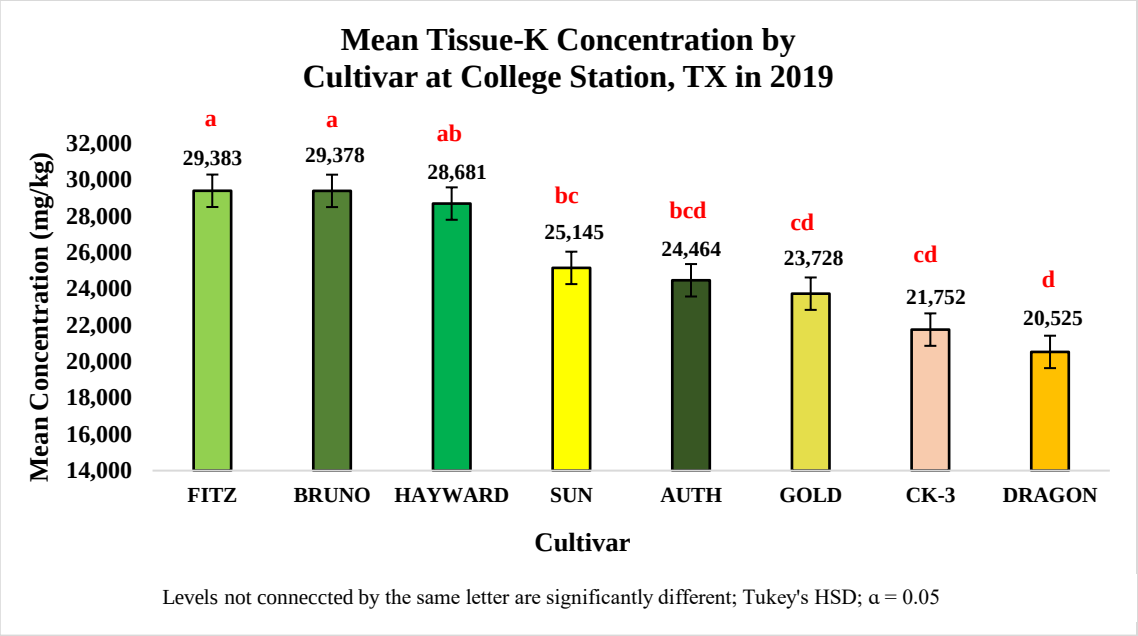


Figure 97 Comparison of mean plant tissue-K concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

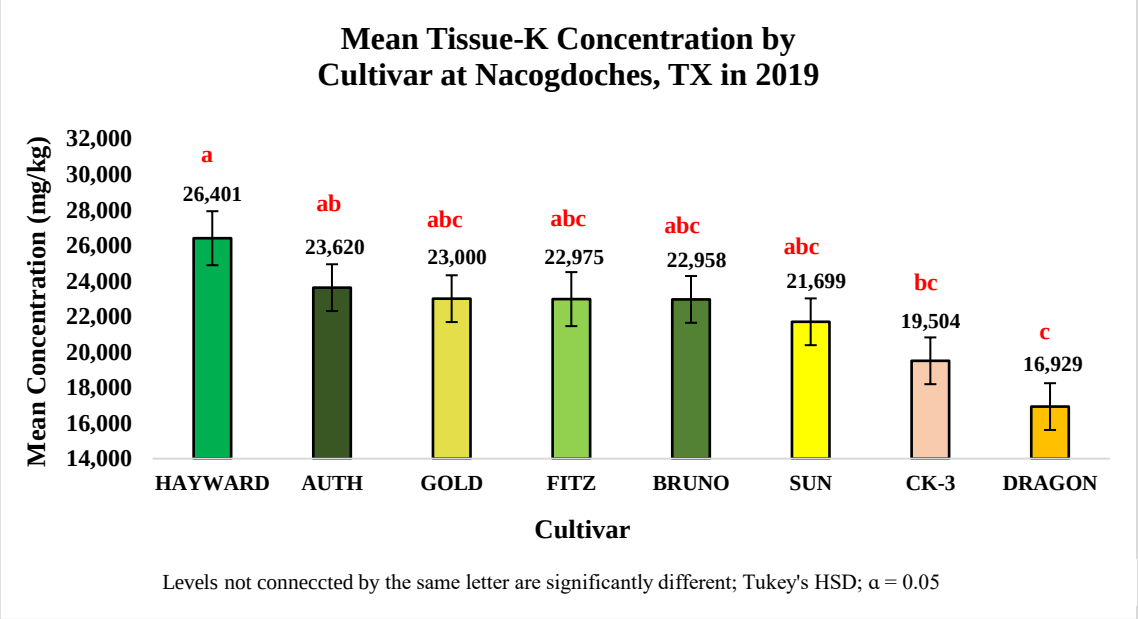


Figure 98 Comparison of mean plant tissue-K concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 39 Comparison of mean tissue-K concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
HAYWARD	30,609	2537.4	812.4	HAYWARD	31,085	4,840.2	1779.1
BRUNO	30,601	2134.2	868.5	FITZ	28,219	5,923.1	1779.1
FITZ	29,571	1592.7	812.4	BRUNO	27,741	5,444.1	1664.2
GOLD	26,736	3643.7	812.4	AUTH	27,588	4,915.6	1664.2
AUTH	26,240	2275.6	812.4	GOLD	26,588	4,485.6	1664.2
SUN	25,997	1294.8	812.4	SUN	24,541	4,065.1	1664.2
CK-3	22,532	2155.1	812.4	CK-3	21,123	2,582.7	1664.2
DRAGON	20,546	1957.9	812.4	DRAGON	20,536	4,863.2	1664.2

Tissue-K response to cultivar significant at College Station ($P<0.0001$) and Nacogdoches ($P=0.0005$)
Significant ($P<0.0001$) site x year interaction present.

Plant Tissue Calcium

Average tissue-Ca at CS in 2018 ($P = 0.0071$) ranged from 14,069 mg/kg to 19,716 mg/kg and comprised three statistical groups in descending order: 1) SUN and CK-3; 2) DRAGON, BRUNO, AUTH, HAYWARD and GOLD; 3) FITZ (Figure 99). At NAC during the same year ($P = 0.0006$) average concentrations ranged between 13,728 mg/kg and 19,757 mg/kg and consisted of seven statistical groups: 1) DRAGON; 2) SUN; 3) CK-3; 4) AUTH and FITZ; 5) BRUNO; 6) GOLD; 7) HAYWARD (Figure 100). At CS during the second year ($P = 0.0019$) average tissue-Ca exhibited a ranged between 11,776 mg/kg and 18,934 mg/kg, consisting of five statistical groups: 1) CK-3; 2) SUN; 3) GOLD and BRUNO; 4) AUTH, HAYWARD, and DRAGON; 5) FITZ (Figure 101). Average concentrations ranged from 9,850 mg/kg to 14,969 mg/kg at NAC

in 2019, however tissue-Ca response was not significant in that environment. Nonetheless, three statistical groups were identified by Tukey's HSD: 1) CK-3; 2) SUN, HAYWARD, AUTH, GOLD, BRUNO, and FITZ; 3) DRAGON (Figure 102). Over years, average tissue-Ca ranged from 12,922 mg/kg to 19,250 mg/kg at CS and between 13,219 mg/kg and 16,531 mg/kg at NAC (Table 40).

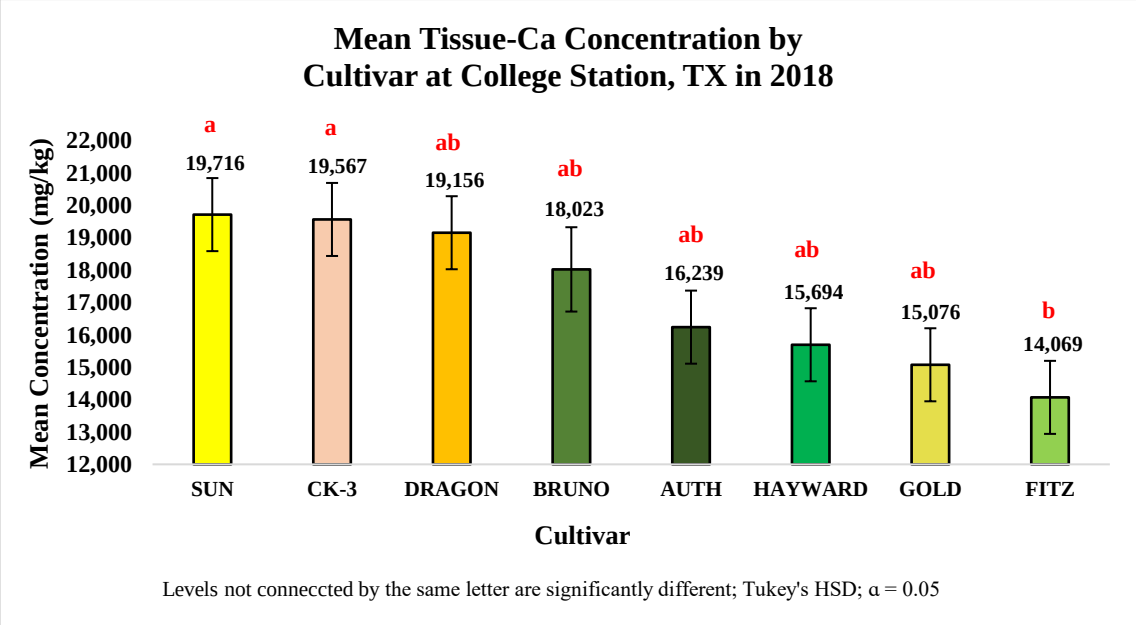


Figure 99 Comparison of mean plant tissue-Ca concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

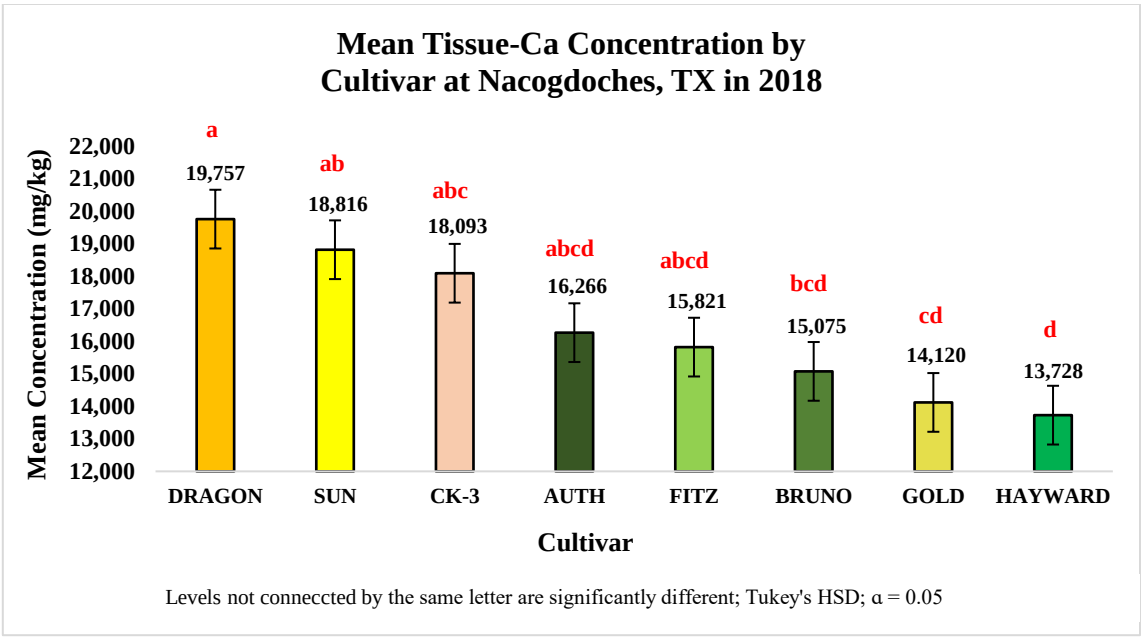


Figure 100 Comparison of mean plant tissue-Ca concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

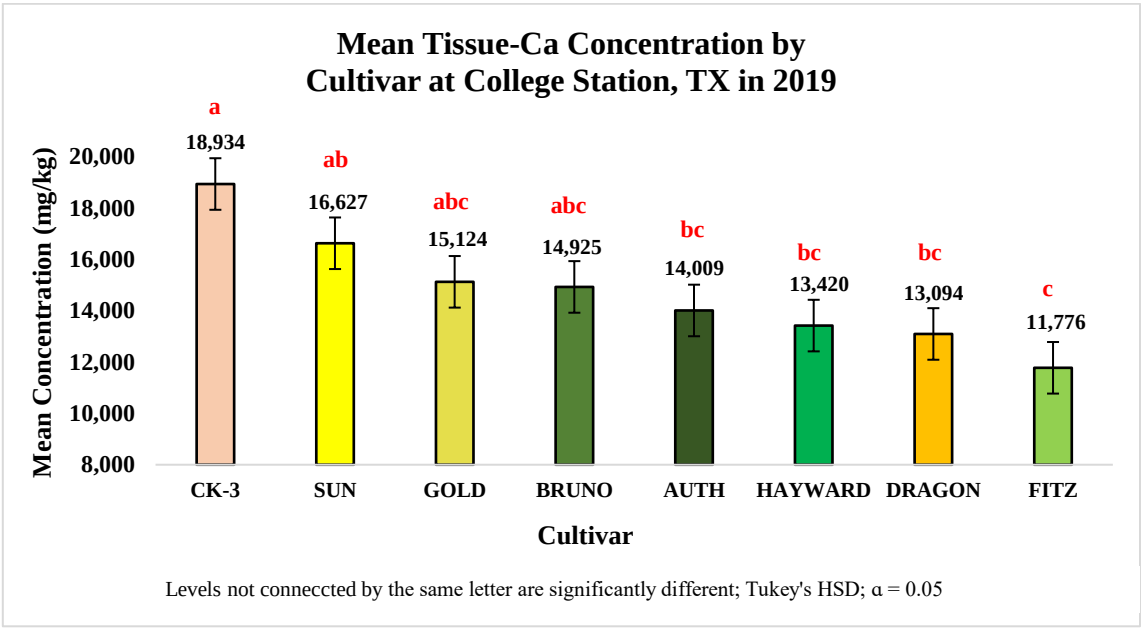


Figure 101 Comparison of mean plant tissue-Ca concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

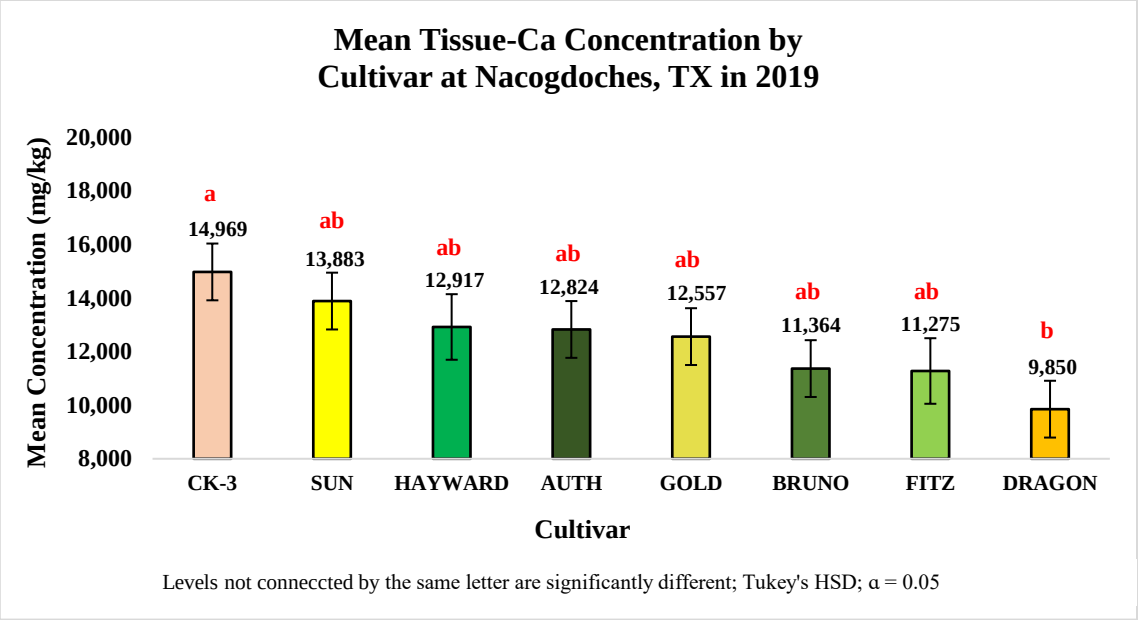


Figure 102 Comparison of mean plant tissue-Ca concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 40 Comparison of mean tissue-Ca concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
CK-3	19,250	3,062.7	926.5	CK-3	16,531	2,609.9	1,107.7
SUN	18,171	2,578.4	926.5	SUN	16,349	3,103.9	1,107.7
BRUNO	16,253	2,293.5	990.5	DRAGON	14,804	5,586.7	1,107.7
DRAGON	16,125	3,408.6	926.5	AUTH	14,545	3,340.4	1,107.7
AUTH	15,124	2,209.7	926.5	FITZ	13,872	2,604.2	1,184.1
GOLD	15,100	1,186.7	926.5	HAYWARD	13,381	1,795.8	1,184.1
HAYWARD	14,557	1,866.2	926.5	GOLD	13,338	1,338.8	1,107.7
FITZ	12,922	3,468.5	926.5	BRUNO	13,219	2,556.2	1,107.7

Tissue-Ca response to cultivar significant at College Station (P=0.0005) only
Significant (P=0.0392) site x year and year x cultivar (P=0.0003) interaction present.

Plant Tissue Magnesium

Average tissue-Mg at CS in 2018 ($P<0.0001$) ranged from 3,136 mg/kg to 4,153 mg/kg and comprised four statistical groups in descending order: 1) SUN; 2) BRUNO; 3) DRAGON, FITZ, GOLD, and HAYWARD; 4) AUTH and CK-3 (Figure 103). At NAC in 2018 ($P = 0.0056$) average concentrations were much higher and ranged from 4,375 mg/kg to 5,901 among cultivars, with three statistical groups: 1) BRUNO; 2) SUN, HAYWARD, GOLD, DRAGON, AUTH, and FITZ; 3) CK-3 (Figure 104). During the second year at CS ($P<0.0001$; block: $P=0.0021$), average concentrations ranged between 2,399 mg/kg and 3,676 mg/kg, consisting of five statistical groups: 1) BRUNO; 2) SUN; 3) HAYWARD and CK-3; 4) AUTH, GOLD, and DRAGON; 5) FITZ (Figure 105). Average tissue-Mg at NAC in 2019 ($P = 0.0296$) ranged from 2,907 mg/kg to 4,045 mg/kg with three statistical groups: 1) HAYWARD; 2) CK-3, BRUNO, SUN, AUTH, GOLD, and FITZ; 3) DRAGON (Figure 106). Over years, concentrations ranged between 2,845 mg/kg and 3,781 mg/kg at CS and between 3,847 mg/kg and 4,769 mg/kg at NAC (Table 41).

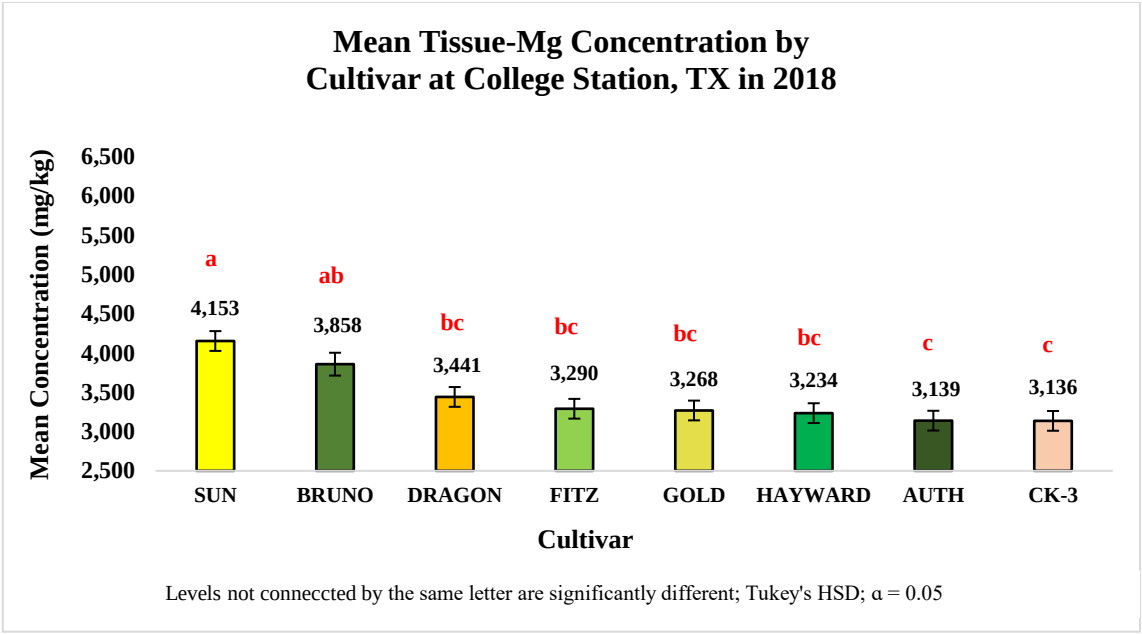


Figure 103 Comparison of mean plant tissue-Mg concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

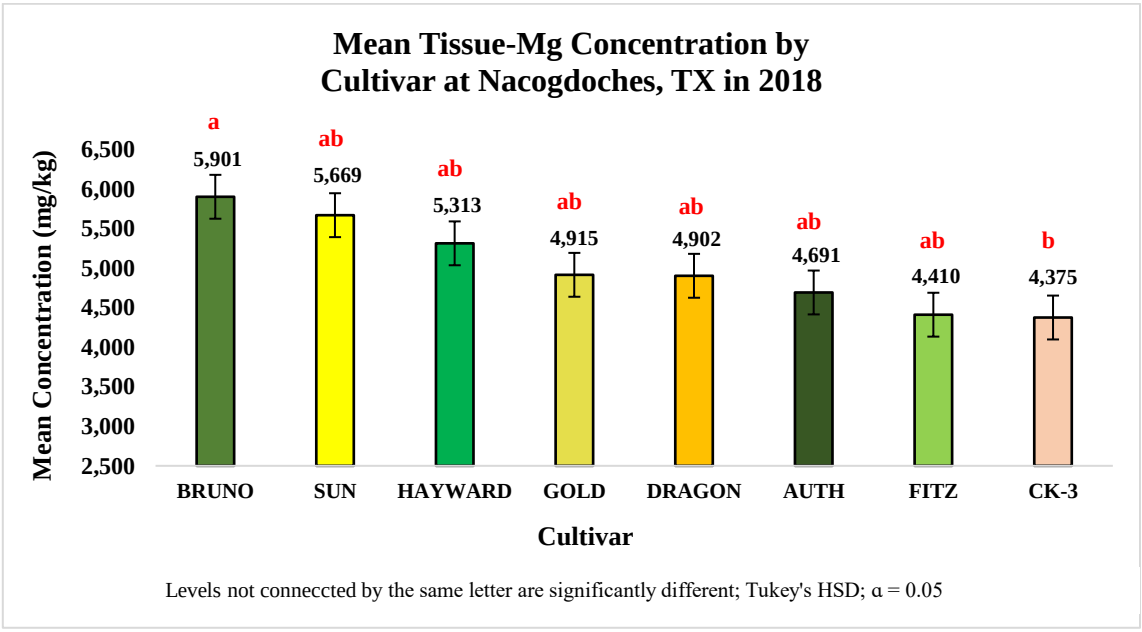


Figure 104 Comparison of mean plant tissue-Mg concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

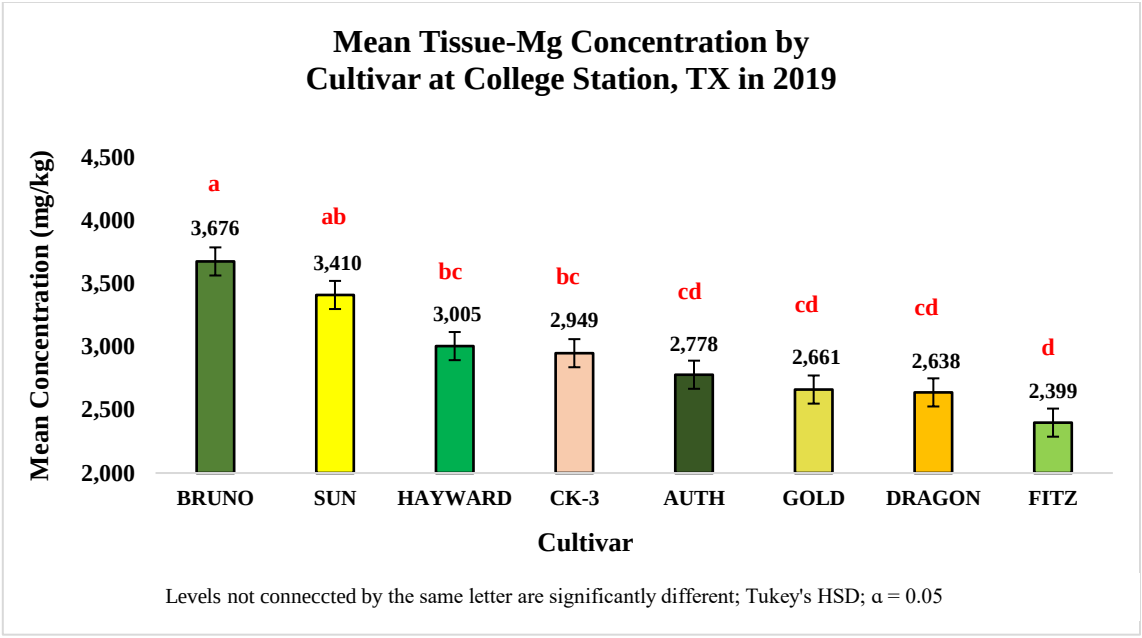


Figure 105 Comparison of mean plant tissue-Mg concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

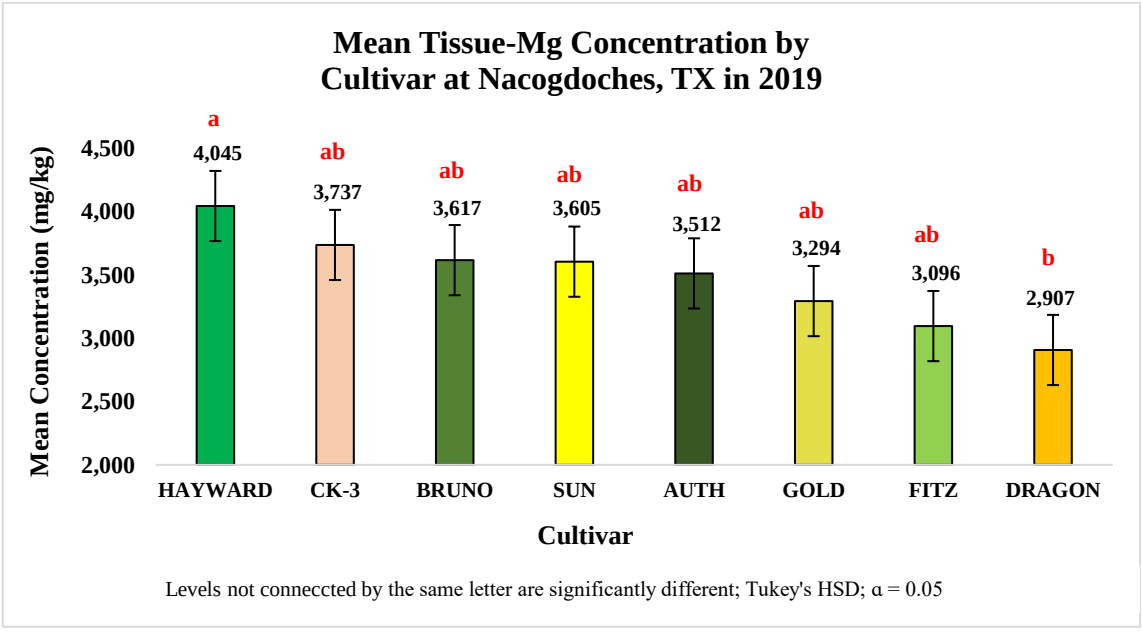


Figure 106 Comparison of mean plant tissue-Mg concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 41 Comparison of mean tissue-Mg concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
SUN	3,781	462.2	140.9	HAYWARD	4,769	822.5	377.1
BRUNO	3,754	395.5	150.63	BRUNO	4,759	1,416.0	352.7
HAYWARD	3,120	363.3	140.9	SUN	4,637	1,128.5	352.7
CK-3	3,042	291.9	140.9	GOLD	4,104	879.1	352.7
DRAGON	3,040	468.2	140.9	AUTH	4,102	862.1	352.7
GOLD	2,965	368.6	140.9	CK-3	4,056	536.8	352.7
AUTH	2,959	276.2	140.9	DRAGON	3,905	1,141.0	352.7
FITZ	2,845	501.6	140.9	FITZ	3,847	912.8	377.1
Tissue-Mg response to cultivar significant at College Station (P<0.0001) only Significant (P<0.0001) site x year and year x cultivar (P=0.0076) interaction present.							

Plant Tissue Sulfur

Average tissue-S at CS in 2018 (P<0.0001) ranged from 2,302 mg/kg to 3,216 mg/kg and comprised four statistical groups in descending order: 1) GOLD; 2) SUN and BRUNO; 3) HAYWARD; AUTH; FITZ, and DRAGON; 4) CK-3 (Figure 107). Concentrations at NAC in 2019 (P = 0.0083) exhibited a range of 2,722 mg/kg and 3,821 mg/kg, consisting of three statistical groups: 1) AUTH; 2) BRUNO, HAYWARD, SUN, FITZ, GOLD, and DRAGON; 3) CK-3 (Figure 108). Average tissue-S at CS during the second year (P<0.0001) ranged between 1,759 mg/kg and 2,502 mg/kg with five statistical groups: 1) SUN and GOLD; 2) AUTH and BRUNO; 3) HAYWARD; 4) DRAGON and FITZ; 5) CK-3 (Figure 109). Lastly, average concentrations at NAC in 2019 (P<0.0001) included a range of 2,119 mg/kg and 2,843 mg/kg with a total of seven

statistical groups: 1) SUN and GOLD; 2) AUTH; 3) BRUNO; 4) HAYWARD; 5) FITZ; 6) DRAGON; 7) CK-3 (Figure 110). Average tissue-S ranged from 2,031 mg/kg to 2,812 mg/kg at CS and from 2,420 mg/kg to 3,258 mg/kg at NAC over the two years (Table 42).

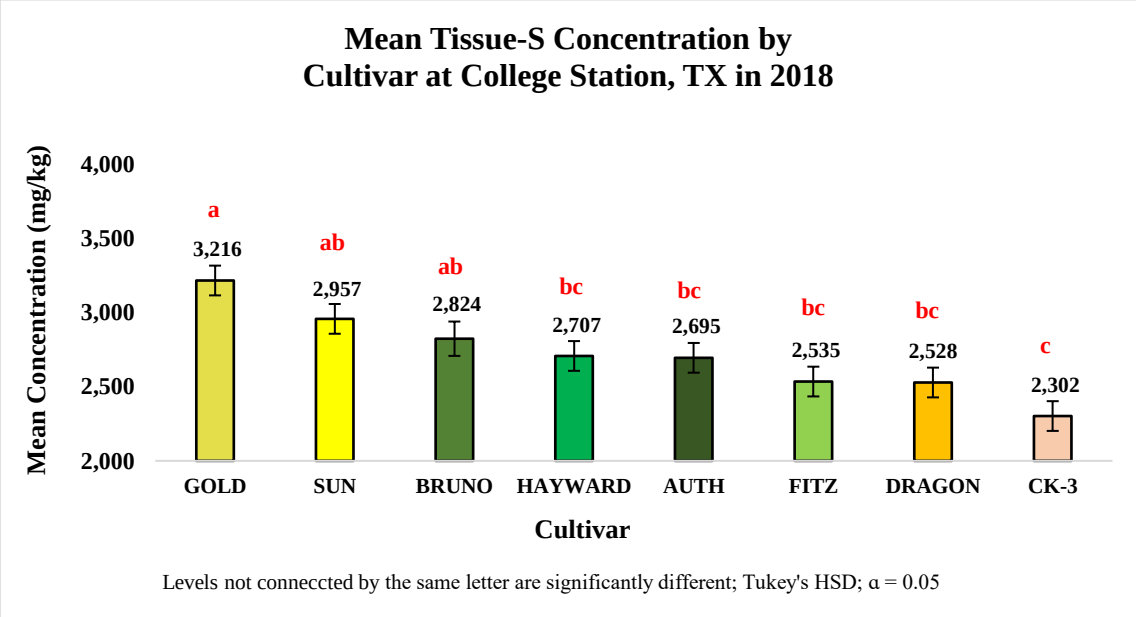


Figure 107 Comparison of mean plant tissue-S concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

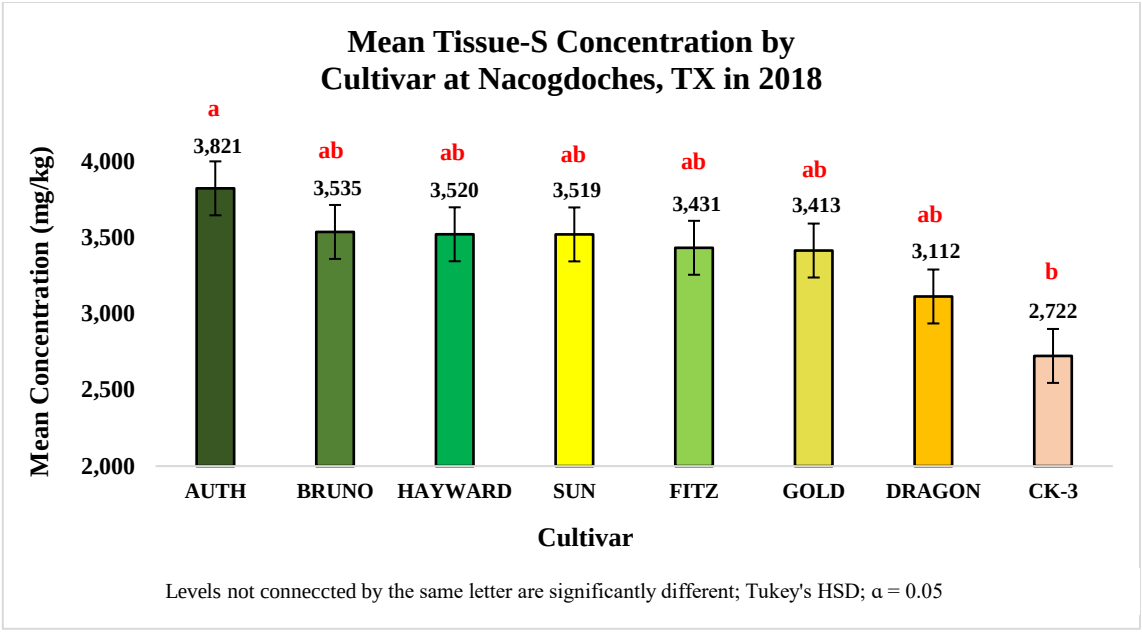


Figure 108 Comparison of mean plant tissue-S concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

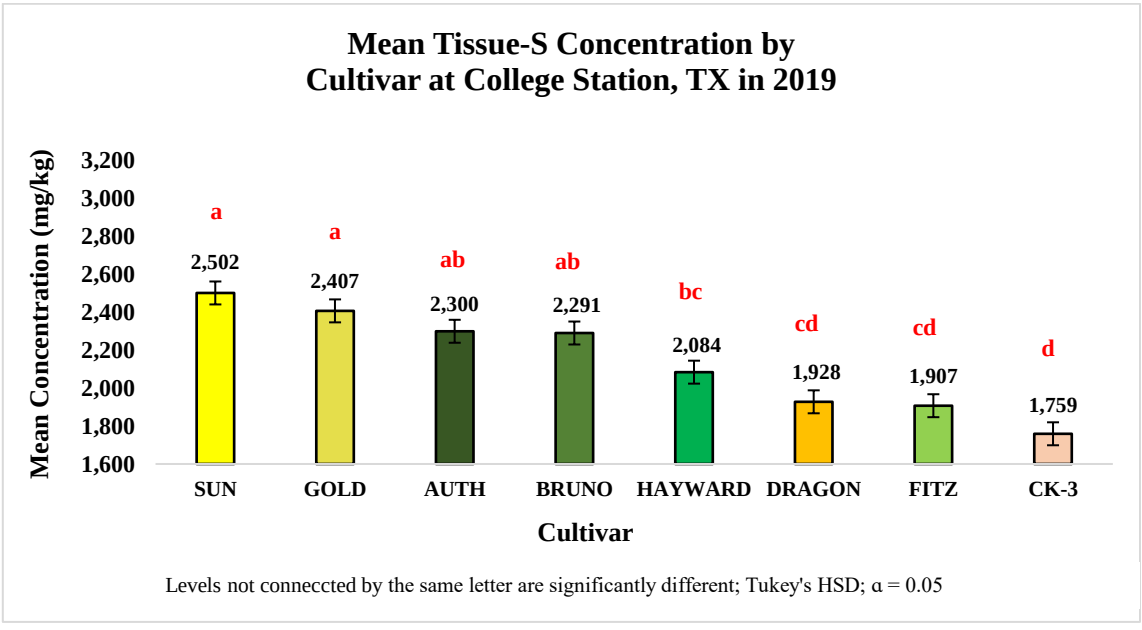


Figure 109 Comparison of mean plant tissue-S concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

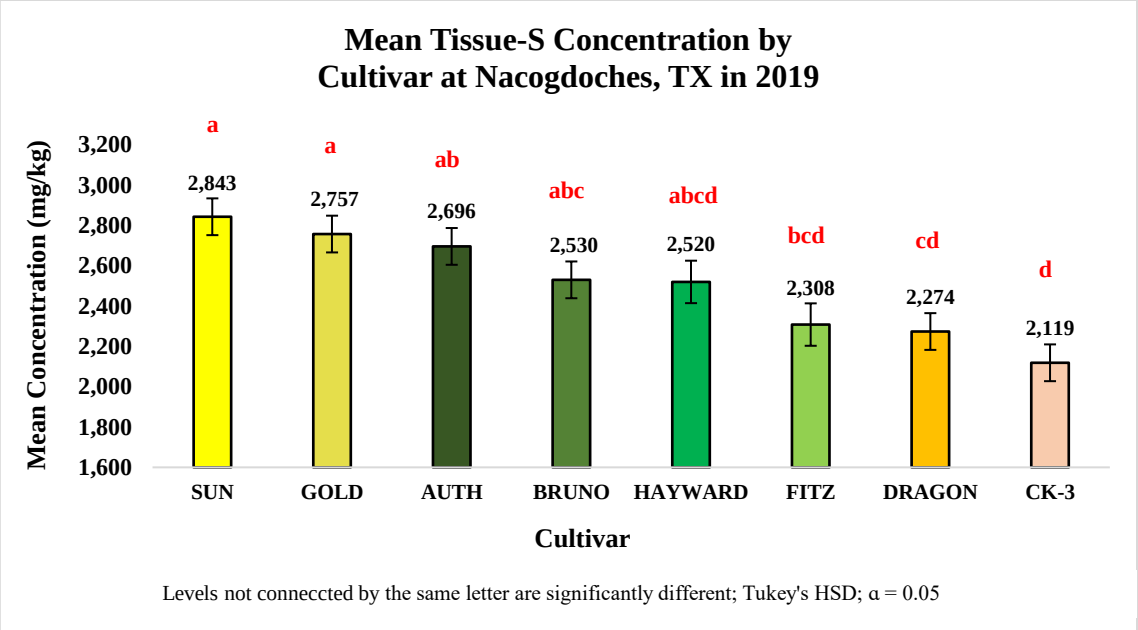


Figure 110 Comparison of mean plant tissue-S concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 42 Comparison of mean tissue-S concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
GOLD	2,812	509.2	123.4	AUTH	3,258	687.4	191.2
SUN	2,730	287.2	123.4	SUN	3,181	415.4	191.2
BRUNO	2,519	293.9	131.9	HAYWARD	3,091	620.8	204.4
AUTH	2,497	275.5	123.4	GOLD	3,085	412.9	191.2
HAYWARD	2,396	376.5	123.4	BRUNO	3,033	582.8	191.2
DRAGON	2,228	335.7	123.4	FITZ	2,950	614.1	204.4
FITZ	2,221	349.4	123.4	DRAGON	2,693	504.3	191.2
CK-3	2,031	298.6	123.4	CK-3	2,420	439.0	191.2
Tissue-S response to cultivar significant at College Station (P<0.0004) only Significant (P=0.0305) site x year interaction present.							

Plant Tissue Sodium

Average tissue-Na response to cultivar was not significant at CS in 2018, despite concentrations ranging from 59.7 mg/kg to 167.3 mg/kg (Figure 111). The same was true at NAC in 2018, where a range of 34.8 mg/kg and 60.3 mg/kg was observed among cultivars (Figure 112). Average concentrations of tissue-Na were generally higher the second year at CS (and NAC), with cultivars ranging between 80.0 mg/kg and 97.9 mg/kg, although this difference was not significant among cultivars (Figure 113). Cultivar response was not significant at NAC in 2019, where an average value of 80.0 mg/kg was reported for all cultivars, resulting from a complication with testing procedure (Figure 114). Over years, average tissue-Na ranged from 81.3 mg/kg to 127.2 mg/kg at CS and between 57.4 mg/kg and 69.8 mg/kg at NAC (Table 43).

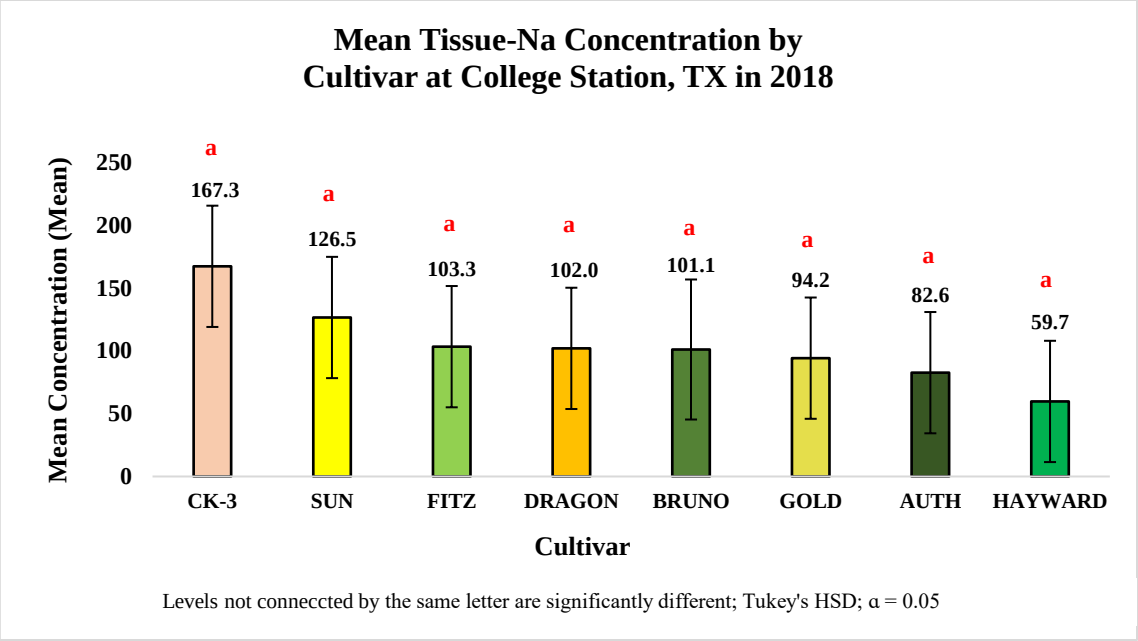


Figure 111 Comparison of mean plant tissue-Na concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

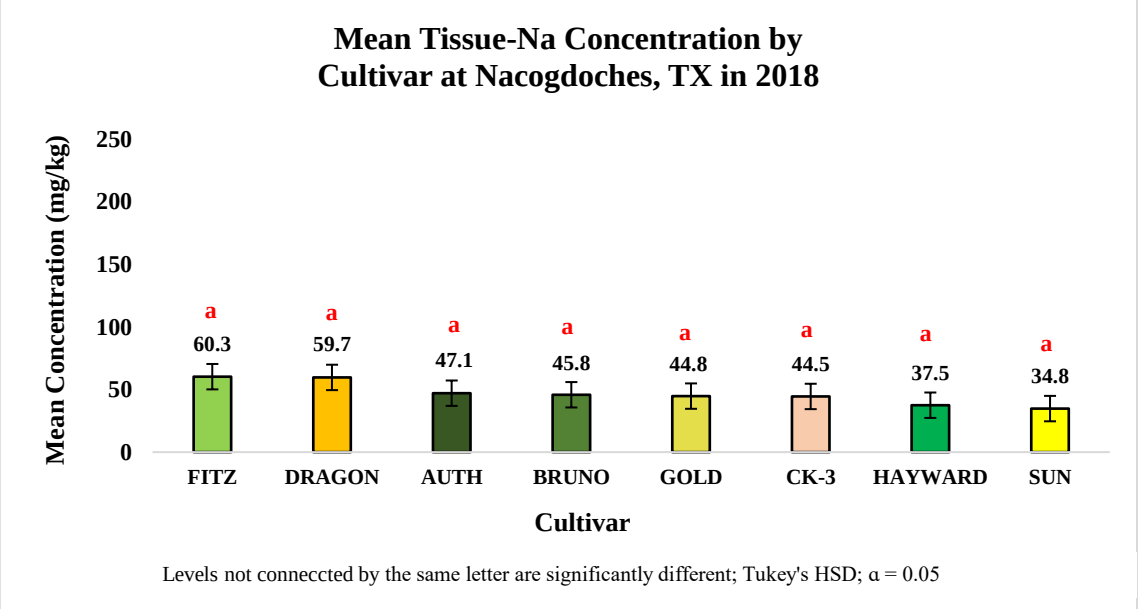


Figure 112 Comparison of mean plant tissue-Na concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

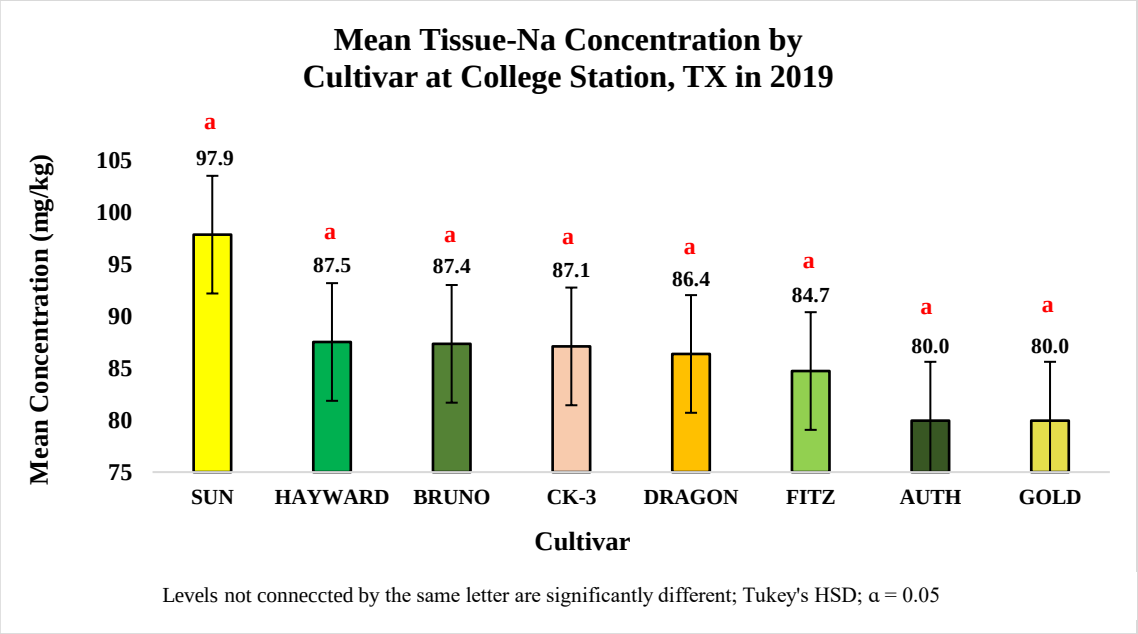


Figure 113 Comparison of mean plant tissue-Na concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

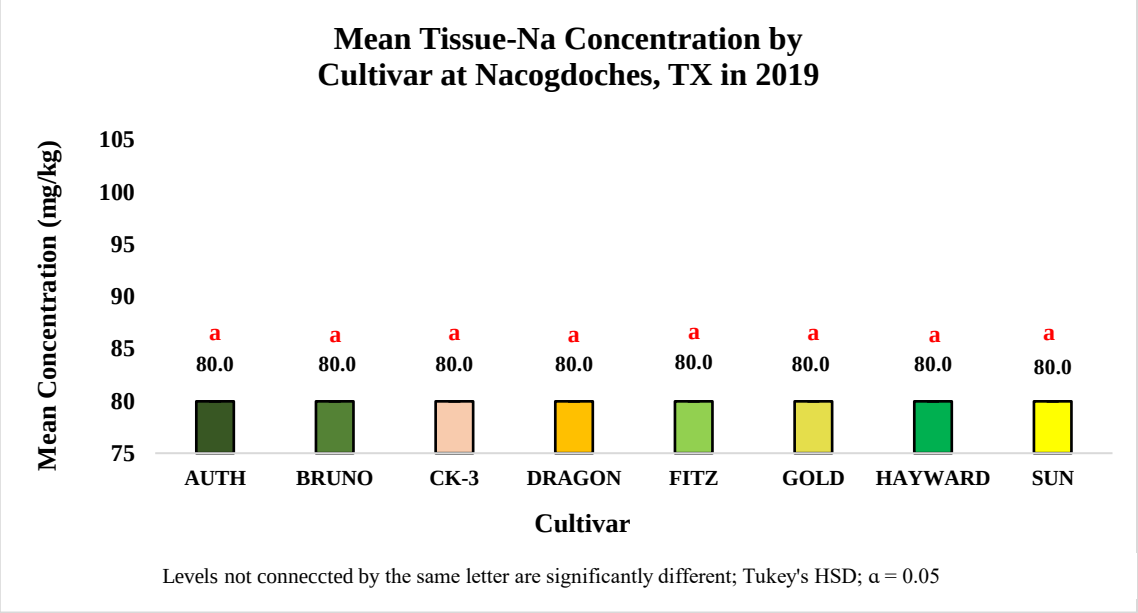


Figure 114 Comparison of mean plant tissue-Na concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 43 Comparison of mean tissue-Na concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
Cultivar	Ave.	St. Dev.	St. Error	Cultivar	Ave.	St. Dev.	St. Error
CK-3	127.2	137.14	23.27	DRAGON	69.8	24.69	8.08
SUN	112.2	74.18	23.27	FITZ	68.7	20.53	8.64
DRAGON	94.2	36.65	23.27	AUTH	63.5	22.30	8.08
FITZ	94.0	52.79	23.27	BRUNO	62.9	24.11	8.08
BRUNO	93.2	25.14	24.88	GOLD	62.4	20.09	8.08
GOLD	87.1	42.13	23.27	CK-3	62.2	22.45	8.08
AUTH	81.3	39.52	23.27	SUN	57.4	24.51	8.08
Tissue-Na response to cultivar not significant at either site Significant (P=0.0025) site x year interaction present.							

Plant Tissue Zinc

Average tissue-Zn at CS in 2018 did not respond significantly to cultivar, despite exhibiting a range of 23.0 mg/kg and 31.6 mg/kg (Figure 115). However, cultivar was significant (P = 0.005) at NAC the same year, where a range of 21.1 mg/kg and 44.8 mg/kg was observed among cultivars, with means separation identifying five statistical groups in descending order: 1) DRAGON, BRUNO, and HAYWARD; 2) FITZ; 3) CK-3 and AUTH; 4) GOLD; 5) SUN (Figure 116). There was no significant response to cultivar again at CS during the second year, where cultivar averages ranged from 15.8 mg/kg to 26.6 mg/kg (Figure 117). Average concentrations at NAC in 2019 (P<0.0001) ranged from 15.3 mg/kg to 30.2 mg/kg, with four statistical groups: 1) BRUNO; 2) HAYWARD; 3) FITZ; 4) DRAGON, AUTH, SUN, GOLD, and CK-3 (Figure 118). A

range of 21.3 mg/kg and 28.7 mg/kg was observed among cultivars at CS, while cultivars ranged from 19.3 mg/kg to 35.5 mg/kg at NAC over the two years (Table 44).

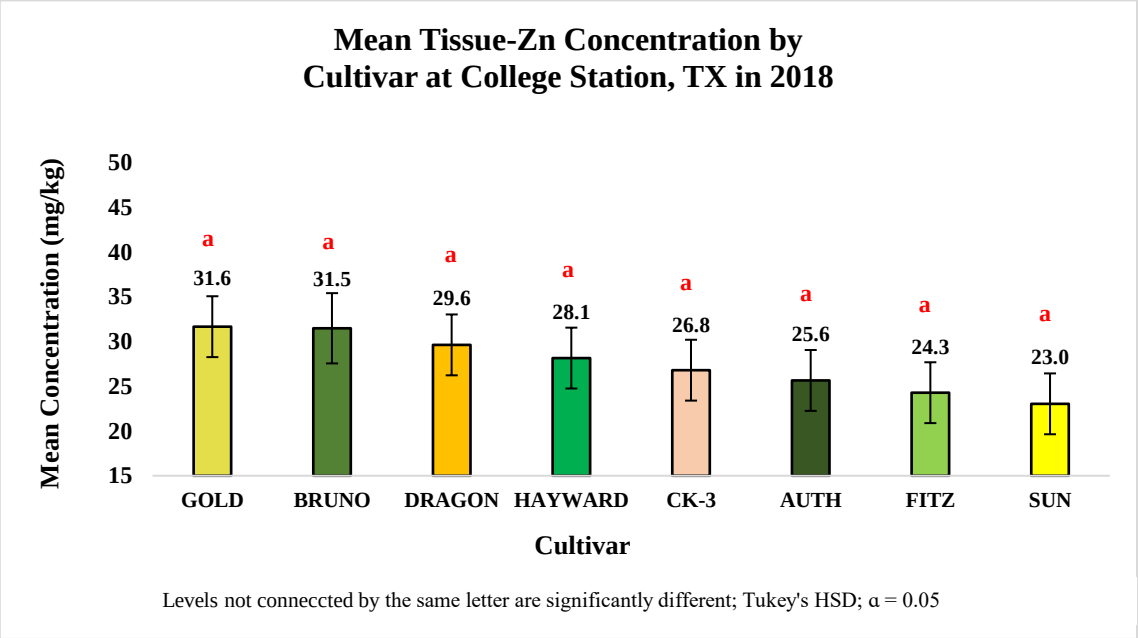


Figure 115 Comparison of mean plant tissue-Zn concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

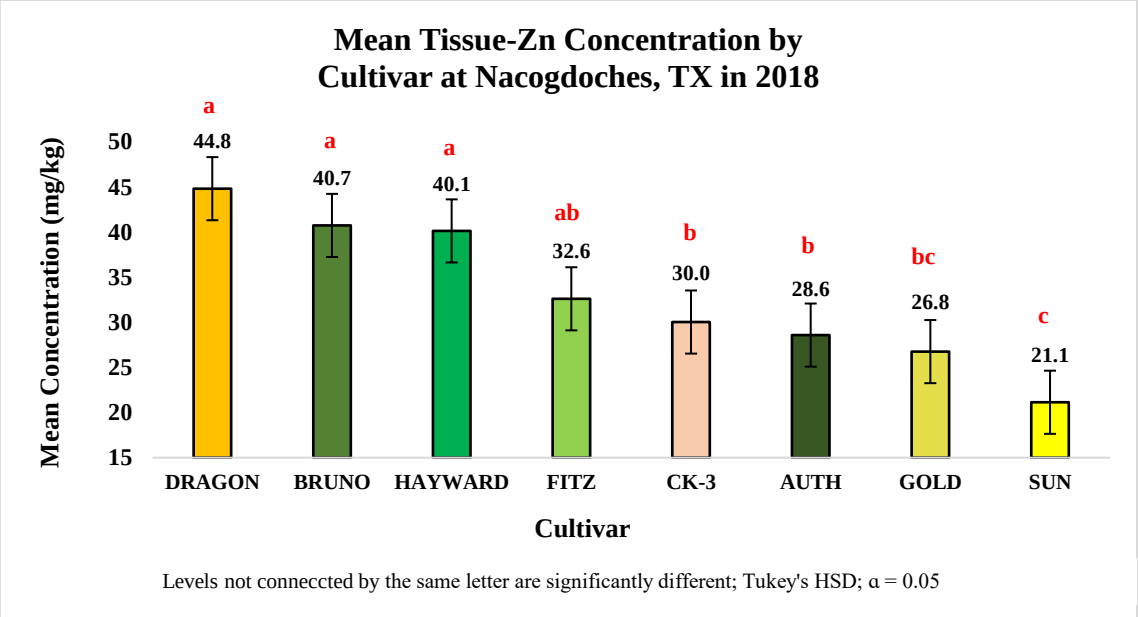


Figure 116 Comparison of mean plant tissue-Zn concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

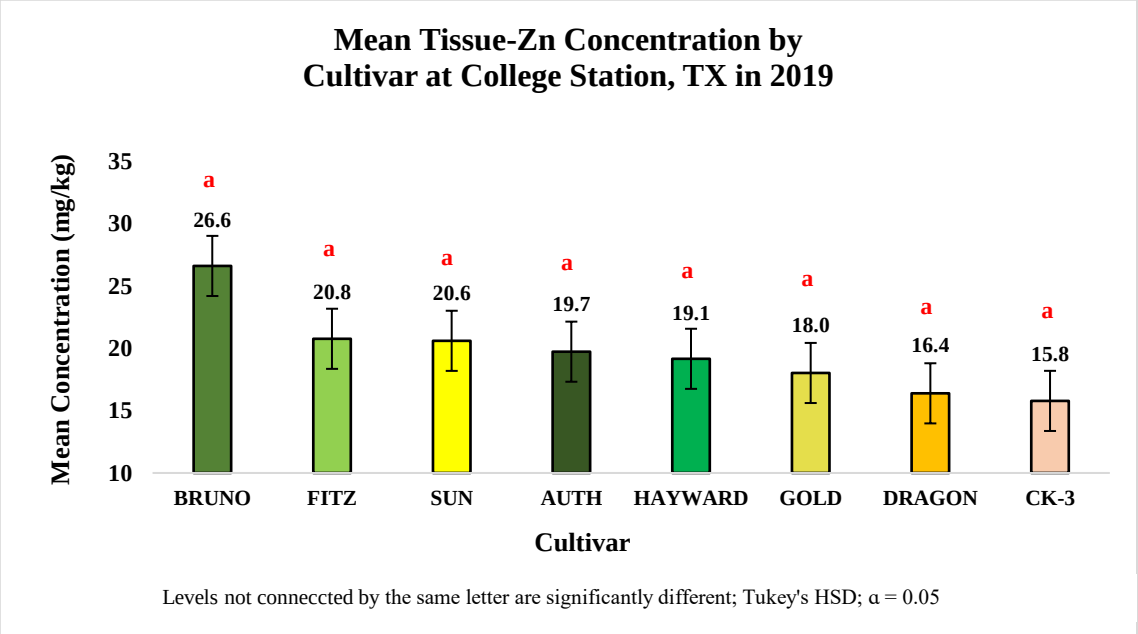


Figure 117 Comparison of mean plant tissue-Zn concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

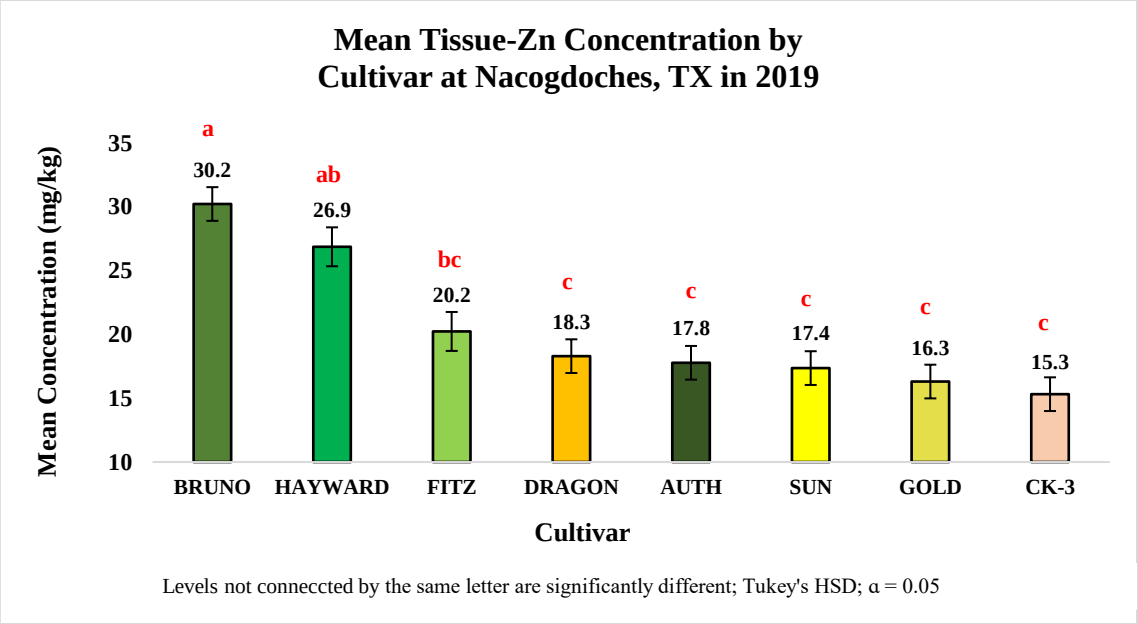


Figure 118 Comparison of mean plant tissue-Zn concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 44 Comparison of mean tissue-Zn concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
BRUNO	28.7	5.44	2.73	BRUNO	35.5	6.69	3.20
GOLD	24.8	11.20	2.55	HAYWARD	34.4	8.06	3.42
HAYWARD	23.6	6.84	2.55	DRAGON	31.6	16.71	3.20
DRAGON	23.0	7.68	2.55	FITZ	27.3	6.88	3.42
AUTH	22.7	6.24	2.55	AUTH	23.2	6.31	3.20
FITZ	22.5	3.69	2.55	CK-3	22.7	11.24	3.20
SUN	21.8	4.77	2.55	GOLD	21.5	6.02	3.20
CK-3	21.3	8.84	2.55	SUN	19.3	3.08	3.20

Tissue-Zn response to cultivar significant (P=0.0014) at Nacogdoches only.
Significant (P=0.0109) site x cultivar and year x cultivar (P=0.002) interaction present.
Analysis of tissue-Zn based on natural log-transformed data.

Plant Tissue Iron

Average tissue-Fe at CS in 2018 (P = 0.0465) ranged from 38.4 mg/kg to 54.9 mg/kg and comprised three statistical groups in descending order: 1) SUN; 2) AUTH, GOLD, BRUNO, CK-3, HAYWARD, FITZ; 3) DRAGON (Figure 119). Response to cultivar was significant at NAC in 2018, where a range of 55.0 mg/kg and 68.8 mg/kg was observed (Figure 120). Average tissue-Fe was significant for cultivar (P = 0.0255) at NAC during the second year with a range of 38.8 mg/kg and 46.6 mg/kg, although means separation failed to identify significant groups (Figure 121). Concentrations at NAC in 2019 (P = 0.0004) ranged from 44.2 mg/kg to 69.4 mg/kg, with three statistical groups identified: 1) AUTH, HAYWARD, BRUNO, SUN, FITZ, and GOLD; 2) DRAGON; 3) CK-3 (Figure 122).

It should be noted that tissue-Fe response to cultivar was significant over years at both locations. Average concentrations ranged from 38.6 mg/kg to 48.3 mg/kg at CS (P = 0.0046) over years, with cultivars separating into three statistical groups: 1) SUN and AUTH; 2) GOLD, BRUNO; HAYWARD, CK-3, and FITZ; 3) DRAGON. At NAC, average tissue-Fe (P = 0.0248) ranged between 50.7 mg/kg and 67.1 mg/kg (Table 45), also with three statistical groups: 1) BRUNO; 2) AUTH, FITZ, HAYWARD, SUN, GOLD, DRAGON; 3) CK-3.

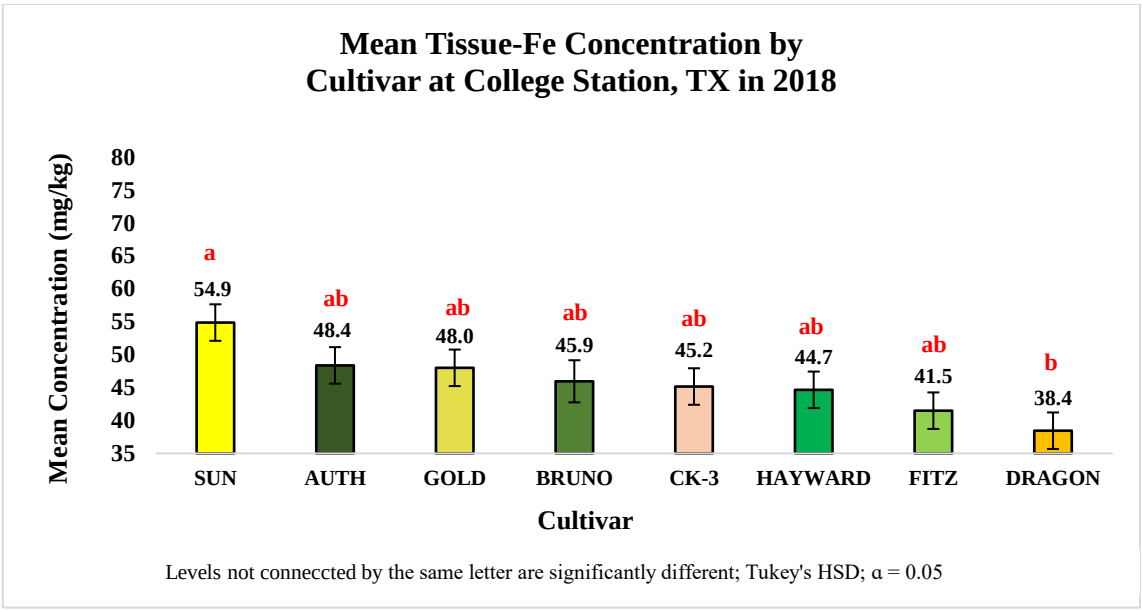


Figure 119 Comparison of mean plant tissue-Fe concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

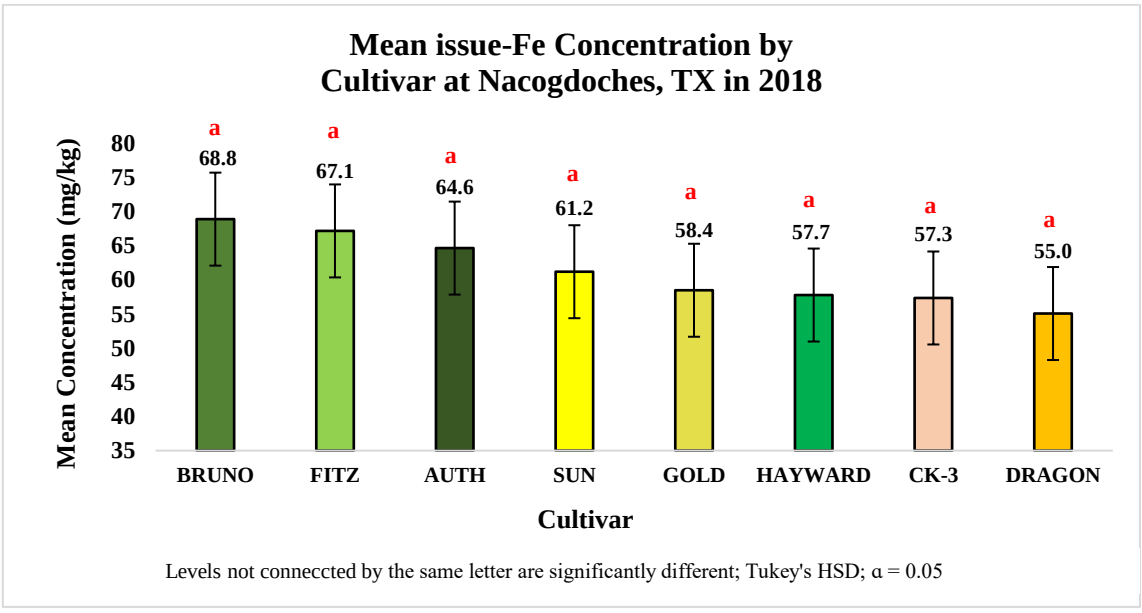


Figure 120 Comparison of mean plant tissue-Fe concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

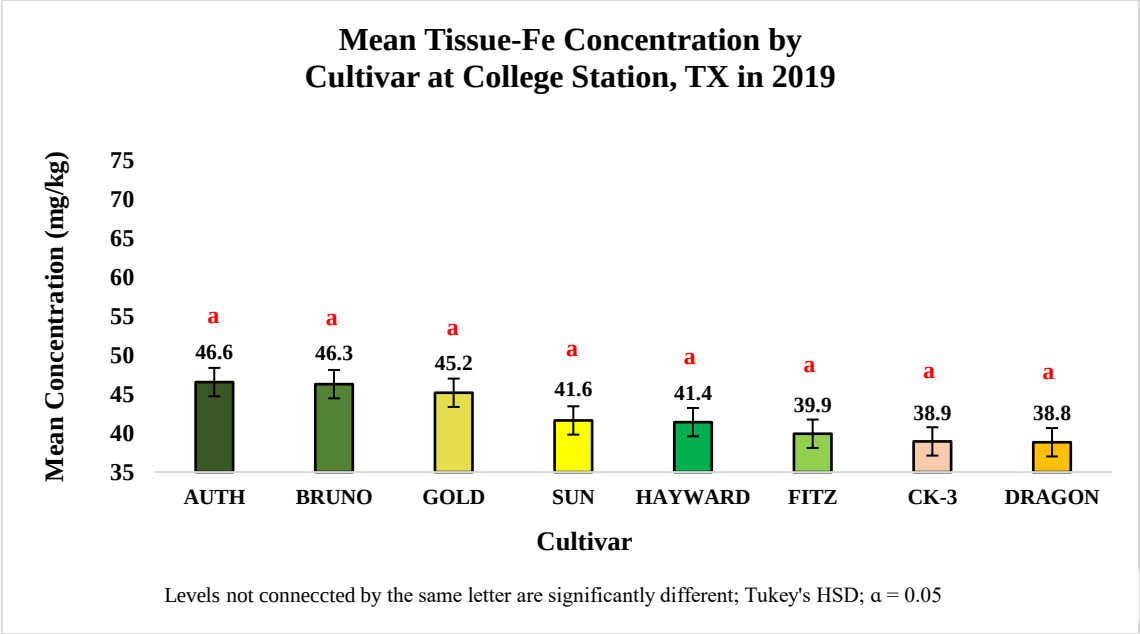


Figure 121 Comparison of mean plant tissue-Fe concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

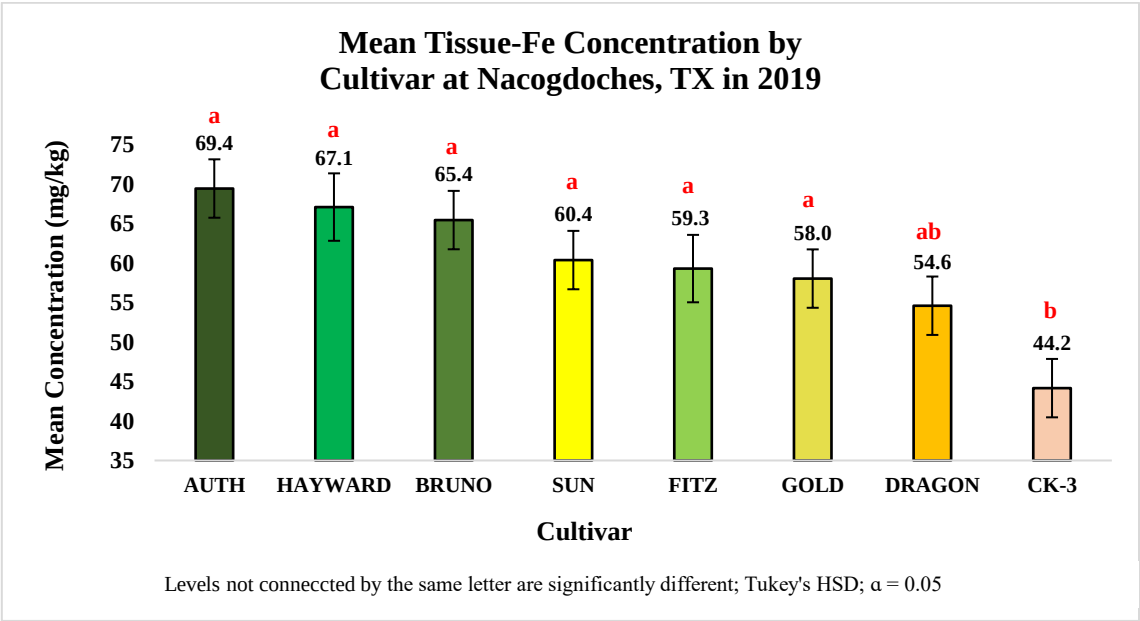


Figure 122 Comparison of mean plant tissue-Fe concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 45 Comparison of mean tissue-Fe concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
SUN	48.3	8.28	1.85	BRUNO	67.1	19.85	3.97
AUTH	47.5	3.34	1.85	AUTH	67.0	11.44	3.97
GOLD	46.6	3.36	1.85	FITZ	63.8	11.40	4.24
BRUNO	46.1	3.77	1.98	HAYWARD	61.7	7.37	4.24
HAYWARD	43.0	2.88	1.85	SUN	60.8	6.71	3.97
CK-3	42.0	5.17	1.85	GOLD	58.2	3.24	3.97
FITZ	40.7	7.52	1.85	DRAGON	54.8	11.17	3.97
DRAGON	38.6	4.48	1.85	CK-3	50.7	10.47	3.97

Tissue-Fe response to cultivar significant at College Station (P=0.0046) and Nacogdoches (P=0.0248) sites. Analysis of tissue-Fe based on natural log-transformed data.

Plant Tissue Manganese

Average tissue-Mn, with a range of 32.2 mg/kg and 41.4 mg/kg, did not vary significantly by cultivar at CS in 2018 (Figure 123). Cultivar response at NAC in 2018 was significant (P = 0.0095), where a range of 39.1 mg/kg and 61.6 mg/kg was observed and three statistical groups were identified in descending order: 1) BRUNO and HAYWARD; 2) AUTH, SUN, GOLD, FITZ, and DRAGON; 3) CK-3 (Figure 124). Average tissue-Mn at CS during second year was significant (P = 0.0047; block: P=0.0233), with a range of 20.5 mg/kg and 32.3 mg/kg, consisting of three statistical groups: 1) AUTH and BRUNO; 2) HAYWARD, SUN, GOLD, and FITZ; 3) DRAGON and CK-3 (Figure 125). Average cultivar concentrations at NAC in 2019 were not

significantly different, despite a range from 74.4 mg/kg to 103.8 mg/kg (Figure 126). Over years, average tissue-Mn ranged from 26.4 mg/kg to 35.5 mg/kg at CS and from 59.8 mg/kg to 82.7 mg/kg at NAC (Table 46).

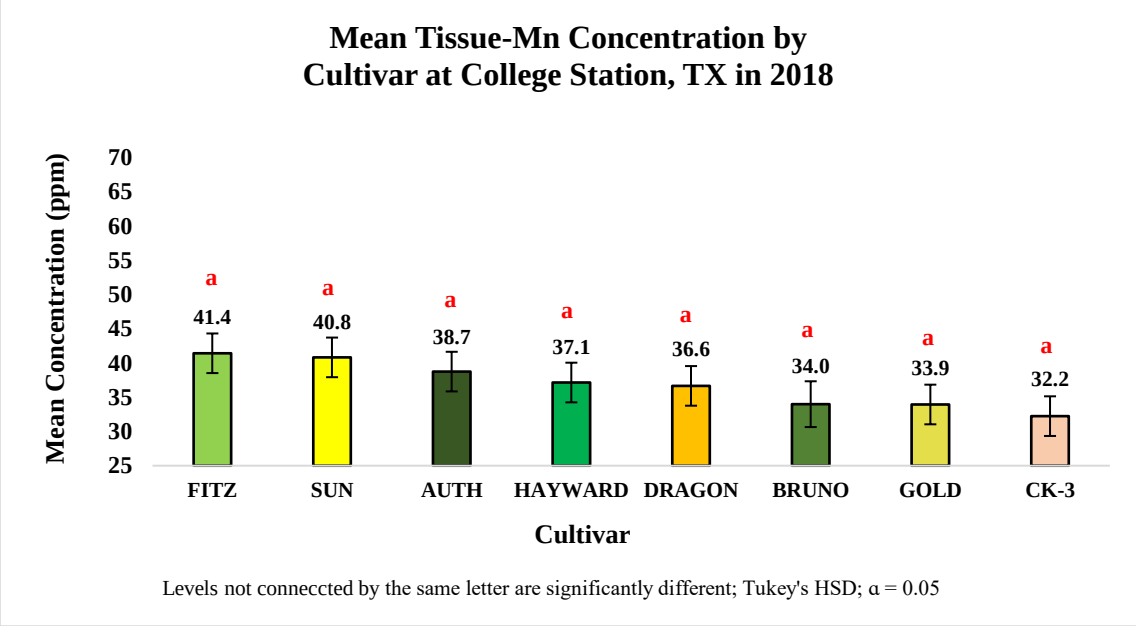


Figure 123 Comparison of mean plant tissue-Mn concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

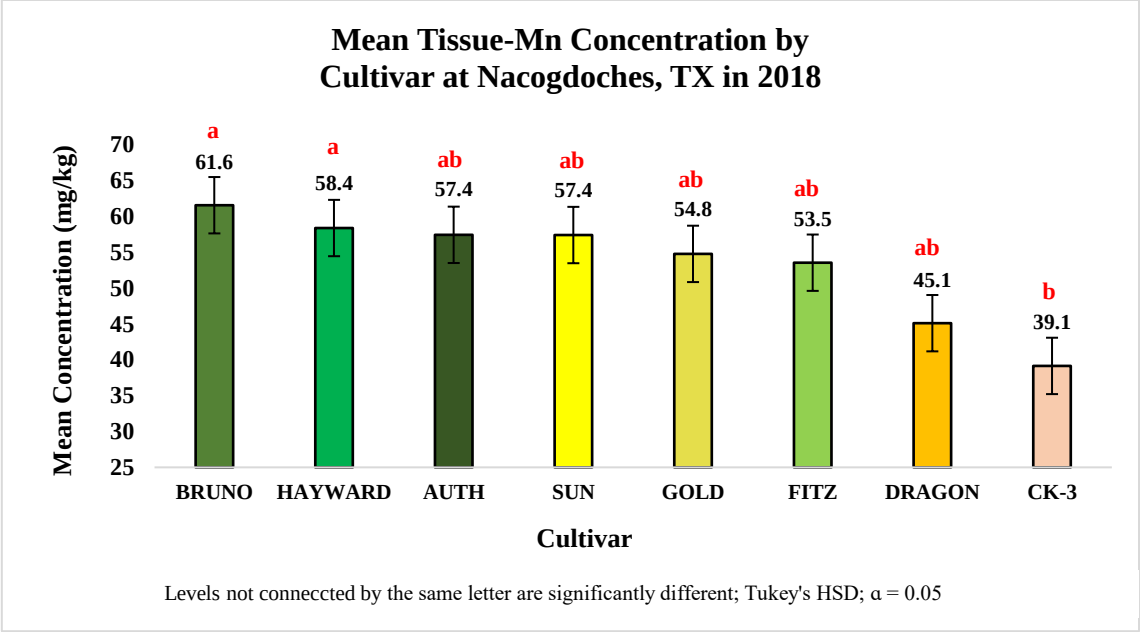


Figure 124 Comparison of mean plant tissue-Mn concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

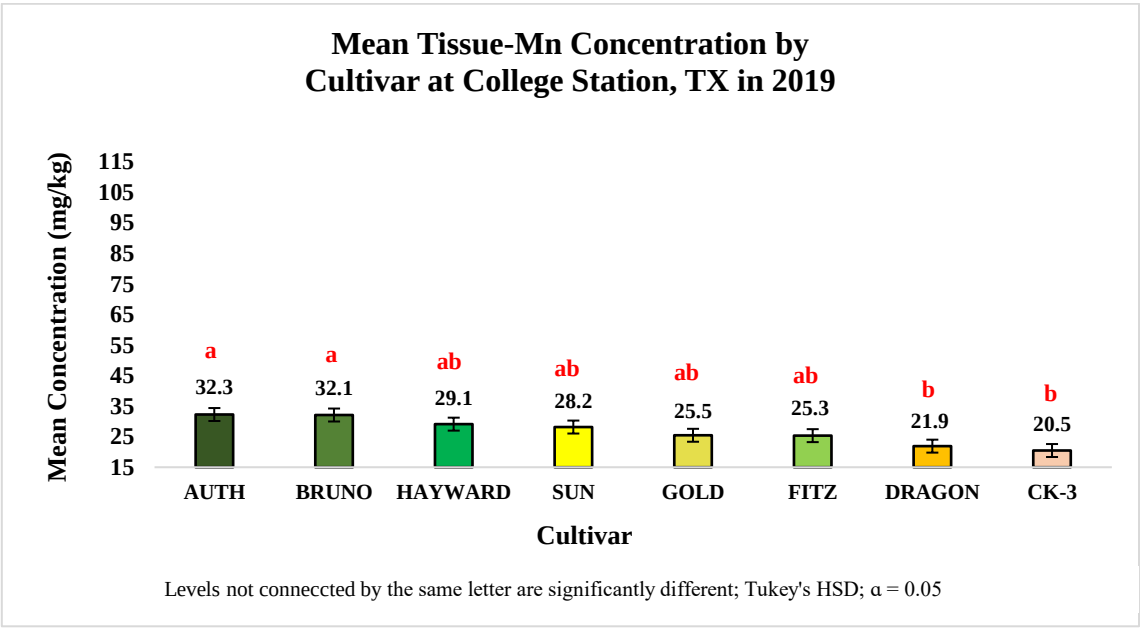


Figure 125 Comparison of mean plant tissue-Mn concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

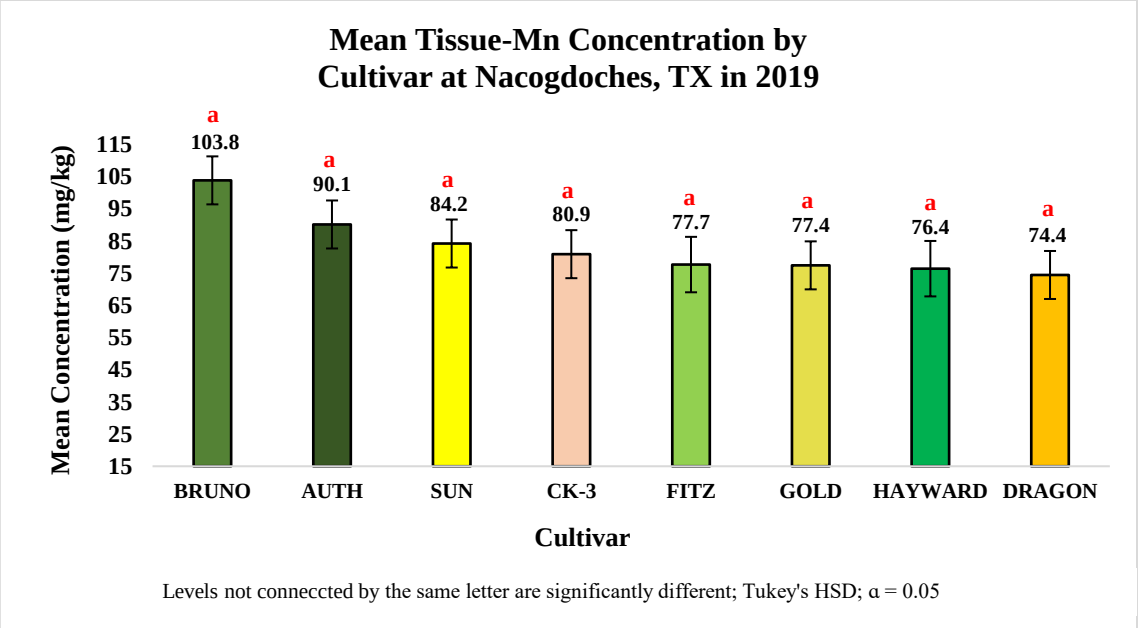


Figure 126 Comparison of mean plant tissue-Mn concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 46 Comparison of mean tissue-Mn concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
AUTH	35.5	7.05	2.72	BRUNO	82.7	30.84	7.03
SUN	34.5	7.04	2.72	AUTH	73.8	18.94	7.03
FITZ	33.4	12.15	2.72	SUN	70.8	16.50	7.03
HAYWARD	33.1	7.39	2.72	HAYWARD	66.1	10.29	7.51
BRUNO	32.9	2.78	2.91	GOLD	66.1	14.10	7.03
GOLD	29.7	5.96	2.72	FITZ	63.9	15.01	7.51
DRAGON	29.3	8.47	2.72	CK-3	60.0	25.63	7.03
CK-3	26.4	7.14	2.72	DRAGON	59.8	18.10	7.03

Tissue-Fe response to cultivar not significant at either site across years.
Significant (P<0.0001) site x year interaction present.

Plant Tissue Copper

At CS in 2018 a range of 4.2 mg/kg and 9.3 mg/kg for average tissue-Cu was observed ($P = 0.0392$). However, Tukey's HSD failed to identify separate statistical cultivar groups, based on means (Figure 127). Average concentrations at NAC in 2018 ($P = 0.0081$) ranged from 8.1 mg/kg to 10.7 mg/kg, which included three statistical groups in descending order: 1) GOLD; 2) DRAGON, CK-3, AUTH, and BRUNO; 3) HAYWARD, FITZ, and SUN (Figure 128). Average tissue-Cu during the second year at CS ($P<0.0001$; block: $P<0.0001$) exhibited a range from 5.1 mg/kg to 8.8 mg/kg and consisted of four statistical groups: 1) GOLD; 2) AUTH and BRUNO; 3) FITZ, CK-3, and SUN; 4) DRAGON (Figure 129). At NAC in 2018 ($P=0.0004$), cultivars ranged from an average of 6.5 mg/kg to 12.2 mg/kg, with three statistical groups: 1) GOLD; 2) BRUNO; 3) CK-3, AUTH, HAYWARD, SUN, DRAGON, and FITZ (Figure 130). Over years, average tissue-Cu range from 4.6 mg/kg to 9.0 mg/kg at CS and from 7.5 mg/kg to 11.4 mg/kg at NAC (Table 47).

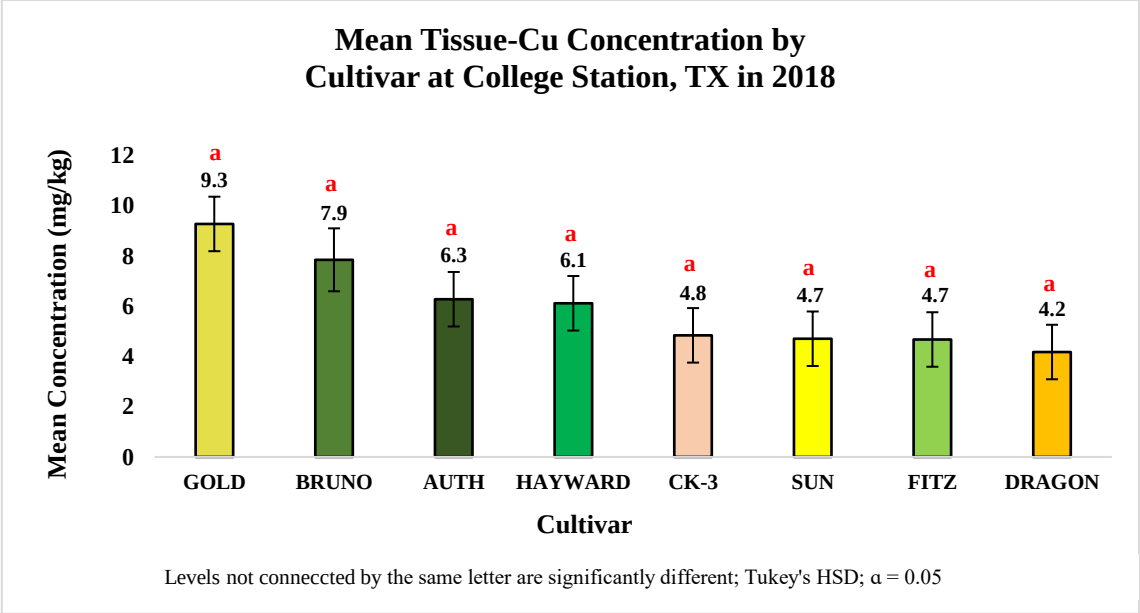


Figure 127 Comparison of mean plant tissue-Cu concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

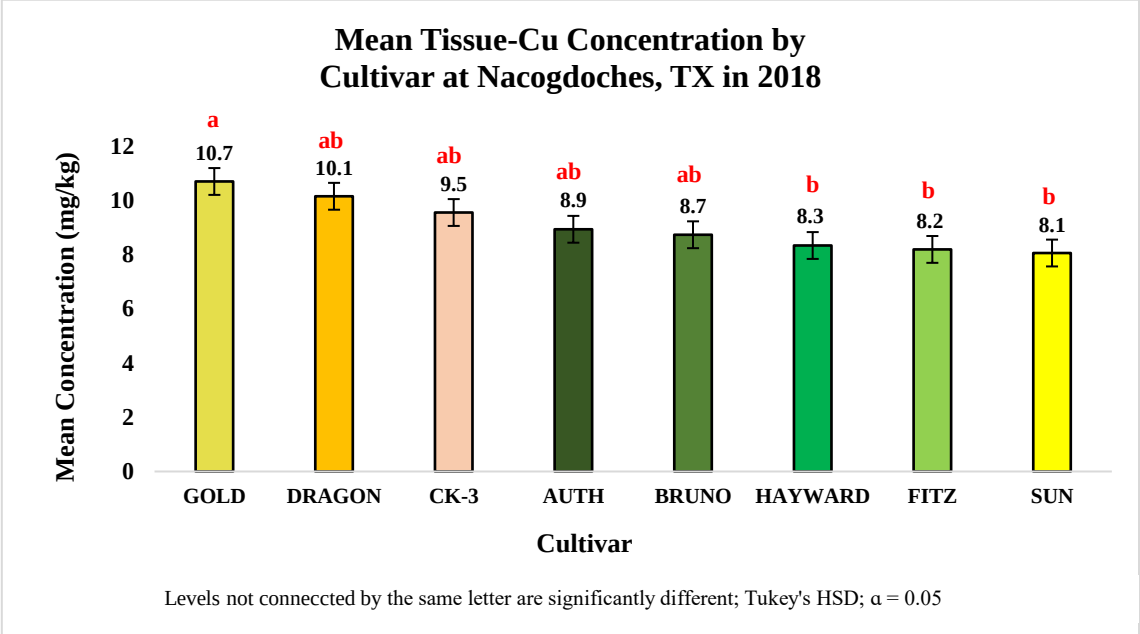


Figure 128 Comparison of mean plant tissue-Cu concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

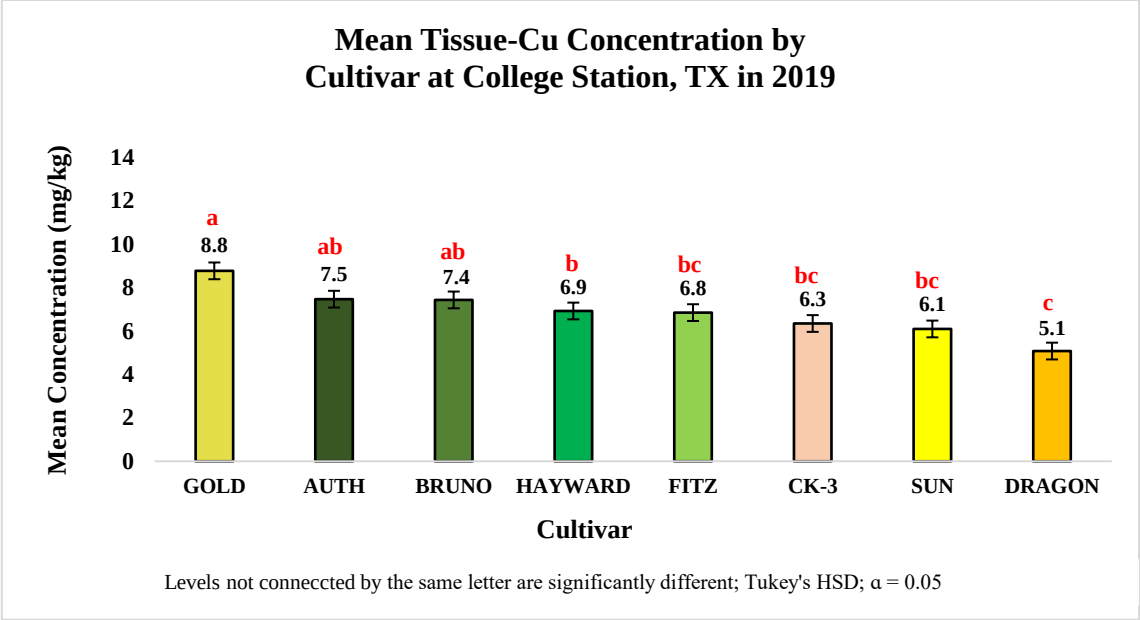


Figure 129 Comparison of mean plant tissue-Cu concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

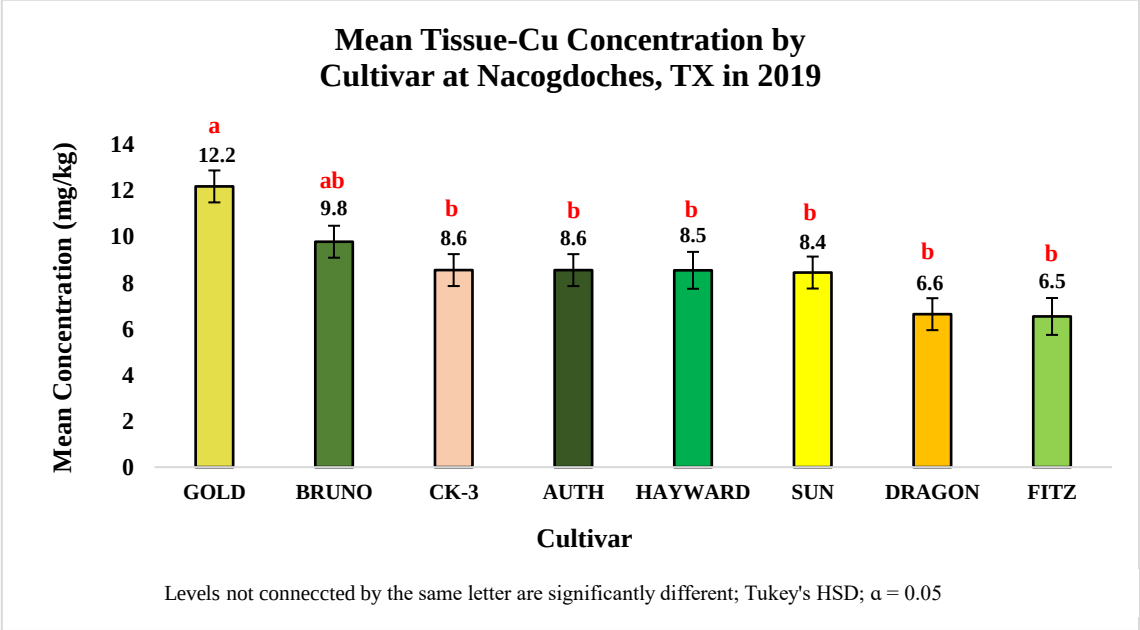


Figure 130 Comparison of mean plant tissue-Cu concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 47 Comparison of mean tissue-Cu concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
GOLD	9.0	2.51	0.64	GOLD	11.4	1.08	0.49
BRUNO	7.6	1.66	0.68	BRUNO	9.3	1.31	0.49
AUTH	6.9	2.09	0.64	CK-3	9.0	1.57	0.49
HAYWARD	6.5	1.52	0.64	AUTH	8.7	0.91	0.49
FITZ	5.8	1.49	0.64	HAYWARD	8.4	0.78	0.52
CK-3	5.6	2.03	0.64	DRAGON	8.4	2.56	0.49
SUN	5.4	1.51	0.64	SUN	8.2	0.57	0.49
DRAGON	4.6	1.34	0.64	FITZ	7.5	1.08	0.52
Tissue-Cu response to cultivar significant at College Station (P=0.0003) and Nacogdoches (P<0.0001) sites. Significant site x year (P=0.0086) and site x year x cultivar (P=0.0141) interaction present.							

Plant Tissue Boron

Average tissue-B at CS in 2018 (P<0.0001) ranged from 24.0 mg/kg to 34.6 mg/kg and comprised eight statistical groups in descending order: 1) CK-3; 2) DRAGON; 3) GOLD; 4) SUN; 5) BRUNO; 6) FITZ; 7) HAYWARD; 8) AUTH (Figure 131). During the same year at NAC (P<0.0001; block: P=0.0348) average concentrations ranged from 25.9 mg/kg to 35.5 mg/kg, with cultivars falling into four statistical groups: 1) GOLD; 2) DRAGON; 3) HAYWARD, CK-3, SUN, and BRUNO; 4) FITZ and AUTH (Figure 132). At CS during the second year, average tissue-B (P<0.0001; block: P<0.0001) exhibited a higher range of 31.9 mg/kg and 46.4 mg/kg with seven statistical groups: 1) GOLD; 2) CK-3; 3) SUN; 4) DRAGON; 5) AUTH; 6) BRUNO; 7) HAYWARD and FITZ (Figure 133). Finally, average concentrations at NAC in 2019 (P

= 0.0004) ranged from 26.3 mg/kg to 40.7 mg/kg and included three statistical groups: 1) GOLD; 2) CK-3, SUN, HAYWARD, and DRAGON 3) AUTH, FITZ, and BRUNO (Figure 134). At CS over years cultivars ranged from 28.6 mg/kg to 40.1 mg/kg and from 26.8 mg/kg to 38.1 mg/kg at NAC (Table 48).

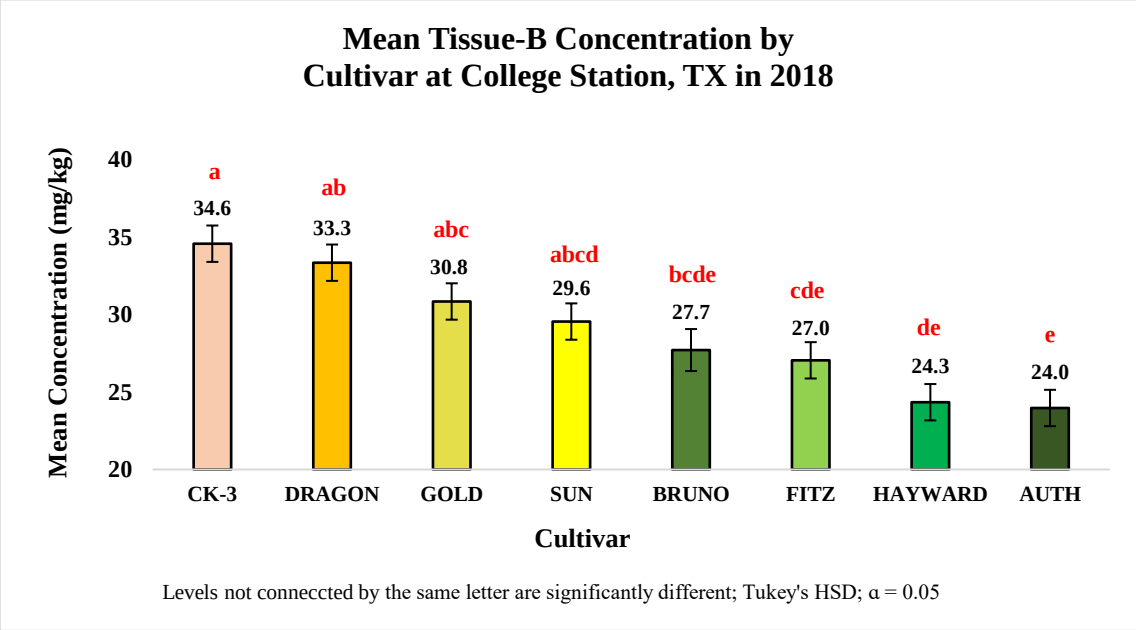


Figure 131 Comparison of mean plant tissue-B concentration by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

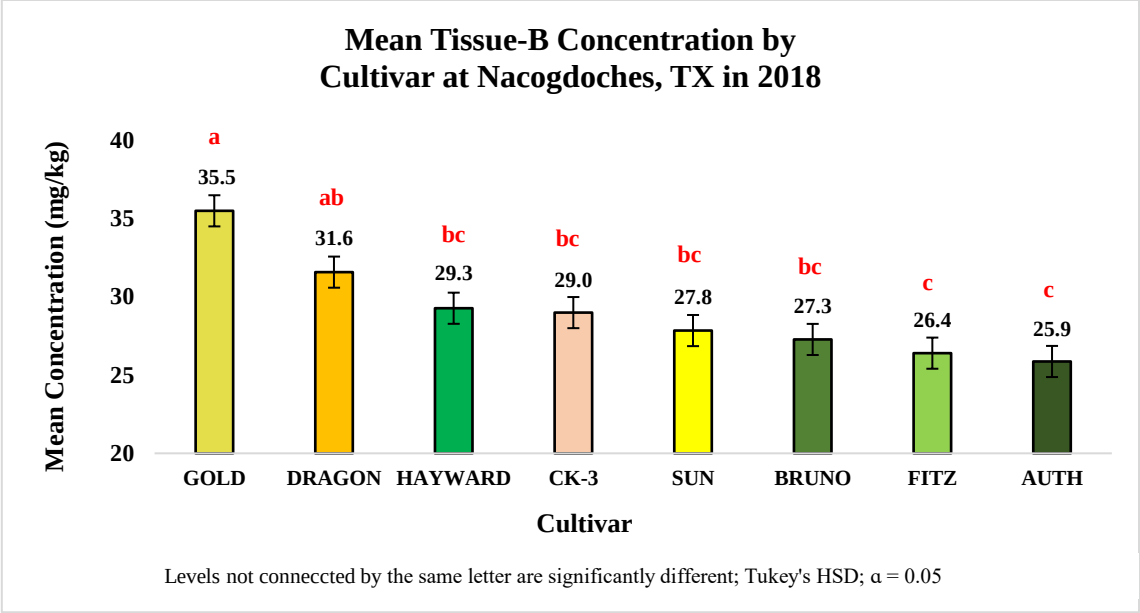


Figure 132 Comparison of mean plant tissue-B concentration by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

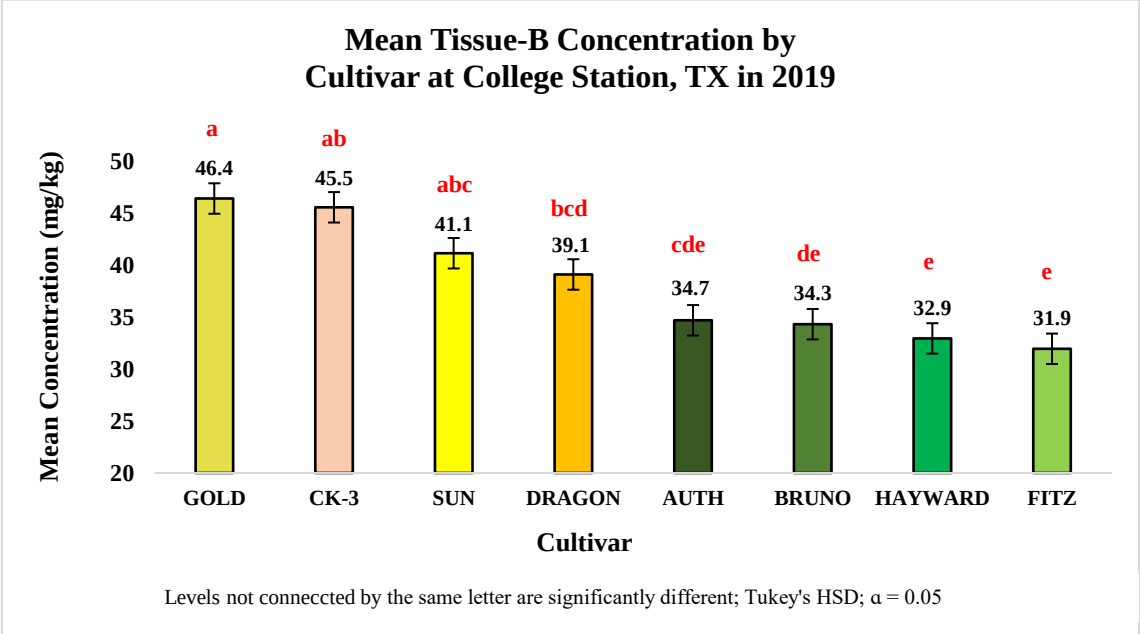


Figure 133 Comparison of mean plant tissue-B concentration by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

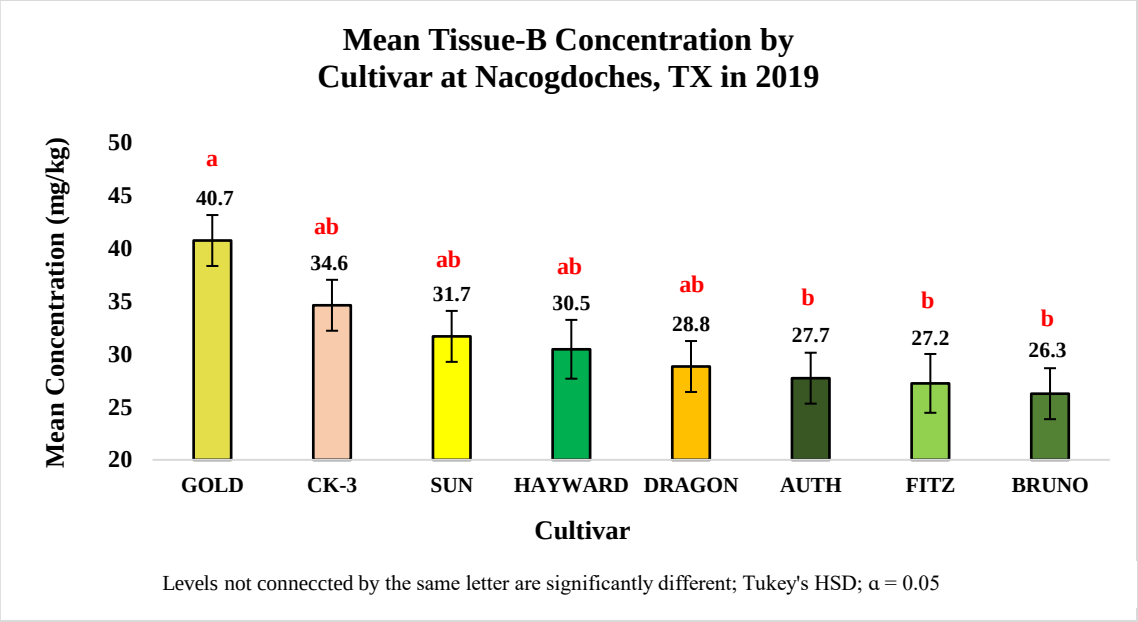


Figure 134 Comparison of mean plant tissue-B concentration by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 48 Comparison of mean tissue-B concentration by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error	Cultivar	Mean (mg/kg)	Standard Deviation	Standard Error
CK-3	40.1	7.89	2.21	GOLD	38.1	4.55	1.37
GOLD	38.6	8.69	2.21	CK-3	31.8	4.87	1.37
DRAGON	36.2	3.93	2.21	DRAGON	30.2	4.60	1.37
SUN	35.3	7.42	2.21	HAYWARD	29.8	3.38	1.46
BRUNO	31.5	4.38	2.36	SUN	29.8	3.82	1.37
FITZ	29.5	3.16	2.21	AUTH	26.8	3.75	1.37
AUTH	29.3	6.40	2.21	BRUNO	26.8	1.96	1.37
HAYWARD	28.6	5.59	2.21	FITZ	26.8	2.99	1.46

Tissue-B response to cultivar significant at College Station (P=0.0008) and Nacogdoches sites (P<0.0001). Significant site x year (P<0.0001), site x cultivar (P=0.006), and year x cultivar (P=0.0228) interaction present. Analysis of Tissue-B based on natural log-transformed data.

Comparison of Visual Responses

Percent Canopy Chlorosis, SPAD Percentage, and Chlorosis Index by Site and Year

Variables used to assess visual symptoms related to chlorosis included percent canopy chlorosis (PCC), SPAD percentage (SPAD-P), and chlorosis index (CI). PCC and CI both showed significant (P<0.0001) site x year interactions. Consequently, comparison of site effect for the two former variables was carried out by individual year. However, site averages were noticeably higher at CS across years for both parameters. SPAD-P was significantly (P<0.0001) higher at NAC (67.5%) as compared to CS (61.8%) across years. Significant block effects were also observed for PCC, SPAD-P, and CI (P = 0.0029, 0.0214, 0.0205, respectively) over years (Figure 135).

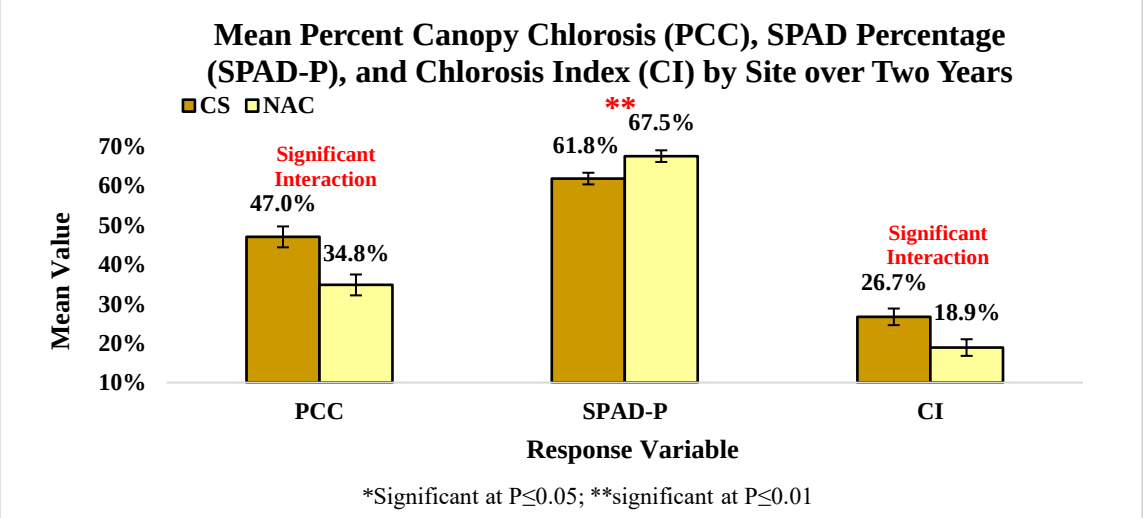


Figure 135 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) by site in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

Single-year analysis of 2018 indicated that PCC was slightly, but not significantly higher on average at NAC (55.9%) as compared to CS (52.5%) and nearly identical for CI (34.0 and 33.9, respectively). In addition to the significant site response across years, SPAD-P was higher ($P = 0.0043$) at NAC (60.4%) than at CS (54.4%) (Figure 136). Conversely, during 2019, PCC and CI were significantly ($P<0.0001$) higher on average at CS (41.5 % and 19.5, respectively) than at NAC (13.8% and 3.9, respectively). Once again, SPAD-P was higher ($P = 0.0351$) at CS (Figure 137). PCC, SPAD-P, and CI also showed significant ($P<0.0001$) year effects. In 2018, average PCC and CI were both higher across sites during the first year, with averages of 54.2% and 34 for both sites in 2018 and 27.7% and 11.7 in 2019. Conversely, average SPAD-H was higher in the second year, with a site average of 57.4% in 2018 and 72.0% in 2019 (Figures 136 & 137).

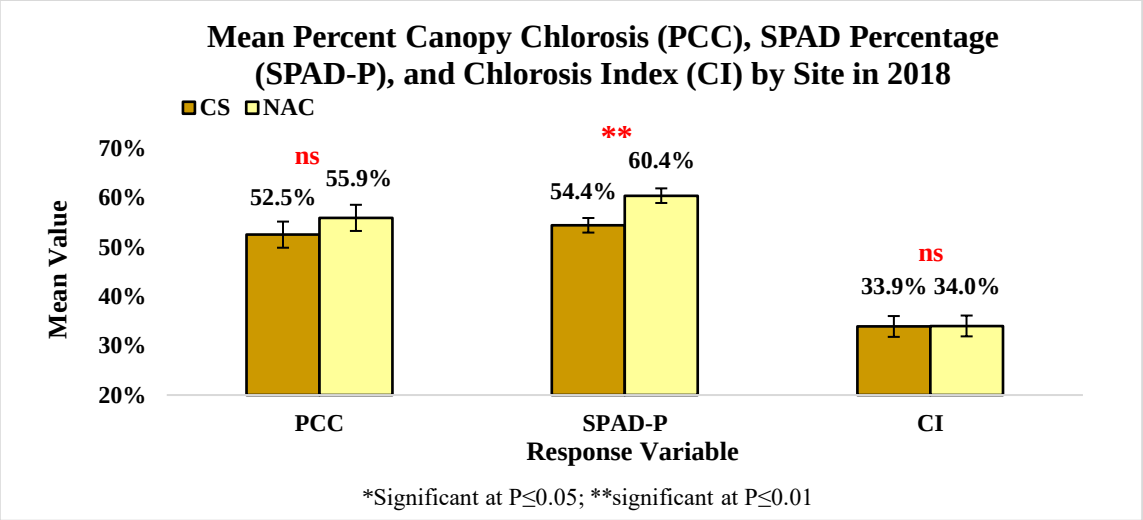


Figure 136 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) by site in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

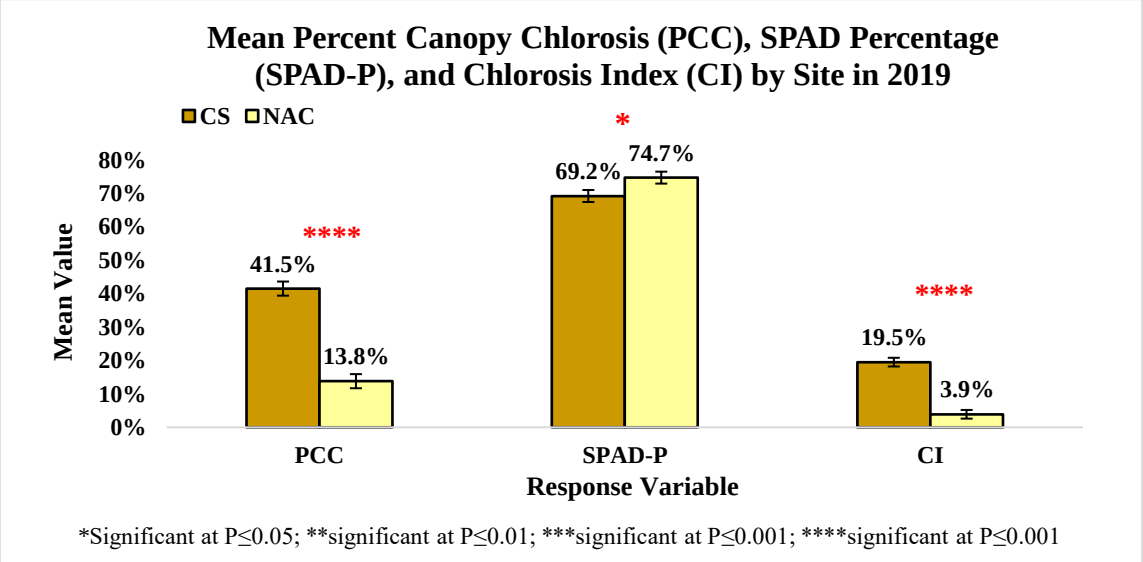


Figure 137 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) by site in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Visual Reponses by Cultivar

Percent Canopy Chlorosis

PCC varied widely among cultivars, with cultivar averages ranging from 23.0% to 53.6% across sites and years. However, significant site x cultivar ($P<0.0001$) and year x cultivar ($P = 0.0029$) interactions necessitated the comparison of cultivars separately by individual site and year.

PCC response to cultivar at CS in 2018 was significant ($P<0.0001$; block: $P=0.0011$) with a range of 23.8% to 77.1% and means separation revealing five statistical groups in descending order: 1) DRAGON; 2) FITZ, CK-3, and BRUNO; 3) HAYWARD and GOLD; 4) SUN; 5) AUTH (Figure 138). Average PCC by cultivar at NAC in 2018 ($P = 0.0322$) ranged from 43.0% to 70.2% and consisted of three statistical groups with decreasing value: 1) BRUNO; 2) SUN, FITZ, DRAGON, HAYWARD, CK-3, and GOLD; 3) AUTH (Figure 139). Average PCC by cultivar ($P<0.0001$) at CS in 2019 ranged from 19.6% to 63.4%, with eight statistical groups: 1) CK-3; 2) DRAGON 3) SUN; 4) FITZ; 5) HAYWARD; 6) BRUNO; 7) GOLD; 8) AUTH (Figure 140). Finally, average PCC at NAC in 2019 ($P<0.0001$) showed a range of only 5.8% to 19.2% and consisted of five statistical groups: 1) DRAGON, FITZ, CK-3; 2) SUN and BRUNO; 3) HAYWARD; 4) GOLD; 5) AUTH (Figure 141).

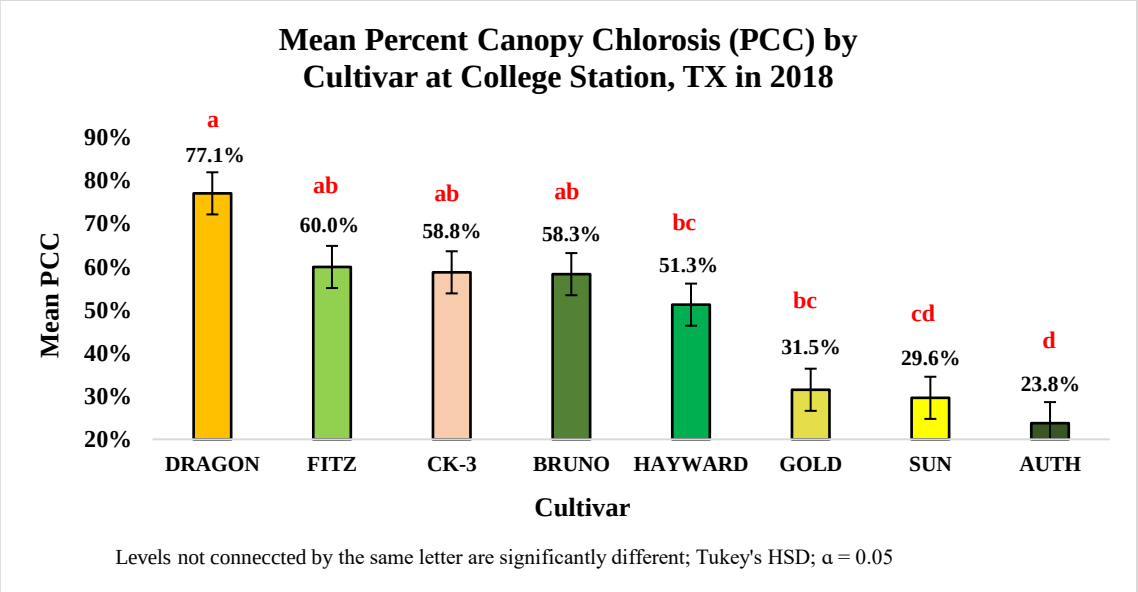


Figure 138 Comparison of mean percent canopy chlorosis (PCC) by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

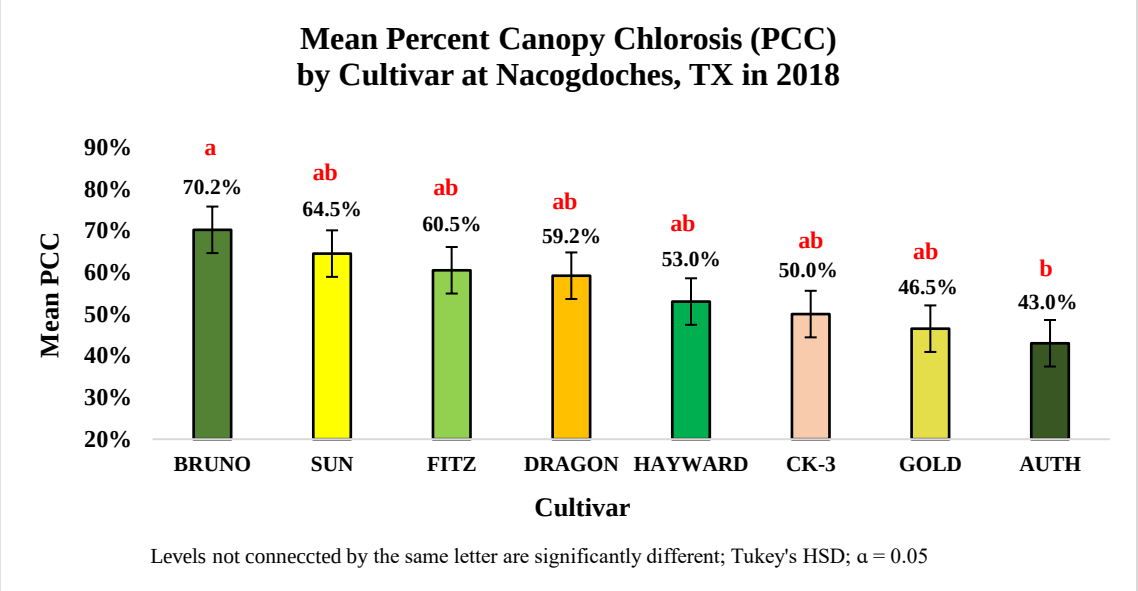


Figure 139 Comparison of mean percent canopy chlorosis (PCC) by cultivar at Nacogdoches in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

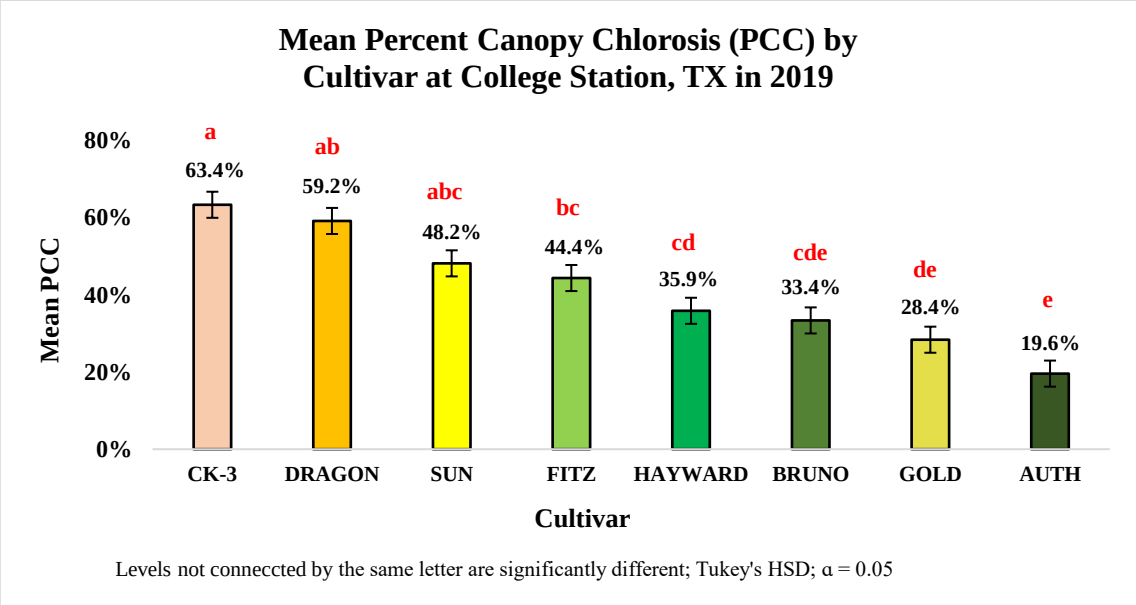


Figure 140 Comparison of mean percent canopy chlorosis (PCC) by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

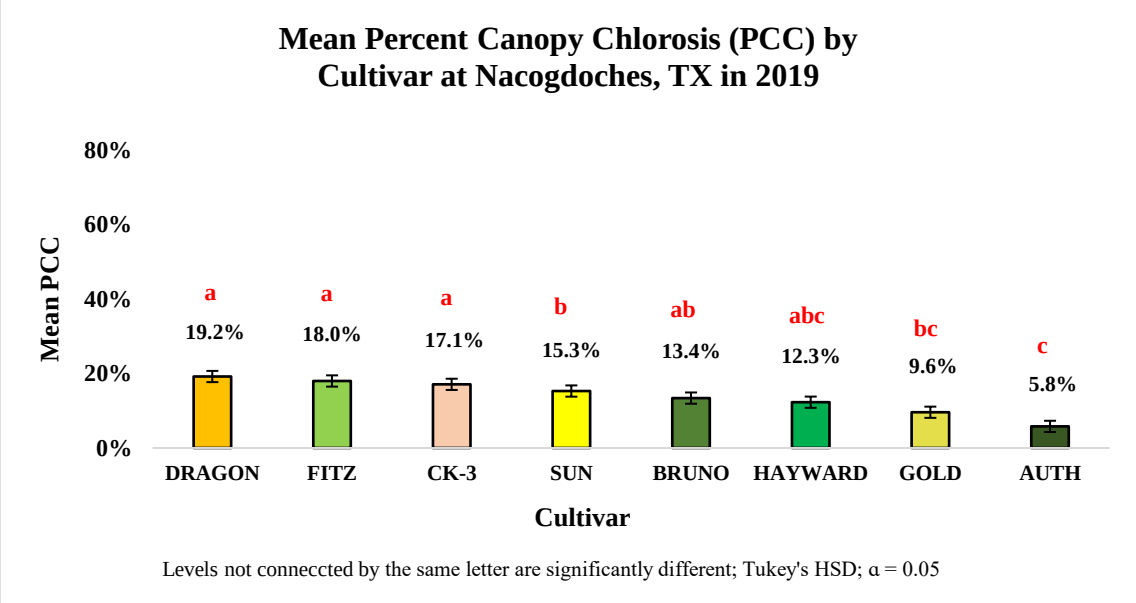


Figure 141 Comparison of mean percent canopy chlorosis (PCC) by cultivar at Nacogdoches in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

SPAD Percentage

In comparison to the previous variable, SPAD-P exhibited less variation, with cultivar means ranging from 69.2% to 80.8% across site and year. Similarly, a significant (P=0.0099) year x cultivar interaction required the comparison of cultivars necessary on a site-by-year basis.

Average SPAD-P at CS in 2018 (P<0.0001; block: P=0.0115) ranged from 38.9% to 67.5% (cultivar average) and consisted of five statistical groups, based on mean separation in descending order: 1) AUTH; 2) BRUNO, GOLD, CK-3; 3) FITZ and SUN; 4) HAYWARD; 5) DRAGON (Figure 142). At NAC in 2018, average SPAD-P (P = 0.0093; block: P=0.0163) ranged from 53.3% to 67.9% and consisted of five statistical groups: 1) CK-3; 2) DRAGON; 3) AUTH, SUN, GOLD, and FITZ; 4) HAYWARD; 5) BRUNO (Figure 143). Average SPAD-P at CS in 2019 (P = 0.0002) ranged from 58.8% to 85.0% and included three statistical groups: 1) AUTH; 2) GOLD, CK-3, and FITZ; 3) BRUNO; SUN; HAYWARD, and DRAGON (Figure 144). Lastly, average SPAD-P response to cultivar at NAC in 2019 (P<0.0001) showed a range of 56.2% to 87.9% and consisted of five statistical groups: 1) DRAGON, FITZ, CK3; 2) SUN and BRUNO; 3) HAYWARD; 4) GOLD; 5) AUTH (Figure 145).

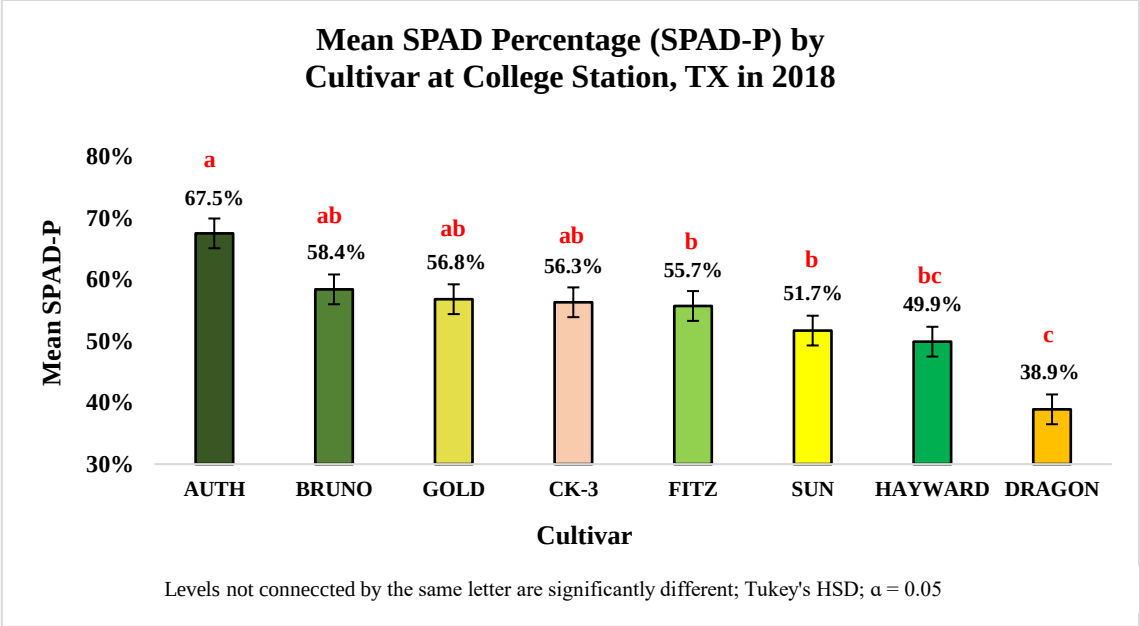


Figure 142 Comparison of mean SPAD percentage (SPAD-P) by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

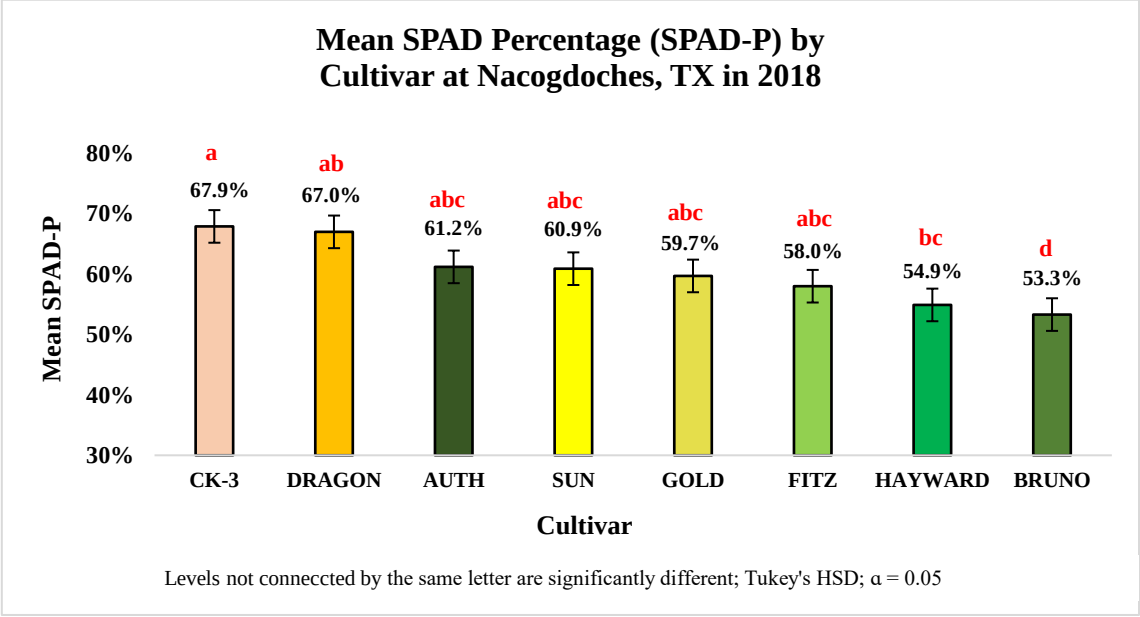


Figure 143 Comparison of mean SPAD percentage (SPAD-P) by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

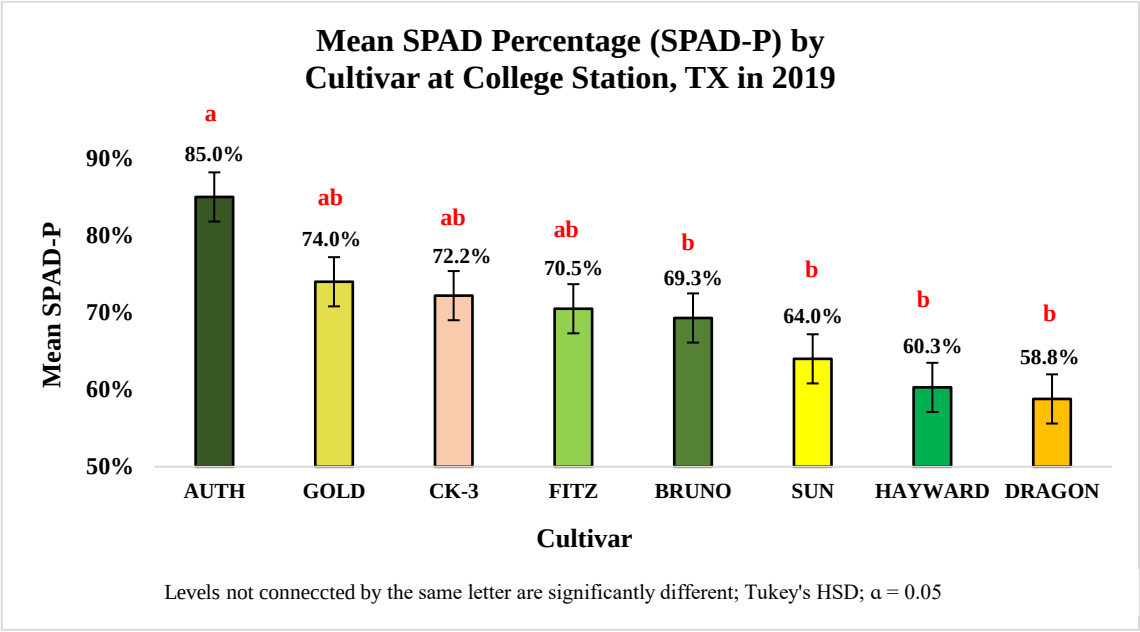


Figure 144 Comparison of mean SPAD percentage (SPAD-P) by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

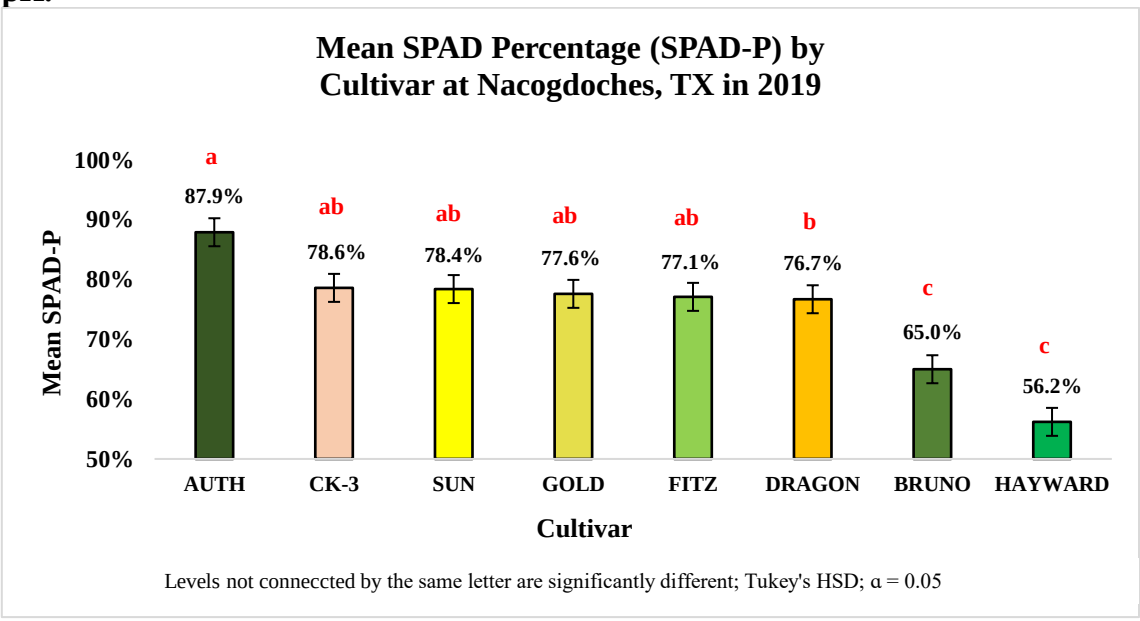


Figure 145 Comparison of mean SPAD percentage (SPAD-P) by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Chlorosis Index

CI exhibited considerable variation among cultivars, with a range of 18.0 and 39.2 across sites and years. However, significant ($P<0.0001$) site x year and site x cultivar interactions required cultivar comparison by individual site and year as with the previous two variables. CI by cultivar at CS in 2018 ($P<0.0001$; block: $P=0.0028$) ranged from 10.8 to 66.3 (cultivar means) and consisted of seven statistical groups in descending order, based on means separation: 1) DRAGON; 2) SUN; 3) CK-3 and FITZ; 4) BRUNO; 5) HAYWARD; 6) GOLD; 7) AUTH (Figure 146). Average CI at NAC in 2018 did not show a significant response to cultivar, ranging from 23.3 to 44.1 (Figure 147). Cultivar effect was significant again at CS in 2019 ($P<0.0001$), with a range of 5.6 to 33.0 and identification of seven statistical groups: 1) DRAGON; 2) SUN; 3) CK-3 and FITZ; 4) HAYWARD; 5) GOLD; 6) BRUNO; 7) AUTH (Figure 148). Even with a more limited range of 0.9 to 6.9, CI response to cultivar was significant ($P<0.0001$) at NAC in 2019, revealing seven statistical groups: 1) DRAGON; 2) SUN and FITZ; 3) CK-3; 4) GOLD; 5) HAYWARD; 6) BRUNO; 7) AUTH (Figure 149).

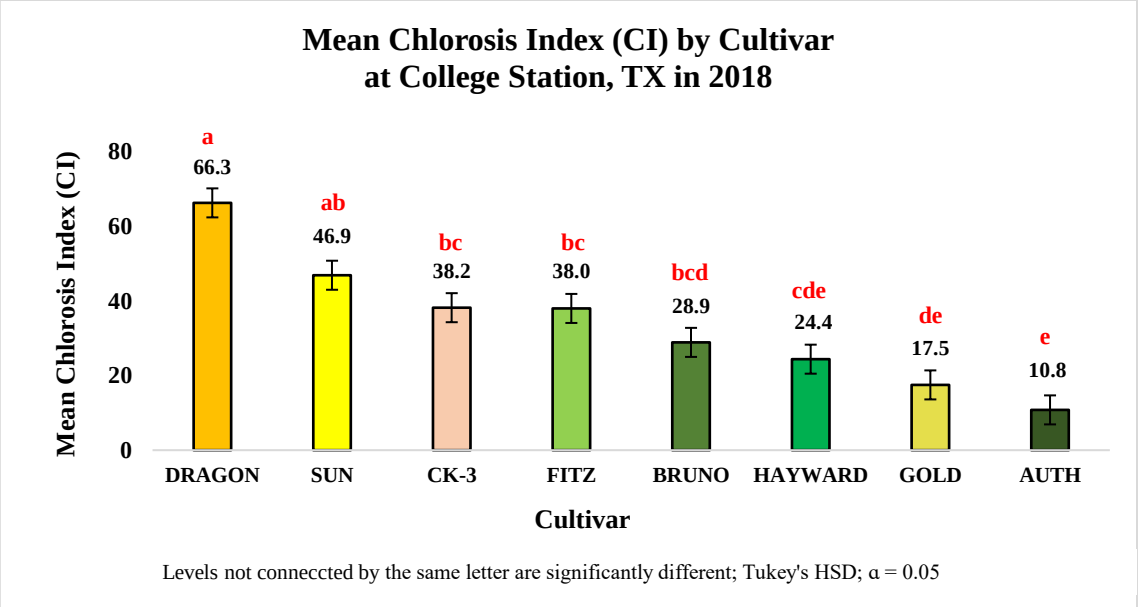


Figure 146 Comparison of mean chlorosis index (CI) by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

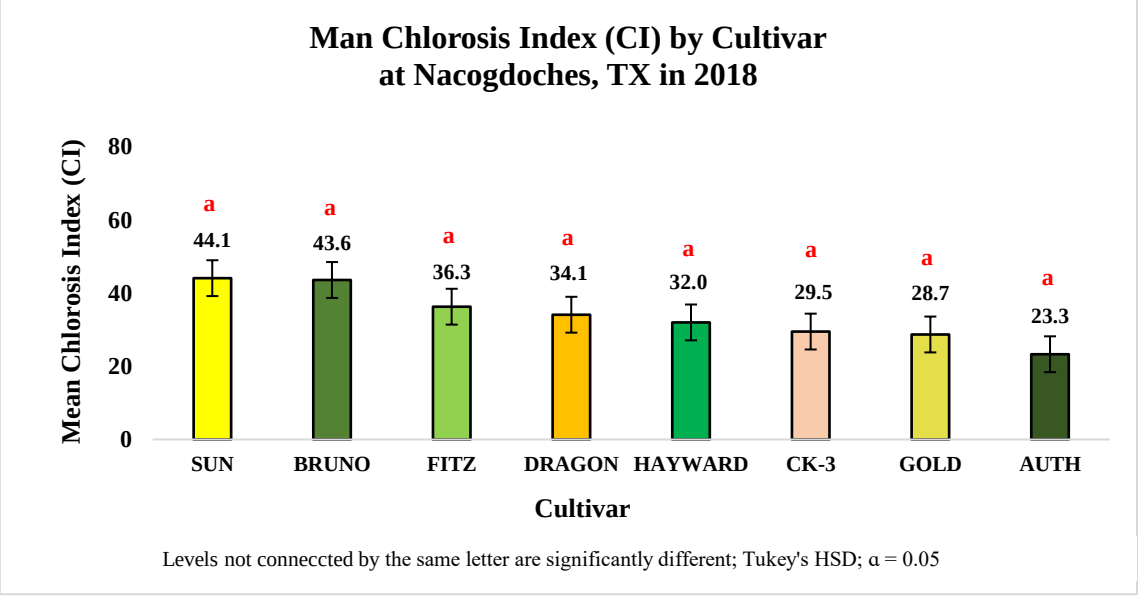


Figure 147 Comparison of mean chlorosis index (CI) by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

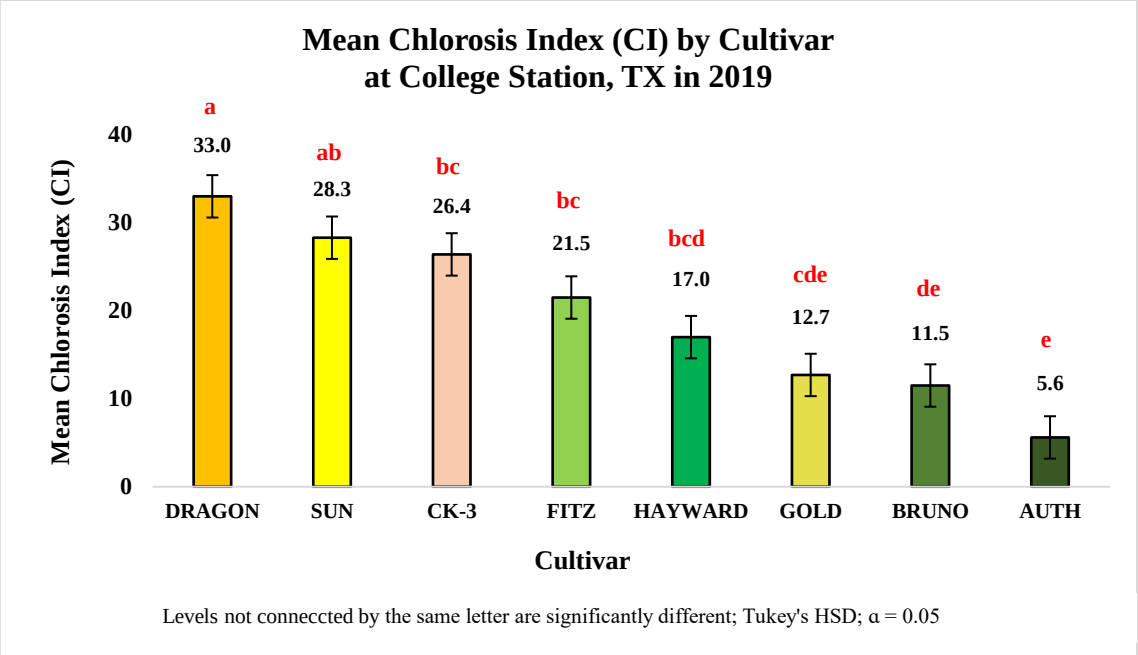


Figure 148 Comparison of mean chlorosis index (CI) by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

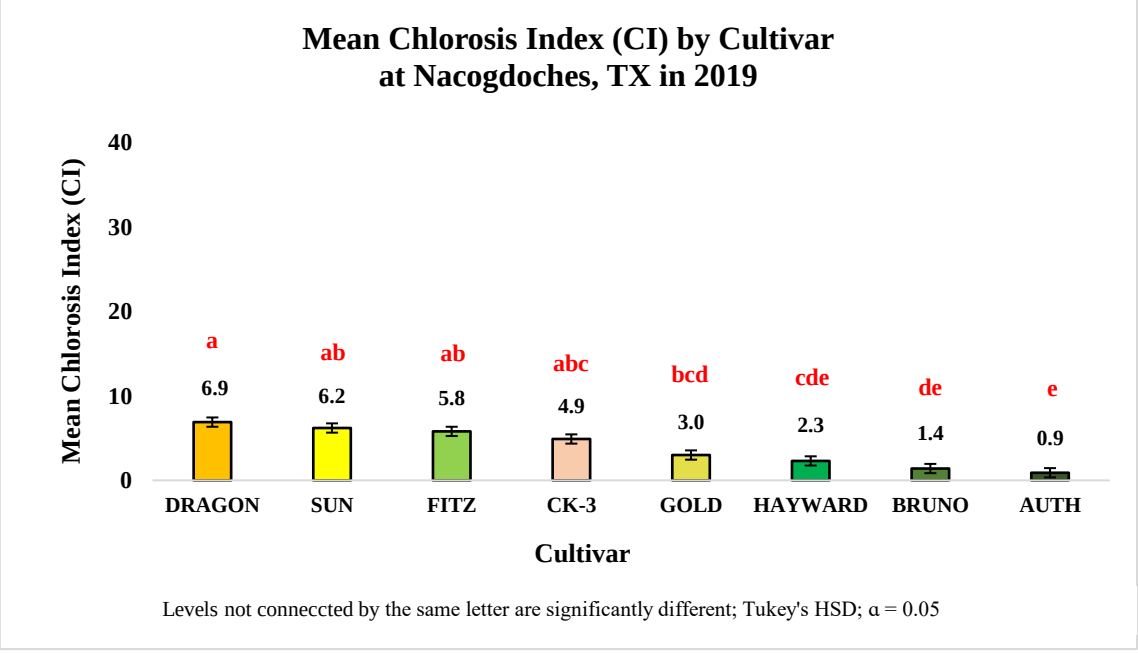


Figure 149 Comparison of mean chlorosis index (CI) by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Visual Responses by Species

Visual responses were assessed on a species level (all cultivars). Visual chlorosis symptoms response to species response to visual chlorosis symptoms over site and year, by site over years, and by site and year can be found in Figures 150-156.

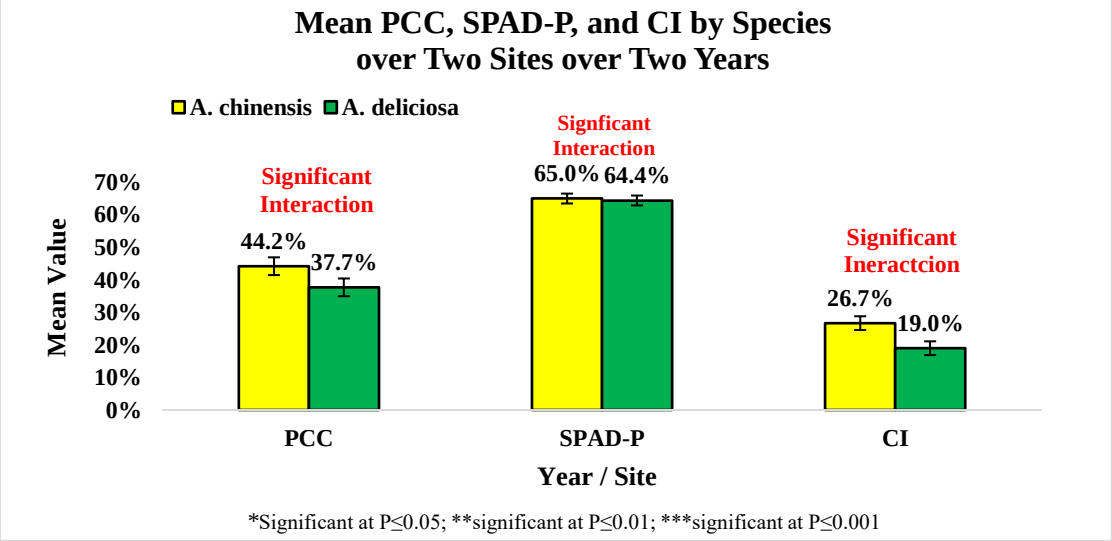


Figure 150 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to species over two sites and two years for eight kiwifruit cultivars in the assessment of response to soil pH.

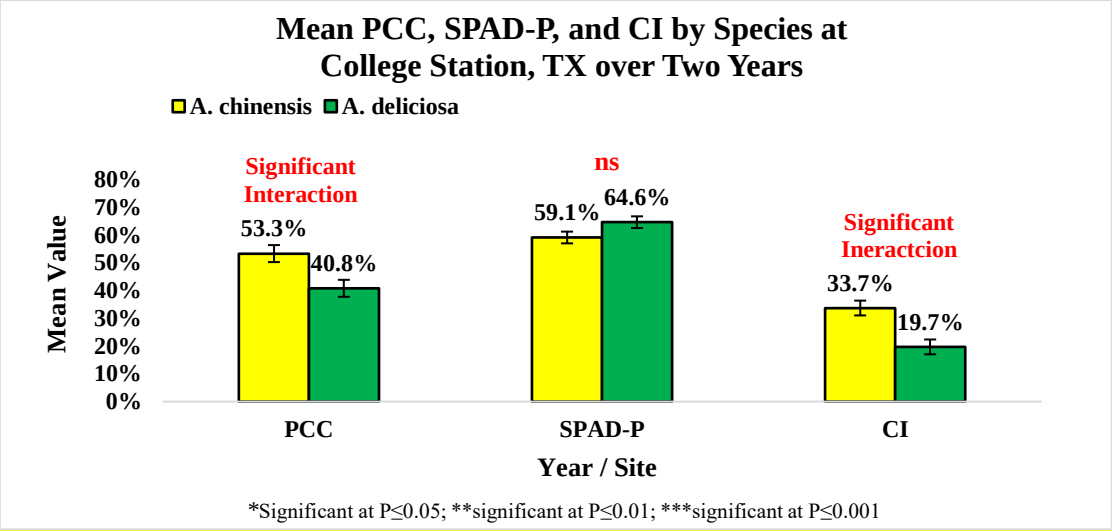


Figure 151 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to species at College Station, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

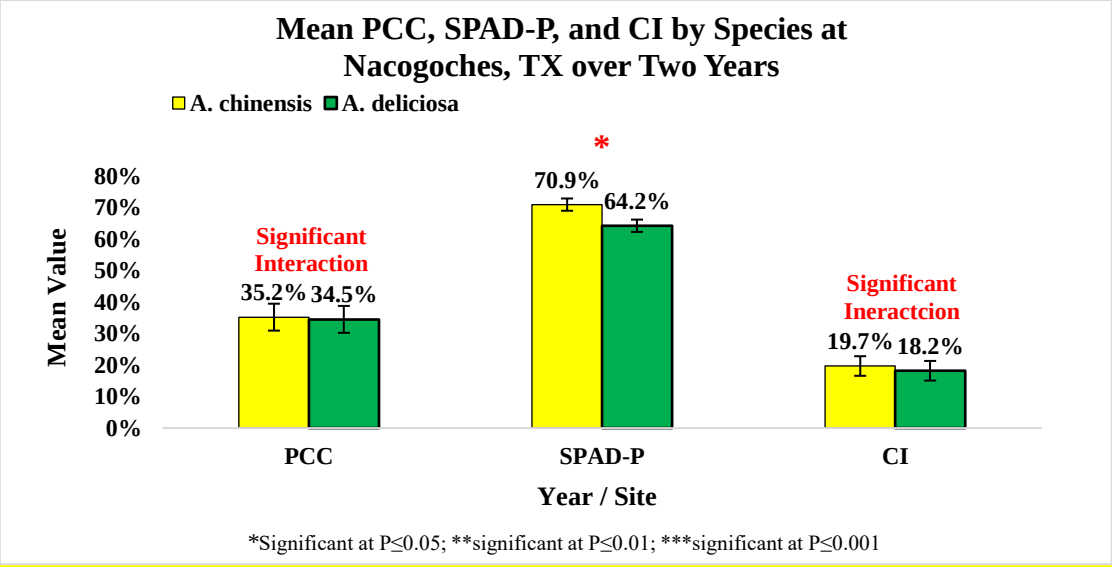


Figure 152 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to species at Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

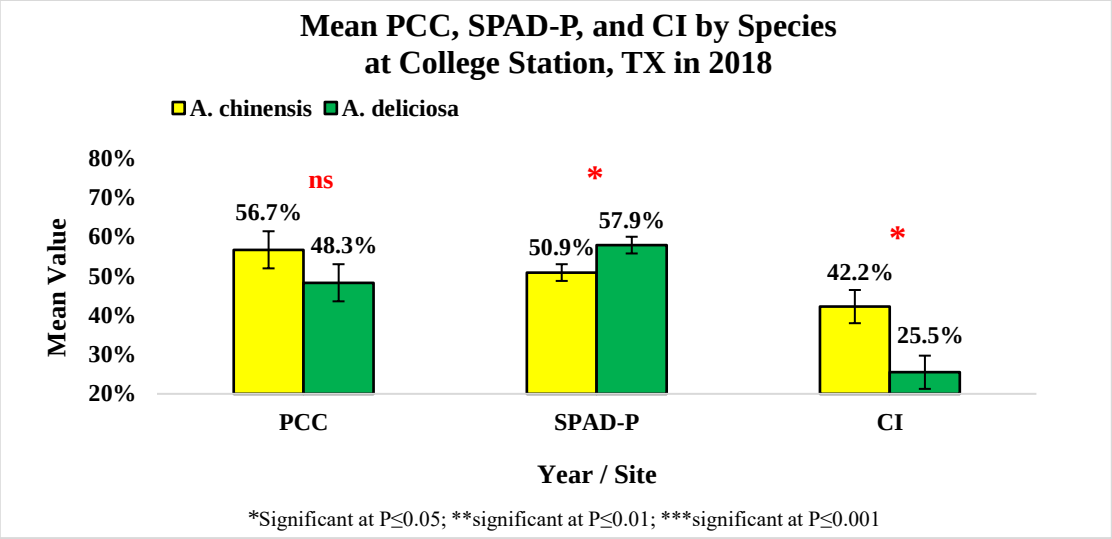


Figure 153 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to species at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

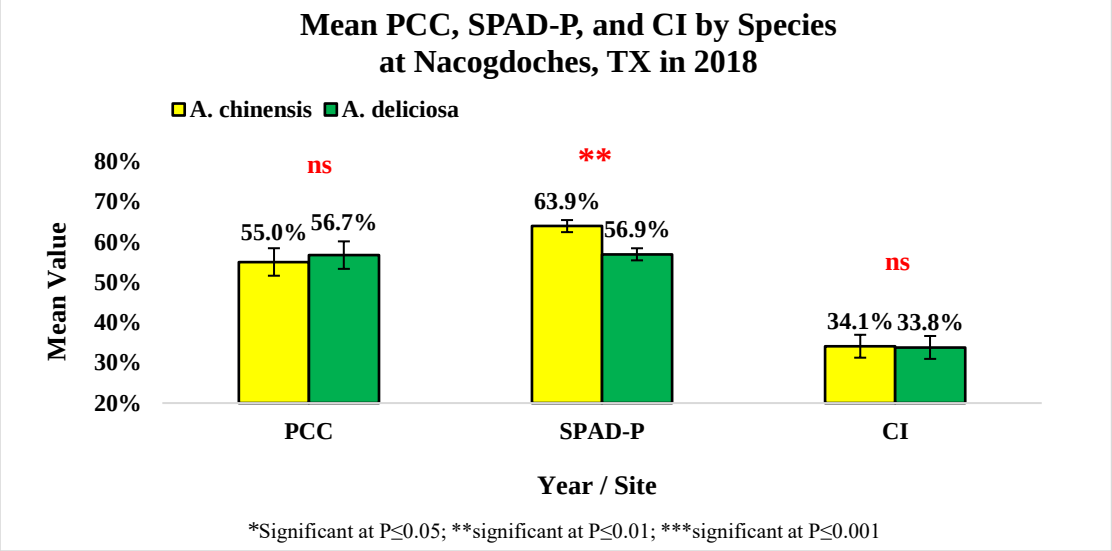


Figure 154 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to species at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

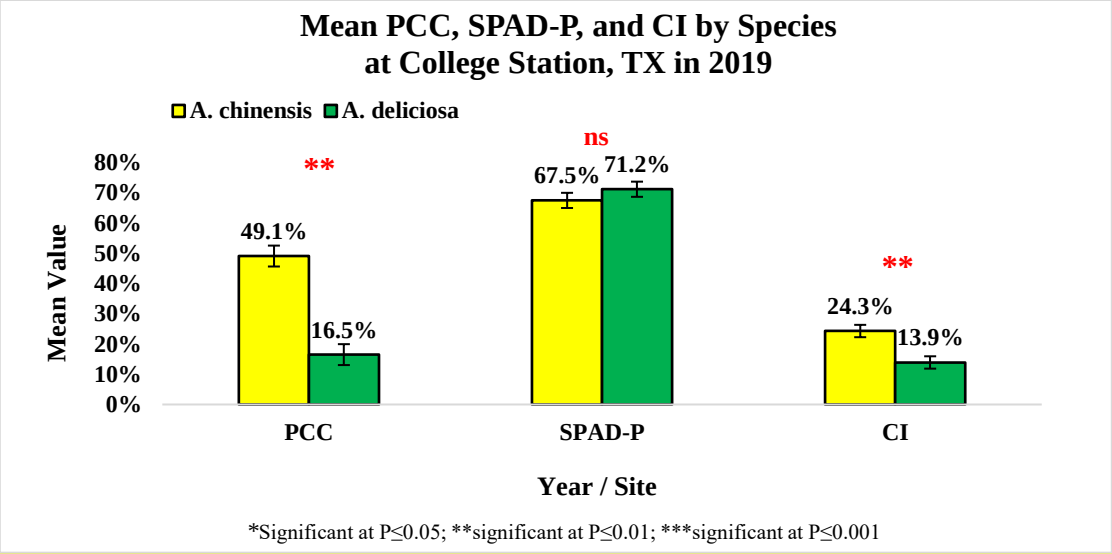


Figure 155 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to species at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

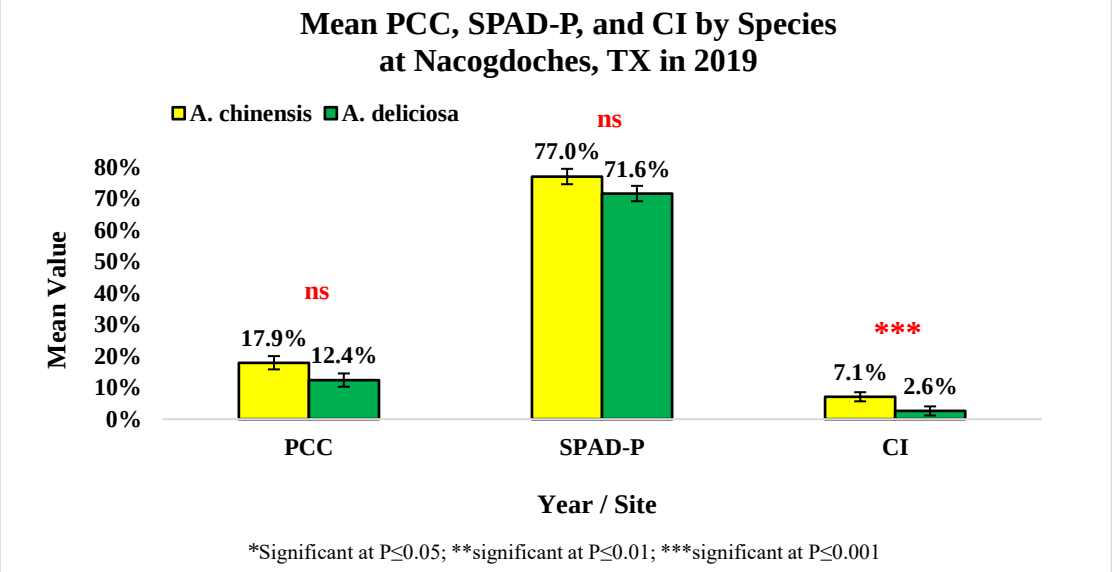


Figure 156 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to species at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Site x species interaction was significant for PCC, SPAD-P, and CI (P<0.0001; <0.0001; 0.0125), requiring comparison by individual site for these variables. Block effect (P = 0.0347) was also significant for PCC. Average SPAD-P was not significantly different between *A. chinensis* (all cultivars) (59.1%) and *A. deliciosa* (all cultivars) (64.6%) over years at CS (Figure 151). However, the average SPAD-P value of 70.9% for *A. chinensis* was significantly (P = 0.0178) higher than 64.2% for *A. deliciosa* at NAC over years 2018 (Figure 152). There was a significant (P<0.0001) site x year interaction present for PCC and CI, requiring comparison by individual year for each site for these variables (Figure 150).

Average values were higher for PCC and significantly (P = 0.0261) for CI in *A. chinensis* (56.7% and 42.2, respectively) than in *A. deliciosa* (48.3% and 25.5, respectively), whereas the 57.9% average SPAD-P for the green kiwifruit was significantly higher (P = 0.0136) than 50.9% for the golden species at CS in 2018 (Figure 153). At NAC in 2018, there was no significant difference between average PCC and CI values between *A. chinensis* (55.0% and 34.1, respectively) and *A. deliciosa* (56.7% and 33.8, respectively). However, average SPAD-P was significantly higher (P = 0.0136; block: P=0.0136) for the gold (63.9%) as compared to green (56.9%) (Figure 154). Year effect was significant for SPAD-P (P<0.0001), as the average value was higher in the second year (71.8%) than the first (64.9%). During the second year at CS, both PCC and CI (P = 0.0023 and 0.0012) were higher in *A. chinensis* (49.1% and 24.3, respectively) than in *A. deliciosa* (16.5% and 13.9, respectively) plants, while the average SPAD-P values of 67.5% and 71.2% for these species were not significantly

different (Figure 155). At NAC in 2019 average PCC and SPAD-P were higher, while average CI was significantly higher (P = 0.0009) higher in *A. chinensis* (17.9%, 77.0%, and 7.1, respectively) than in *A. deliciosa* (12.4%, 71.6%, and 2.6, respectively) (Figure 156).

Visual Responses by Propagation Method

Propagation method (clonal vs. sexual) was also compared for visual response to soil alkalinity. Visual chlorosis symptoms response to species response to visual chlorosis symptoms over site and year, by site over years, and by site and year can be found in Figures 157-163.

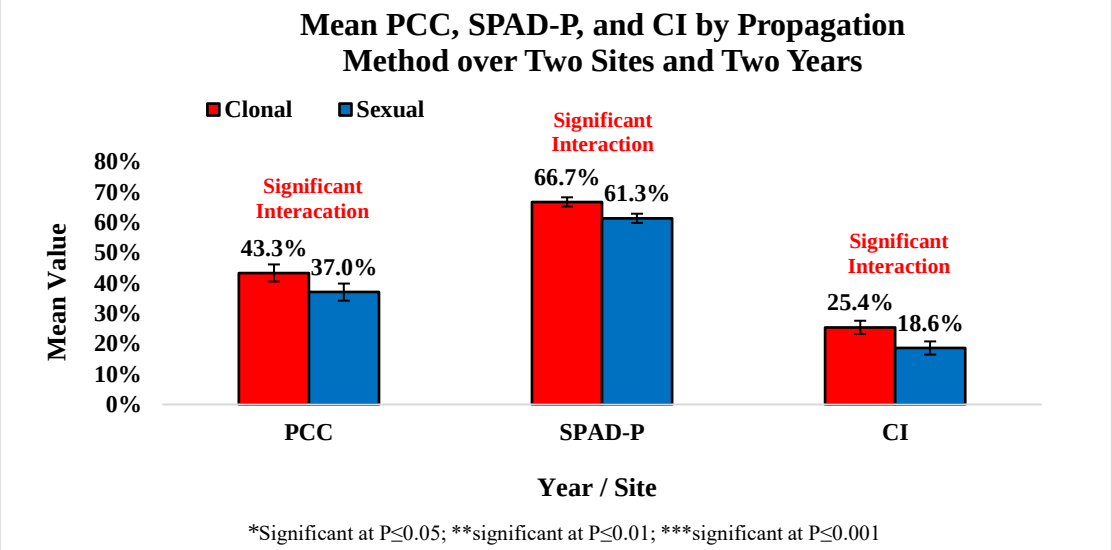


Figure 157 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to propagation method over two sites and two years for eight kiwifruit cultivars in the assessment of response to soil pH.

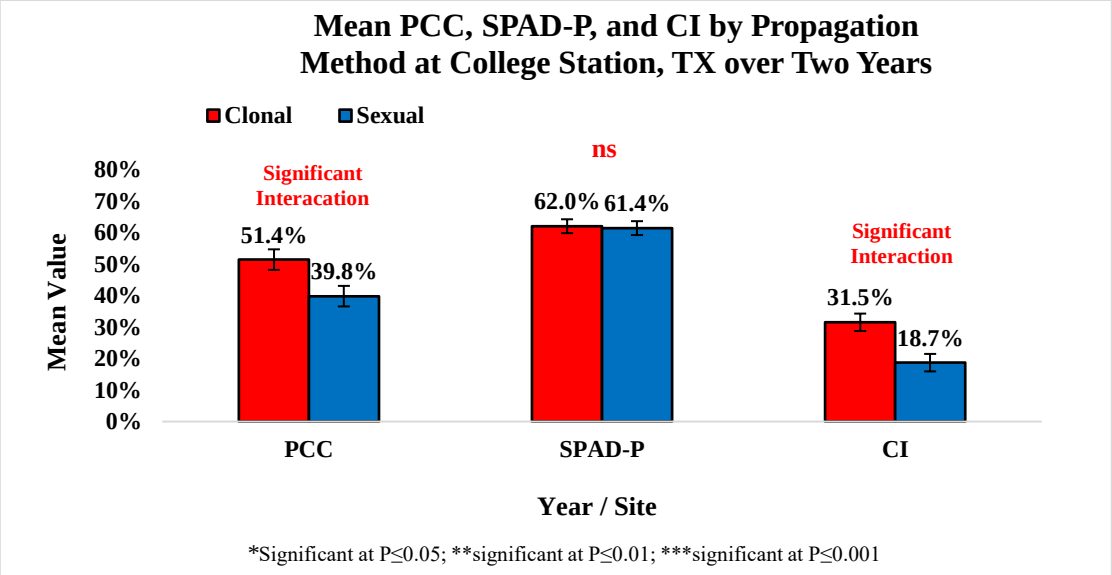


Figure 158 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to propagation method at College Station, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

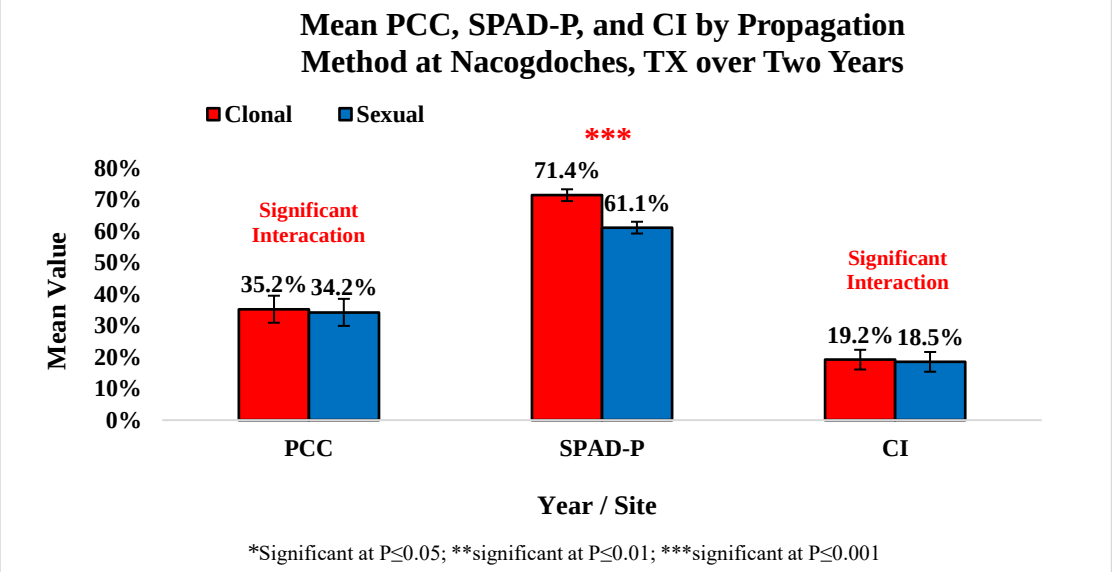


Figure 159 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to propagation method at Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

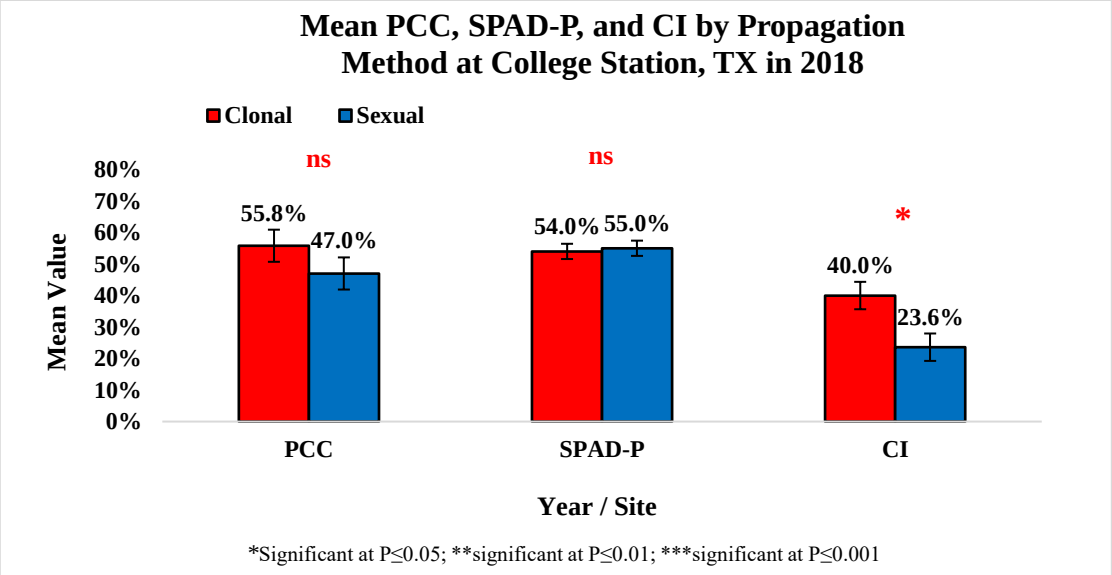


Figure 160 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to propagation method at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

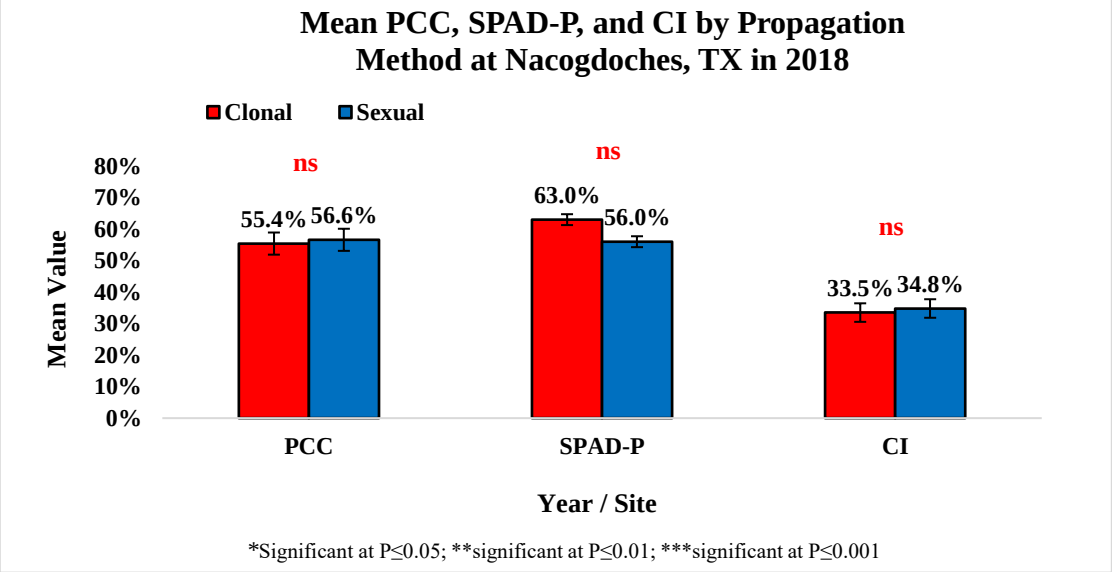


Figure 161 Figure 161. Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to propagation method at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

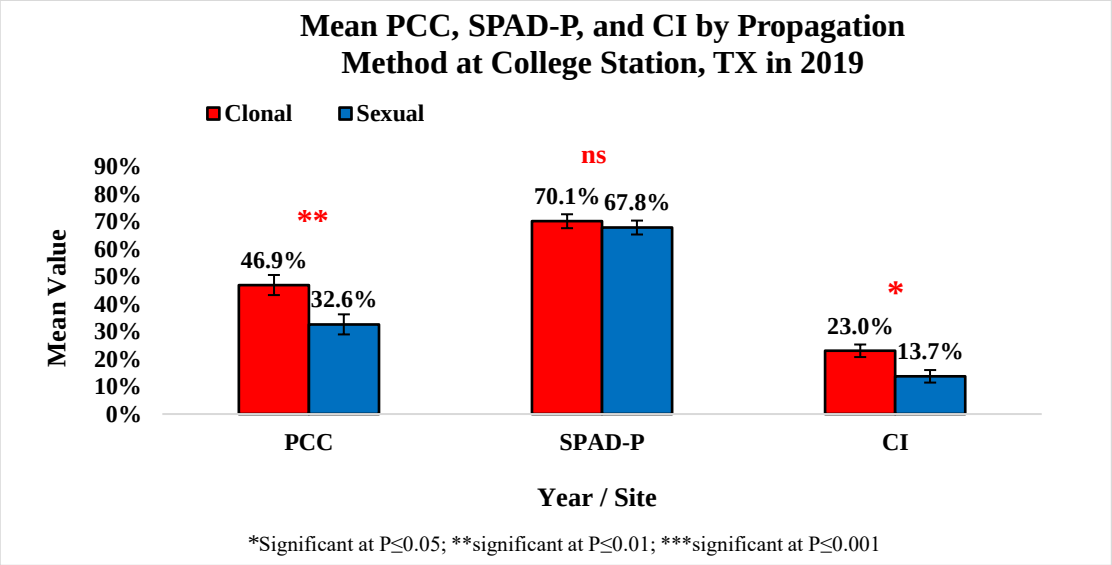


Figure 162 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to propagation method at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

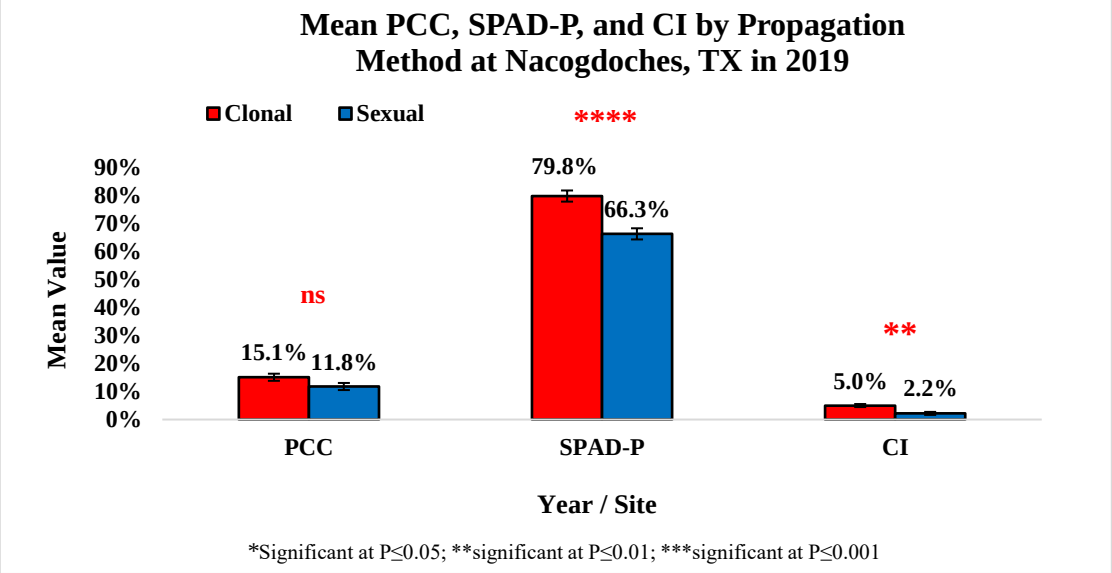


Figure 163 Comparison of mean percent canopy chlorosis (PCC), mean SPAD percentage (SPAD-P), and mean chlorosis index (CI) response to propagation method at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Comparison of Physiological Responses

As discussed earlier, gas exchange measurements were conducted only on the cultivars AUTH and DRAGON. There was a significant site x year interaction for photosynthesis (*PS*) and transpiration (*E*) (P = 0.0325; <0.0001), requiring comparison of site by individual year (Figure 164). Additionally, site x cultivar interaction was present for stomatal conductance (*gs*) and *E* (P = 0.0077; 0.002), requiring that comparisons for these parameters be made between cultivar. Lastly, there was a significant (P = 0.0268) site x year x cultivar observed for *E*. *PS* was significantly (P<0.0001; 0.026) higher at CS in 2018 and 2019 (16.27 and 15.10 µmol CO_2 m^{-2} s^{-1}, respectively) as compared to NAC (11.40 and 12.72 µmol CO_2 m^{-2} s^{-1}), respectively) (Figures 165 & 166).

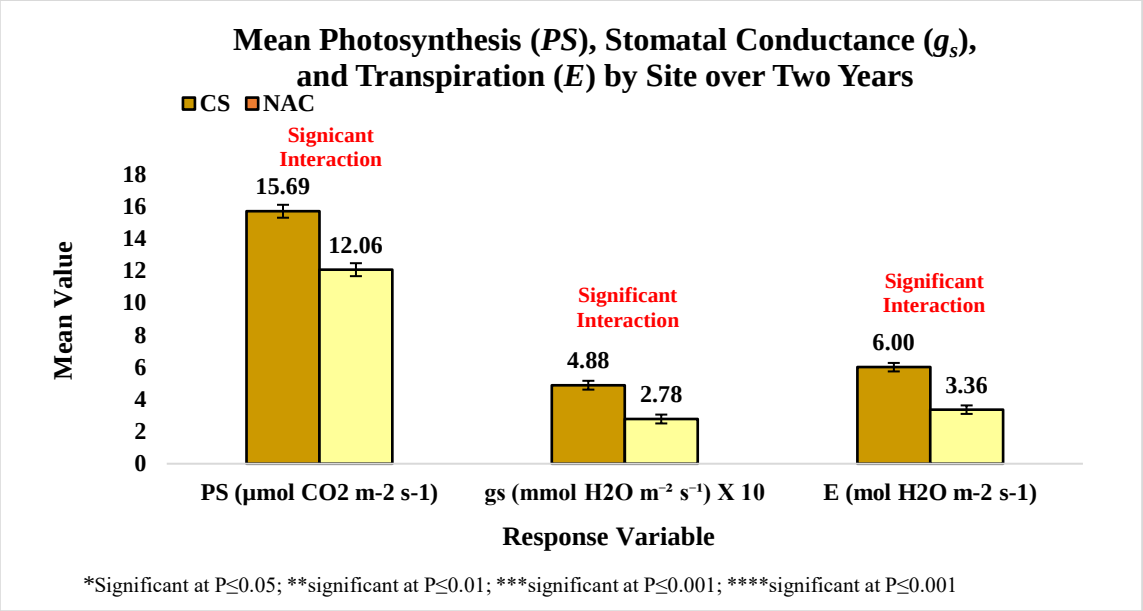


Figure 164 Comparison of mean photosynthesis (PS), mean stomatal conductance (gs), and mean transpiration (E) by site for two kiwifruit cultivars over two years in the assessment of response to soil pH.

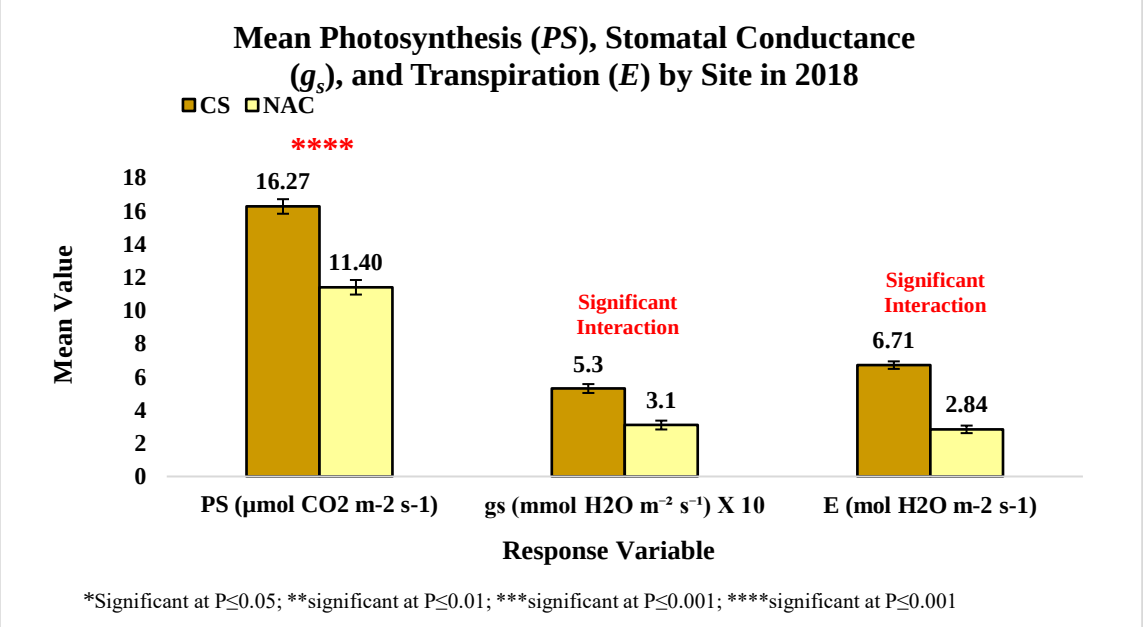


Figure 165 Comparison of mean photosynthesis (PS), mean stomatal conductance (gs), and mean transpiration (E) by site for two kiwifruit cultivars in 2018 in the assessment to response of soil pH.

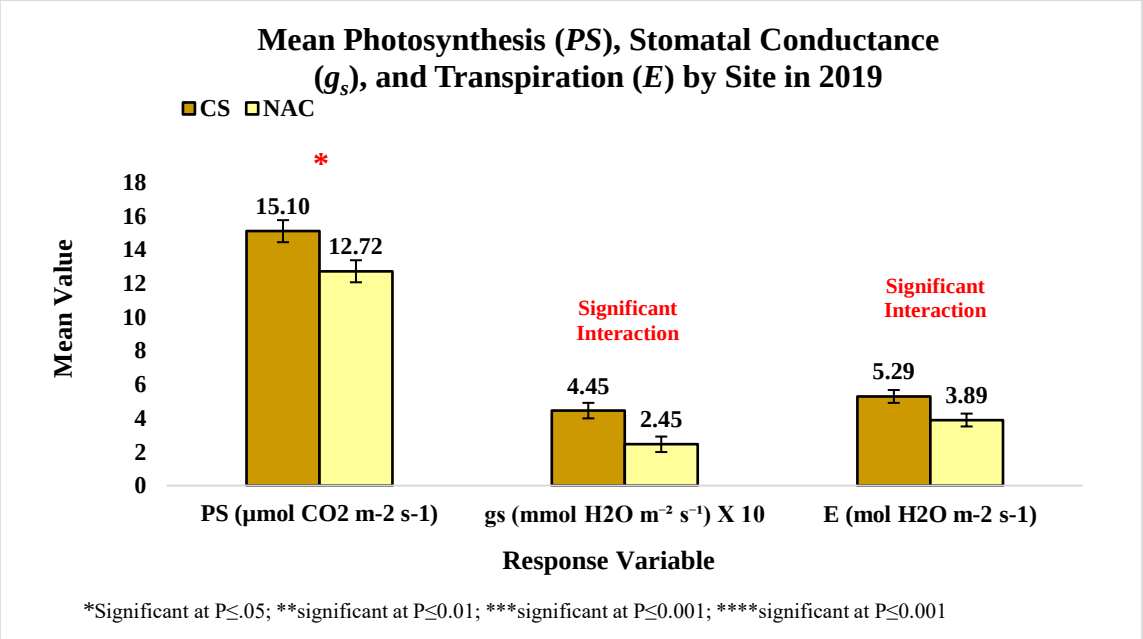


Figure 166 Comparison of mean photosynthesis (PS), mean stomatal conductance (gs), and mean transpiration (E) by site for two kiwifruit cultivars in 2019 in the assessment of response to soil pH.

There was no significant response to cultivar for *PS* or *E* at either site during either year (Tables 49 & 50). The same was also true for g_s, except for at CS in 2019 where AUTH produced a significantly (P = 0.0022) lower value (0.320 mol H_2O m^{-2} s^{-1}) as compared to DRAGON (0.570 mol H_2O m^{-2} s^{-1}) (Table 51).

Table 49 Comparison of mean photosynthesis (*PS*) between two kiwifruit cultivars across four environments in the assessment of response to soil pH.

Environment (Site / Year)	'AU Authur'		'AU Golden Dragon'		Standard Error	Significance
	Mean (μmol CO_2 m^{-2} s^{-1})	Standard Deviation	Mean (μmol CO_2 m^{-2} s^{-1})	Standard Deviation		
CS / 2018	16.09	0.547	16.45	0.547	0.387	ns
NAC / 2018	11.35	1.072	11.45	1.072	0.758	ns
CS / 2019	14.95	0.879	15.25	0.879	0.621	ns
NAC / 2019	13.68	0.960	11.76	0.960	0.679	ns
Student t-Test between cultivar for each environment (α = 0.05).						

Table 50 Comparison of mean transpiration (*E*) between two kiwifruit cultivars across four environments in the assessment of response to soil pH.

Environment (Site / Year)	'AU Authur'		'AU Golden Dragon'		Standard Error	Significance
	Mean (mmol H_2O m^{-2} s^{-1})	Standard Deviation	Mean (mmol H_2O m^{-2} s^{-1})	Standard Deviation		
CS / 2018	6.52	0.629	6.91	0.629	0.445	ns
NAC / 2018	2.91	0.334	2.77	0.334	0.237	ns
CS / 2019	4.40	0.284	6.18	0.284	0.201	ns
NAC / 2019	4.36	0.480	3.41	0.480	0.339	ns
Student t-Test between cultivar for each environment (α = 0.05).						

Table 51 Comparison of mean stomatal conductance (g_s) between two kiwifruit cultivars across four environments in the assessment of response to soil pH.

Environment (Site / Year)	'AU Authur'		'AU Golden Dragon'		Standard Error	Significance
	Mean (mol H_2O m^{-2} s^{-1})	Standard Deviation	Mean (mol H_2O m^{-2} s^{-1})	Standard Deviation		
CS / 2018	0.526	0.0453	0.535	0.0453	0.0321	ns
NAC / 2018	0.318	0.0328	0.303	0.0328	0.0232	ns
CS / 2019	0.320	0.0361	0.570	0.0361	0.0255	P=0.0022
NAC / 2019	0.275	0.0252	0.215	0.0252	0.0178	ns
Student t-Test between cultivar for each environment (α = 0.05). Analysis based on natural log-transformed data.						

Comparison of Plant Growth Responses

Leaf Weight and Pruning Weight by Site

Comparison of average leaf weight (LW) was complicated by significant (P<0.0001) site x year, site x cultivar year x cultivar, and site x year x cultivar interactions (Figure 167). There was also a significant (P = 0.002) site x year interaction present for pruning weight (PW), requiring comparison of site by individual year. Significant block effect (P = 0.0471) was also present for PW. Ave PW (dry weight) was significantly greater in 2018 and 2019 (P = 0.0016; 00003) at NAC (40.2 g and 152.9 g, respectively) than at CS (23.0 g and 101.2 g, respectively) (Figure 168).

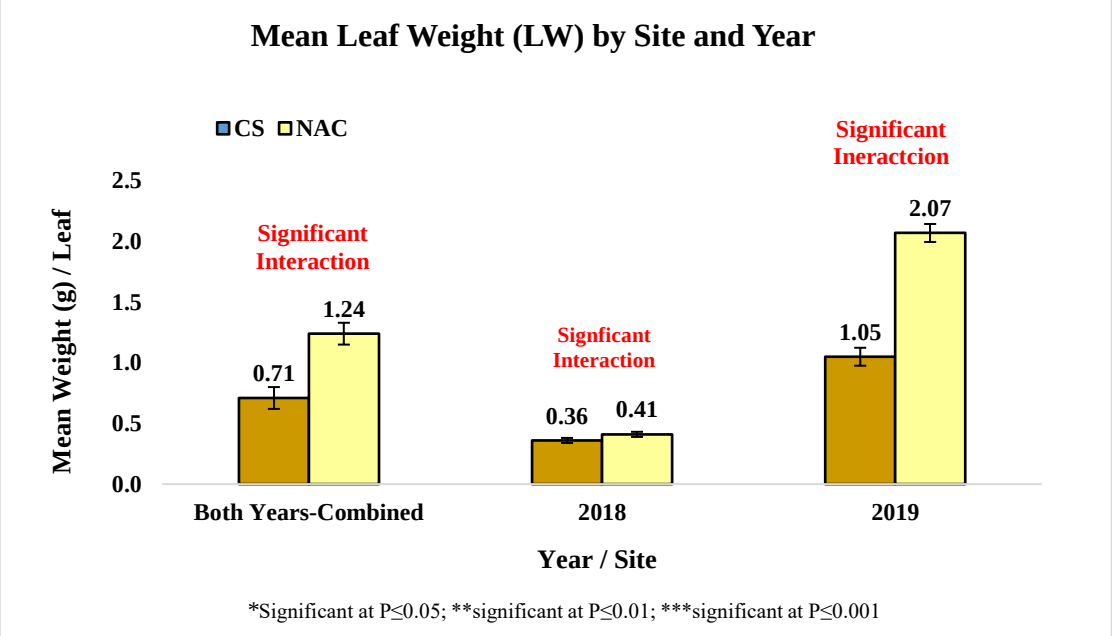


Figure 167 Comparison of mean leaf weight (LW) by site for 2018-2019, 2018, and 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

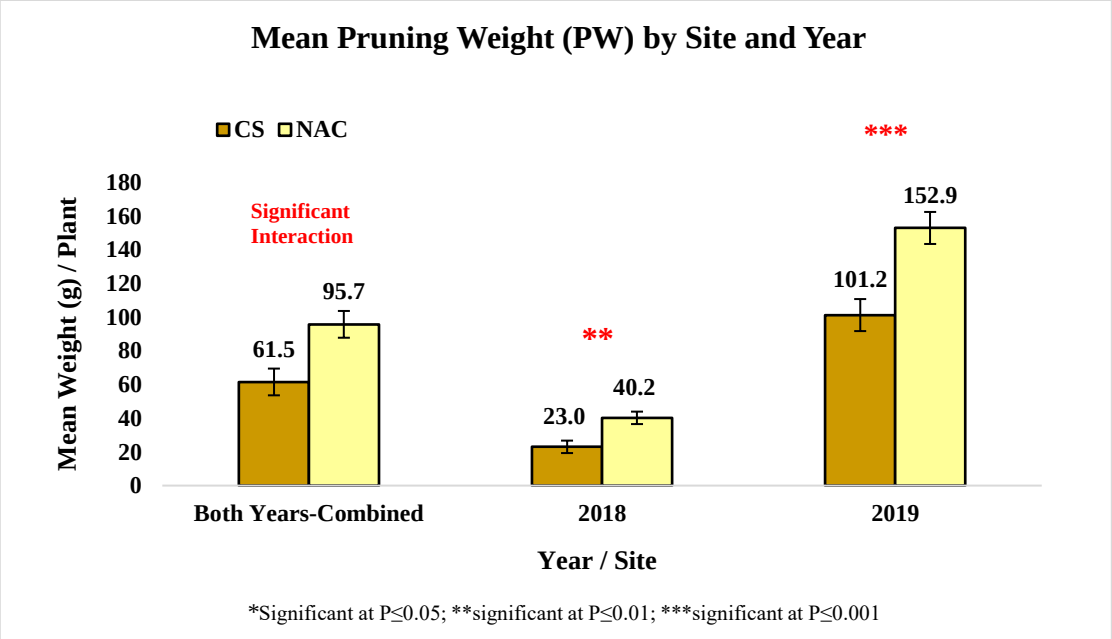


Figure 168 Comparison of mean pruning weight (PW) by site for 2018-2019, 2018, and 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Leaf and Pruning Weight by Cultivar

Leaf Weight

Average LW at CS in 2018 (P<0.0001) ranged from 0.18 g to 0.52 g (cultivar average) and consisted of five statistical groups in descending order, based on means separation: 1) GOLD and HAYWARD; 2) BRUNO and FITZ; 3) AUTH; 4) SUN and DRAGON; 5) CK-3 (Figure 169). At NAC during the same year, average LW (P<0.0001) had a range of 0.26 g and 0.52 g and separated into four statistical groups: 1) HAYWARD, GOLD, and BRUNO; 2) FITZ, AUTH, and SUN; 3) DRAGON; 4) CK-3 (Figure 170). Leaf-size was much larger during the second year. Ave LW ranged from 0.68 g to 1.44 g at CS in 2019 (P<0.0001) with five statistical groups: 1) GOLD; 2)

HAYWARD, BRUNO, AUTH; 3) FITZ; 4) SUN; 5) CK-3 and DRAGON (Figure 171). At NAC during the second year average LW (P = 0.0002; block: P=0.0487) had a range of 1.67 g and 2.76 g and separated into two statistical groups: 1) BRUNO, HAYWARD; 2) AUTH, FITZ, GOLD, SUN, CK-3, and DRAGON (Figure 172). Over years, average LW ranged from 0.44 g to 0.98 g at CS and from 0.99 g to 1.63 g at NAC for individual cultivars (Table 52).

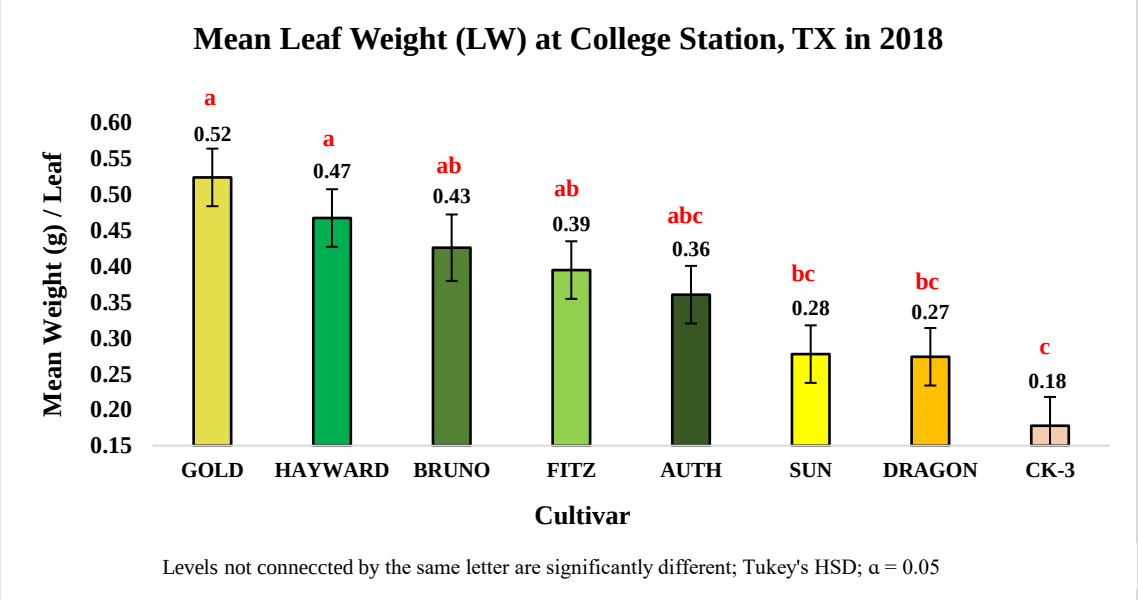


Figure 169 Mean leaf weight (LW) by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

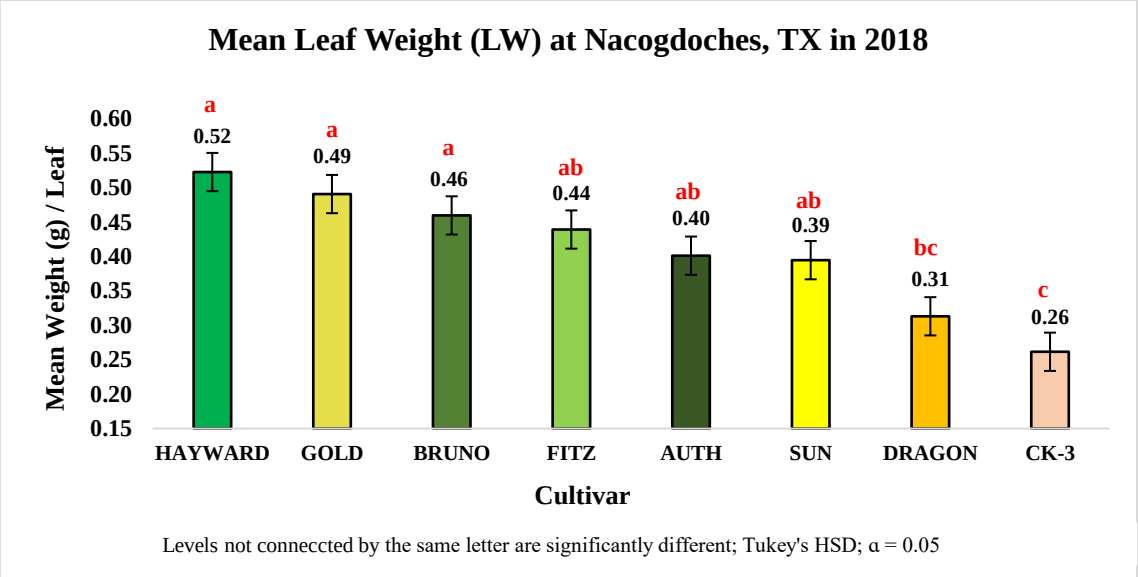


Figure 170 Mean leaf weight (LW) by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

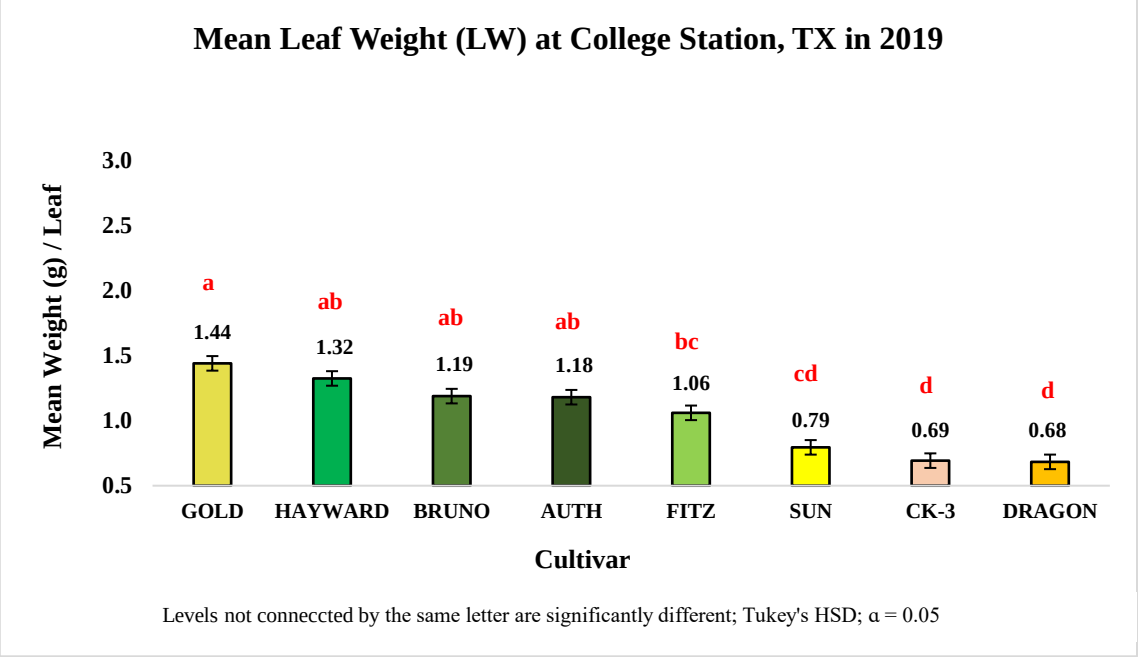


Figure 171 Mean leaf weight (LW) by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

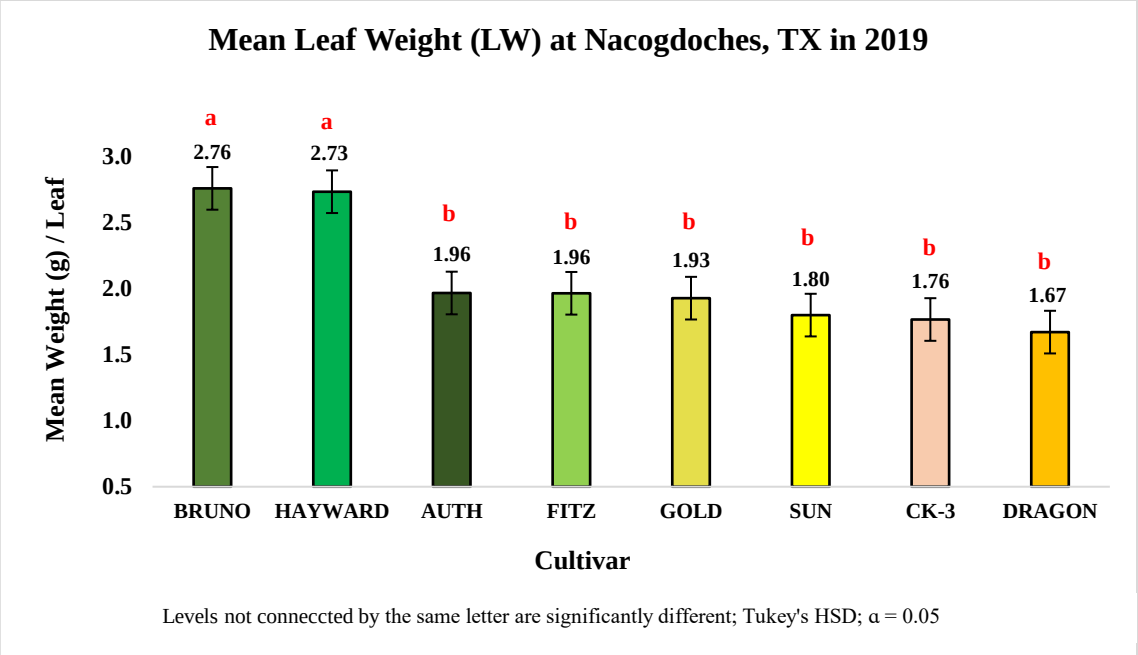


Figure 172 Mean leaf weight (LW) by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 52 Comparison of mean leaf weight (LW) by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean	Standard Deviation	Standard Error	Cultivar	Mean	Standard Deviation	Standard Error
GOLD	0.98 g	0.501	0.137	HAYWARD	1.63 g	1.204	0.339
HAYWARD	0.90 g	0.468	0.137	BRUNO	1.61 g	1.254	0.339
BRUNO	0.86 g	0.418	0.146	GOLD	1.21 g	0.787	0.339
AUTH	0.77 g	0.456	0.137	FITZ	1.20 g	0.847	0.339
FITZ	0.73 g	0.364	0.137	AUTH	1.18 g	0.862	0.339
SUN	0.54 g	0.284	0.137	SUN	1.10 g	0.766	0.339
DRAGON	0.48 g	0.223	0.137	CK-3	1.01 g	0.861	0.339
CK-3	0.44 g	0.284	0.137	DRAGON	0.99 g	0.739	0.339

Significant (P<0.0001) site x year interaction; significant (P<0.0001) site x cultivar interaction; significant (P<0.0001 year x cultivar interaction; significant (P<0.0001) site x year x cultivar interaction present for leaf weight.

Pruning Weight

Average PW at CS in 2018 (P<0.0001; block: P=0.003) ranged from 6.6 g to 47.7 g per plant and consisted of four statistical groups in descending order: 1) GOLD; 2) AUTH and BRUNO; 3) FITZ, HAYWARD, CK-3, and SUN; 4) DRAGON (Figure 173). At NAC in 2018 average PW (P = 0.0118) had a range of 18.7 g and 75.2 g and consisted of three statistical groups: 1) GOLD; 2) AUTH, HAYWARD, FITZ, and DRAGON; 3) CK-3, BRUNO, and SUN (Figure 174). The average dry weight per plant at CS in 2019 (P = 0.0028) ranged from 56.3 g to 17.6 g and consisted of three statistical groups: 1) GOLD; 2) AUTH, BRUNO, and HAYWARD; 3) CK-3, FITZ, SUN, and DRAGON (Figure 175). Ave PW did not significantly vary by cultivar at NAC during the second year, although cultivar averages varied from 102.4 g to 212.0 g (Figure 176). PW response to cultivar was not significant at either site across years, where average cultivar values ranged from 27.9 g to 113.6 g at CS and from 60.5 g to 143.6 g at NAC (Table (53).

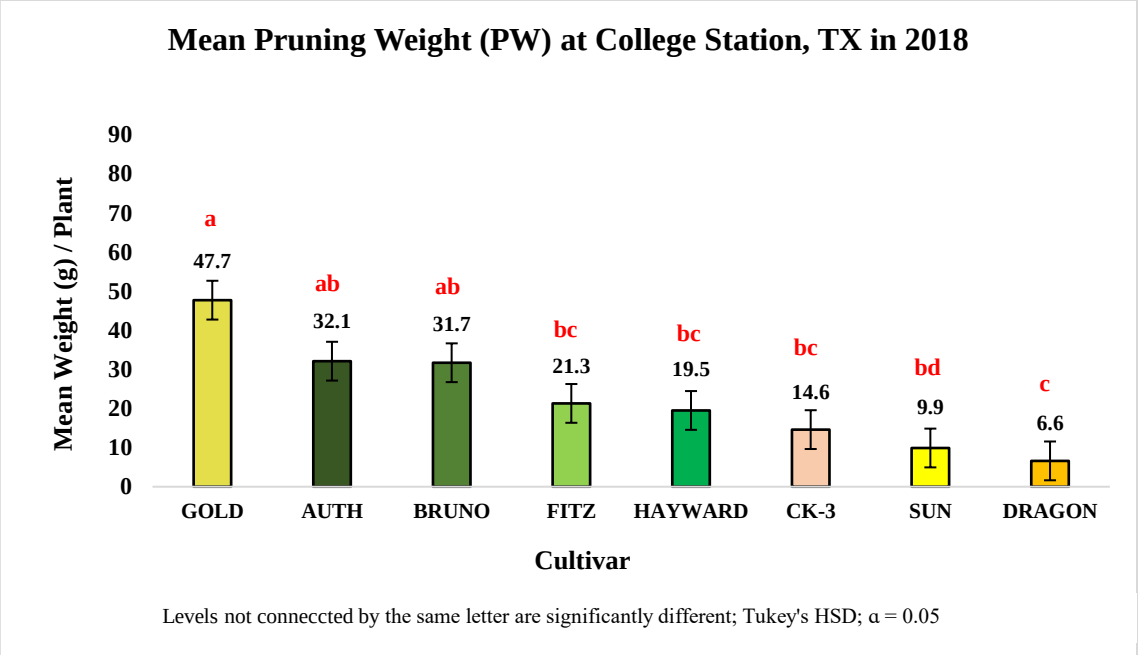


Figure 173 Mean pruning weight (PW) by cultivar at College Station, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

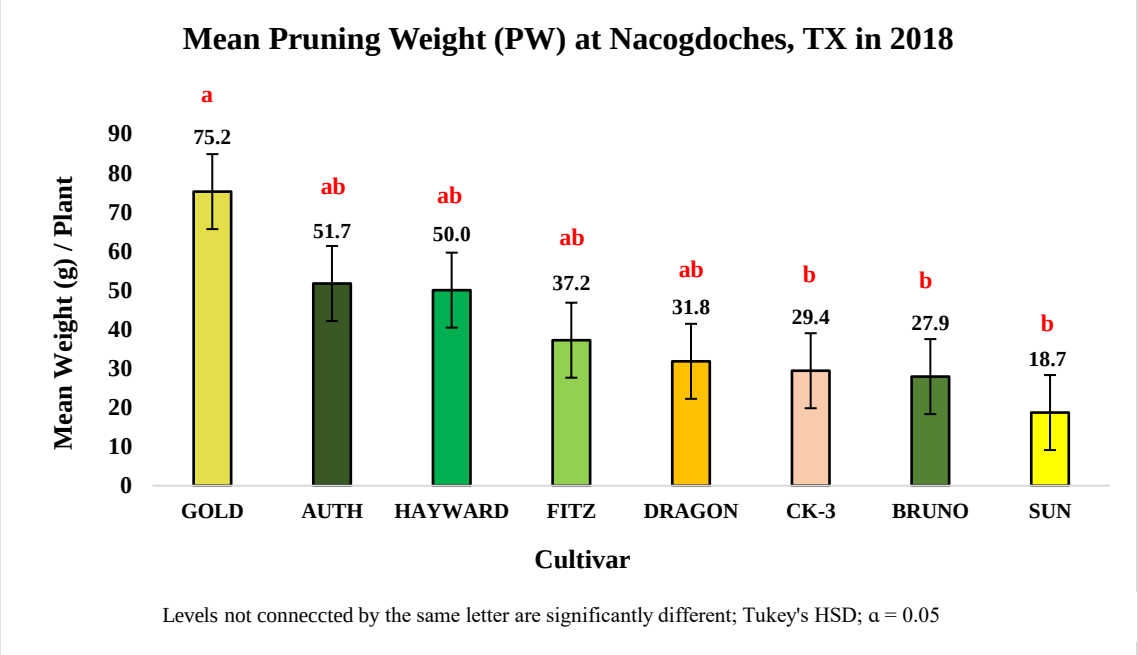


Figure 174 Mean pruning weight (PW) by cultivar at Nacogdoches, TX in 2018 for eight kiwifruit cultivars in the assessment of response to soil pH.

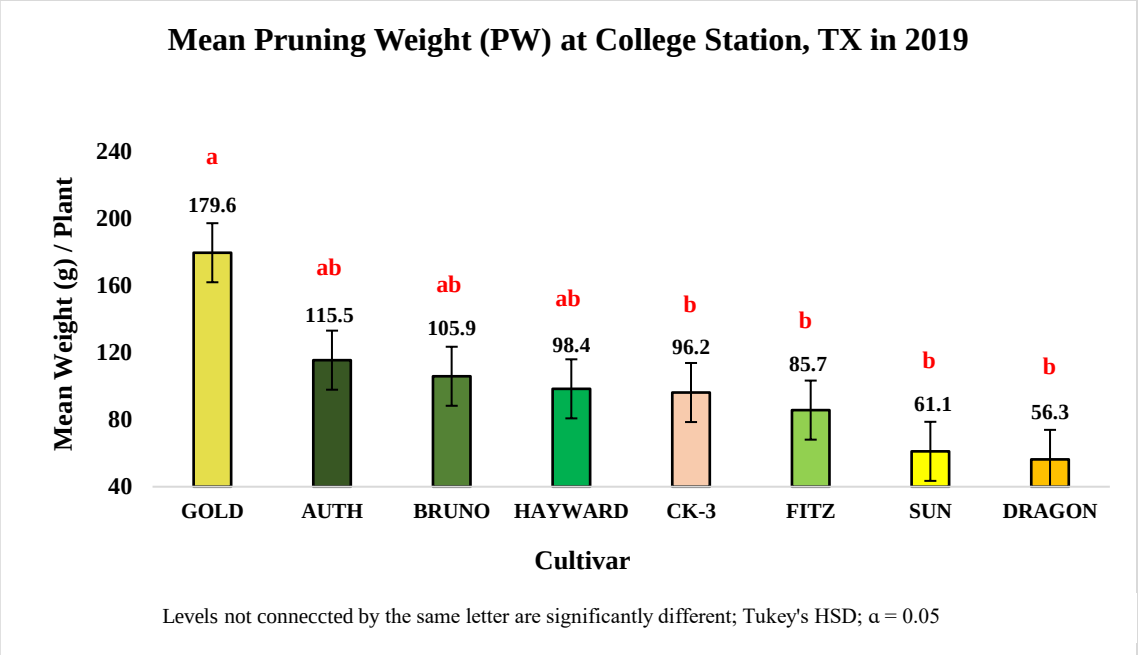


Figure 175 Mean pruning weight (PW) by cultivar at College Station, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

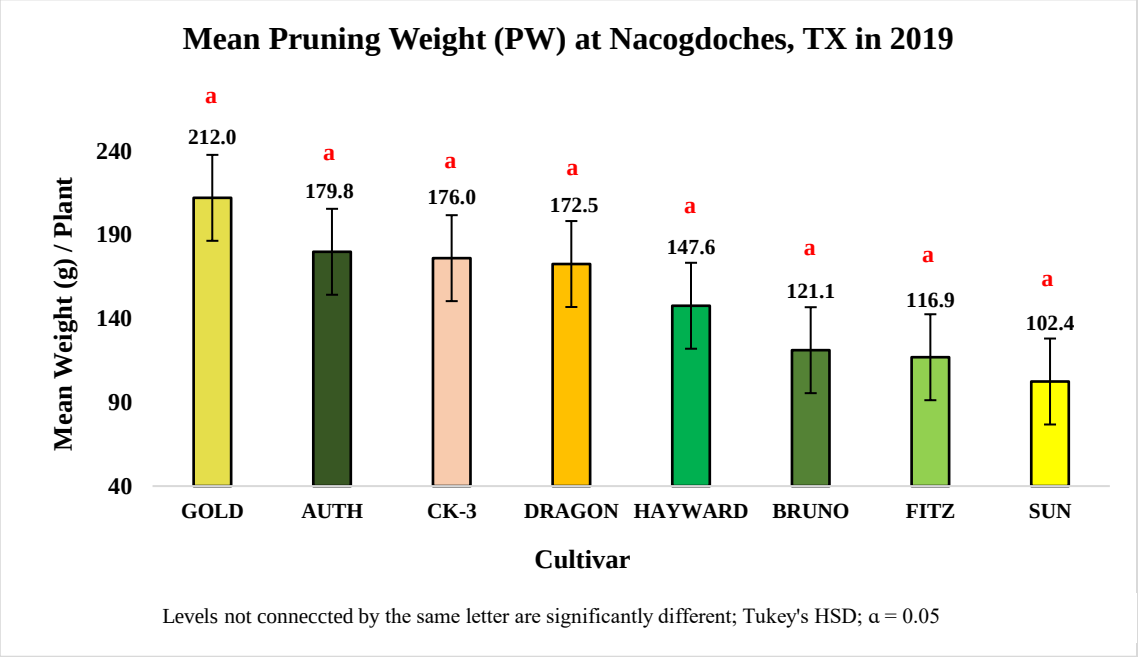


Figure 176 Mean pruning weight (PW) by cultivar at Nacogdoches, TX in 2019 for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 53 Comparison of mean pruning weight (PW) by cultivar at College Station, TX and Nacogdoches, TX over two years for eight kiwifruit cultivars in the assessment of response to soil pH.

College Station, TX				Nacogdoches, TX			
Cultivar	Mean	Standard Deviation	Standard Error	Cultivar	Mean	Standard Deviation	Standard Error
GOLD	113.6 g	71.74	17.56	GOLD	143.6 g	78.32	25.21
AUTH	73.8 g	48.05	17.56	AUTH	115.8 g	88.97	25.21
BRUNO	68.8 g	61.03	17.56	CK-3	102.7 g	80.01	25.21
HAYWARD	59.0 g	43.81	17.56	HAYWARD	98.8 g	58.02	25.21
CK-3	55.4 g	53.71	17.56	DRAGON	92.1 g	99.93	26.96
FITZ	53.5 g	37.31	17.56	FITZ	77.1 g	50.30	25.21
SUN	35.5 g	34.51	17.56	BRUNO	74.5 g	54.50	25.21
DRAGON	27.9 g	30.80	18.77	SUN	60.5 g	45.66	25.21
Significant (P=0.002) site x year interaction present for pruning weight.							

Principle Component Analysis

Model Parameters

Principle Component Analysis (PCA) was used in attempt to explain and identify relationships in the data collected. The whole model identified a total of five PCA's with Eigenvalues ≥ 1.0. However, the first three explained approximately 43.3%, 24.4%, and 6.7% (collectively 74.3% of the total variance). Additionally, successive PCA's explained an increasingly small amount of variance. Consequently, only the first three were retained in the model (Figure 177).

Rotational factor analysis using Principle Component/Varimax and Principle Component/Promax identified two variables with significant loading factors < 0.50: tissue-Na and tissue-B whereas the non-rotated loading matrix included all the variables,

therefore these were not included in further analysis. Soil-COM was also excluded from the final analysis (data not shown).

Partial contribution of variables for each PCA was well distributed among the 33 variables and relatively small for most. However, individual variables such as tissue-N, *PS*, PCC, soil-Na, *E*, and tissue-Mn collectively accounted for approximately 34% of the total contribution to each of the three PCA's, on average. PCA partial contributions for each variable are listed in (Table 54).

Eigenvalues

Number	Eigenvalue	Percent	20 40 60 80	Cum Percent
1	14.3	43.229		43.229
2	8.05	24.393		67.622
3	2.21	6.692		74.314
4	1.73	5.246		79.561
5	1.39	4.220		83.781
6	0.86	2.605		86.386
7	0.69	2.079		88.464
8	0.63	1.919		90.383
9	0.51	1.554		91.938

Figure 177 Principle component analyses with eigenvalues ≥0.50 considered for eight kiwifruit cultivars in the assessment of response to soil pH.

Table 54 List of partial contribution of 33 variables to three Principle Component Analyses for eight kiwifruit cultivars in the assessment of response to soil pH.

Variable	PCA 1	PCA 2	PCA 3
PCC	0.42	7.59	6.44
SPAD-P	0.26	6.51	1.16
CI	0.29	8.04	2.00
PS	4.24	0.00	10.45
gs	3.80	1.54	2.75
E	4.72	0.18	8.02
LW	0.55	8.90	1.67
PW	0.27	8.48	0.34
Tissue-N	0.09	1.63	33.13
Tissue-P	0.34	7.33	3.06
Tissue-K	0.01	3.30	0.00
Tissue-Ca	0.18	4.08	0.27
Tissue-Mg	2.84	4.11	0.40
Tissue-Zn	0.59	4.64	0.31
Tissue-Fe	3.56	0.02	1.70
Tissue-Cu	2.32	0.24	0.23
Tissue-Mn	4.05	1.30	6.56
Tissue-S	2.43	4.43	0.77
Soil-pH	5.90	0.92	0.16
Soil-Cond.	2.88	5.50	1.13
Soil-Nitrate	0.09	9.55	0.47
Soil-P	5.47	0.40	5.31
Soil-K	6.46	0.41	0.51
Soil-Ca	6.54	0.23	0.29
Soil-Mg	5.76	0.60	0.70
Soil-S	3.83	3.27	2.11
Soil-Na	3.20	2.79	7.39
Soil-Fe	6.52	0.10	0.57
Soil-Zn	3.99	0.07	1.41
Soil-Mn	5.85	0.03	0.02
Soil-Cu	0.23	3.35	0.16
Soil-B	5.86	0.46	0.44
Soil-OM	6.48	0.01	0.07
Total	100.0	100.0	100.0

Principle Component Analysis Associations

PCA 1 and PCA 2

PCA 1 and PCA2 together explained approximately 67.6% of the total variance. PCC, CI, tissue-Ca, soil-Cu, which were closely aligned, along with g_s, soil-pH, soil-K, soil-Ca, and *E* were all positively associated with PCA 1 and PCA 2. Soil OM and *PS* were positively associated with PCA 1, but showed a weak or neutral association with

PCA 2. Soil-B, soil-Na, soil-S, soil-conductivity, and soil-nitrate were positively associated with PCA 1, but negatively associated with PCA2. Tissue-N, SPAD-P, PW, and LW were closely clustered together and, along with tissue-Cu and tissue-Mn, were negatively associated with both PCA 1 and PCA2. Tissue-Fe, soil-Fe, soil-Mn and soil-Zn were closely aligned, showing a negative association with PCA1 and a weak, but nonetheless positive association with PCA 2. Additionally, tissue-Mg, tissue-S, tissue-Zn, tissue-P, and tissue-K were all negatively associated with PCA 1 and positively associated with PCA 2.

Soil-pH, soil-Mg, soil-K, soil-Ca, soil-OM, soil-B, soil-S, soil-conductivity, soil-nitrate, LW, soil-Mn, and soil-Fe appeared to show the strongest influence in the association between PCA 1 and PCA 2. Trends associated with cultivar and species scores were not readily discernable from the score plots for PCA 1 & PCA 2. However, in terms of site, it was clear that values represented by CS were positively associated with PCA 1 (Figure 178).

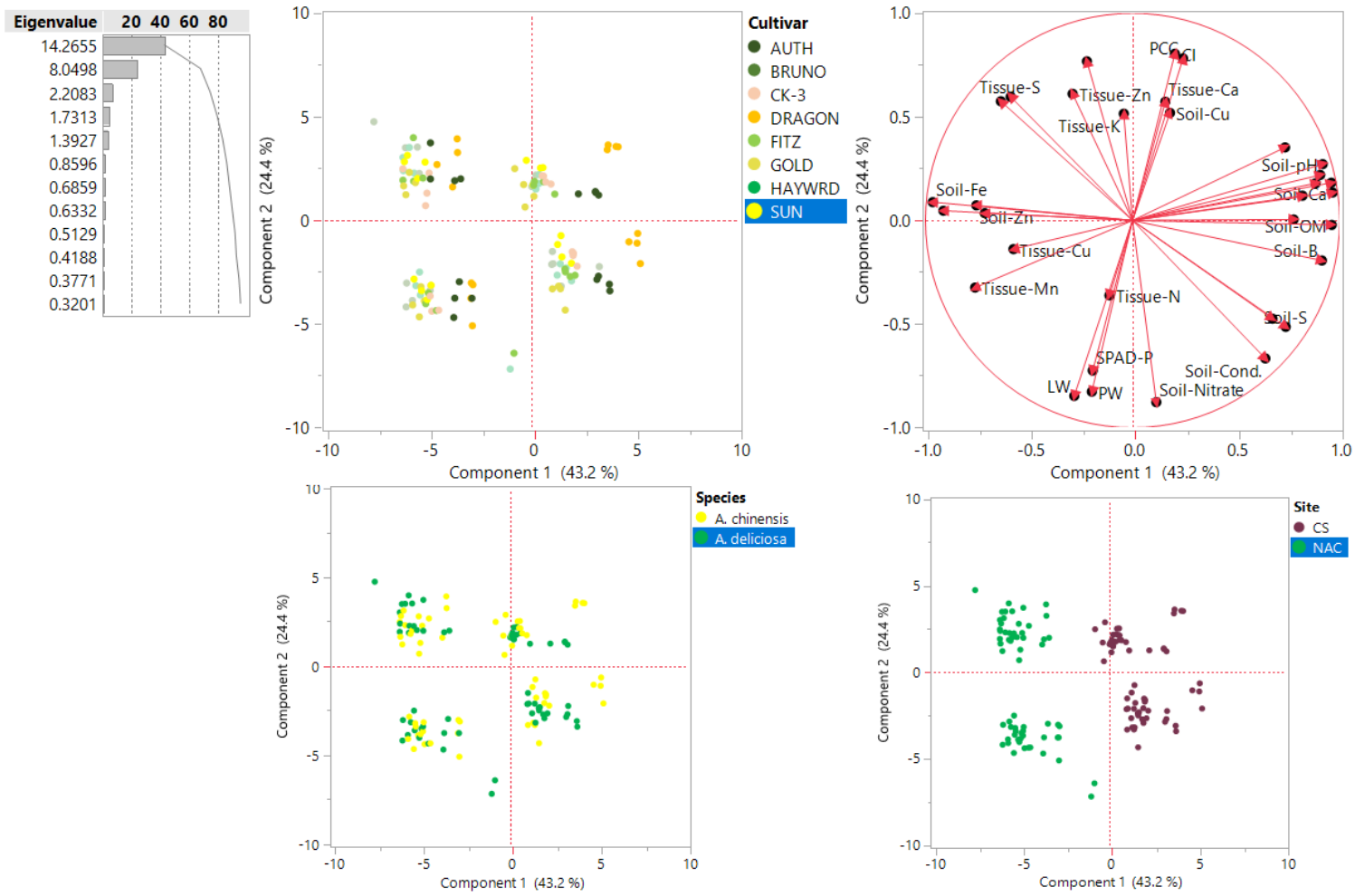


Figure 178 Principle component analysis on correlations with eigenvalues showing summary plot and score plots for cultivar, species, and site method for PCA 1 & PCA 2 in the assessment of kiwifruit response to soil pH.

PCA 1 and PCA 3

PCA 1 and PCA 3 together explained approximately 50% of the total variance observed. Most of the association with the two vectors appeared to be more strongly associated with PCA 1 more so than PCA 3. *PS*, *E*, g_s soil-Cu, and soil-P all exhibited positive associations with both PCA 1 and PCA 3. However, soil-Mg, soil-K, soil-Ca, and soil-OM all were positively associated with PCA 1, but showed a weaker yet still positive association with PCA3. Soil-pH and soil-B were positively associated with PCA 1, but appeared only weakly associated with PCA 3 in a negative manner. Comparatively, tissue-Ca, soil-conductivity, soil-S, soil-nitrate, soil-Na, CI, and PCC were all positively associated with PCA 1 and clearly negatively associated with PCA 3. Only SPAD-P showed a clearly negative association with both PCA 1 and PCA 2, whereas tissue-Mg, soil-Fe, and especially soil-Mn were all negatively associated with PCA 1 but appeared have a weaker negative association with PCA 3. Tissue-P, LW, PW, tissue-Mn, tissue-Zn, tissue-Fe, soil-Zn, and to a lesser degree, tissue-Cu, were positively associated with PCA 3 while showing a negative association with PCA 1. Tissue-N, PS, E, soil-P, soil-Mg, soil-K, soil-Ca, soil-OM, soil-pH, soil-B, soil-S, soil-Na, soil-Fe, soil-Mn, and tissue-Mn appeared to be most influential in the associations between PCA 1 and PCA 3. Again, trends among individual cultivars and between species were not clearly evident from the score plots. However, values associated with CS were generally positively associated with PCA 1 and negatively associated with PCA 3, whereas those from NAC had a negative association with PCA 3 and as well as PCA 1 (Figure 179).

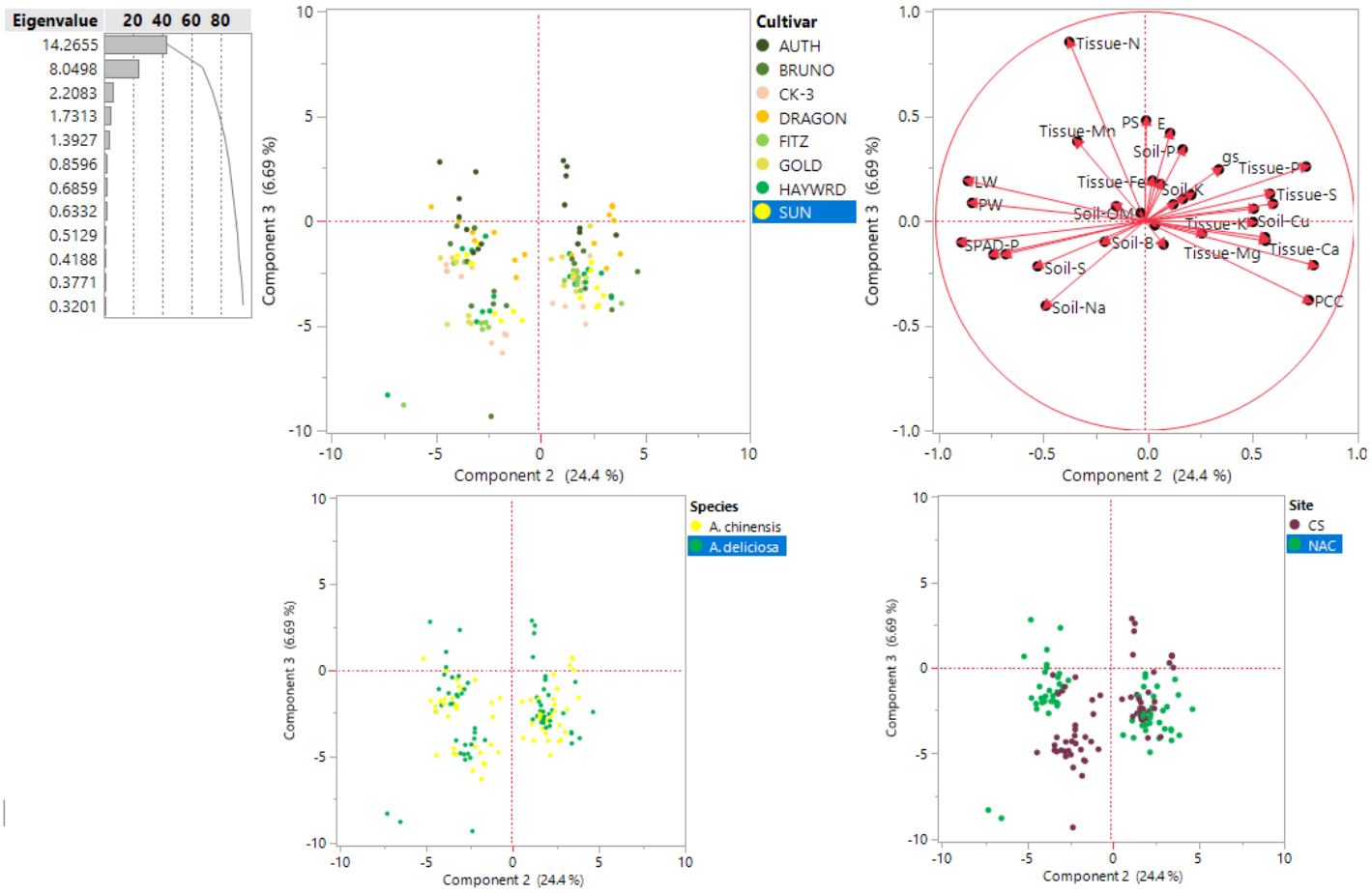


Figure 179 Principle component analysis on correlations with eigenvalues showing summary plot and score plots for cultivar, species, and site for PCA 1 & 3 PCA in the assessment of kiwifruit response to soil pH.

PCA 2 and PCA 3

Collectively, PCA 2 and PCA 3 explained approximately 31% of the total variance observed in this study. Tissue-Fe, E, soil-Zn, soil-P, soil-Ca, g_s, soil-Mg, soil-K, tissue-P, tissue-S, tissue-Zn, and soil-Cu were positively associated with PCA 2 and PCA 3. Of these, only tissue-P appeared to have much influence. Tissue-K was positively associated with PCA 2, but showed a neutral association with PCA 3. Tissue-Ca, tissue-Mg, soil-pH, CI, soil-Mn, PCC, and soil-Fe were positively associated with PCA 2, while exhibiting a negative association with PCA 3. Of these, only CI and PC appeared to contribute meaningfully. Soil-Na, soil-B, soil S, soil-conductivity, SPAD-P, and soil-nitrate were all negatively associated with both PCA 2 and PCA 3. SPAD-P, soil-NA, and especially soil-nitrate were noticeable. PW, LW, tissue-Cu, soil-OM, tissue-Mn, and tissue-N showed a negative association with PCA 2 and positive association with PCA 3. PW, LW, and tissue-N appeared to have the most influence. *PS* showed a clear positive association with PCA 3, but no association with PCA 2.

Several trends were evident from the score plots for PCA 2/PCA 3. Cultivars AUTH and BRUNO appeared to be positively associated with PCA 3, with no clear pattern with respect to PCA 2. With respect to species, *A. deliciosa* also appeared to show the same associations. Values from both sites (CS and NAC) were clearly more negatively associated with PCA 3, with those from CS possibly more negatively associated with PCA 2 as well (Figure 180).

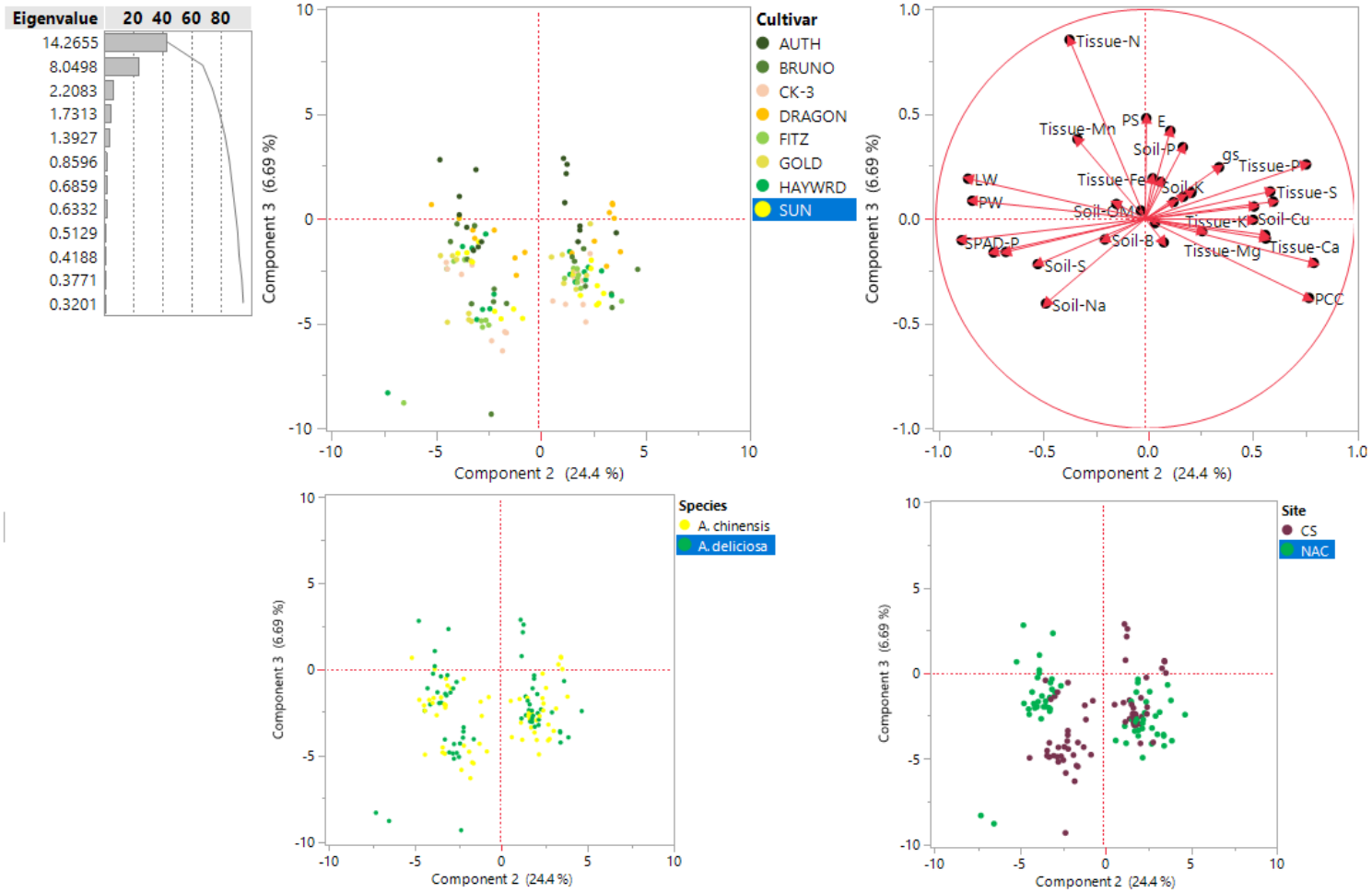


Figure 180 Principle component analysis on correlations with eigenvalues showing summary plot and score plots for cultivar, species, and site for PCA 1 & 3 PCA in the assessment of kiwifruit response to soil pH.

Correlations

Correlation values were based on data from both sites over both years. Correlation coefficients among all dependent variables and correlation probabilities are listed in Tables 55-57. PCC was negatively correlated with SPAD-P, g_s, LW, PW, tissue-N, tissue-Cu, and tissue Mn, while strongly and positively correlated with CI. SPAD-P was negatively correlated with CI, g_s, *E*, tissue-P, tissue-K, and tissue-Zn, but positively correlated with LW and PW. *PS* showed negative correlations with tissue-Mg, tissue-Cu, tissue-Mn, and tissue-S, whereas was positively correlated with g_s and *E*. The variable g_s was negatively correlated with LW, PW, tissue-Fe, tissue-Cu, and tissue-Mn, but positively correlated with *E*. LW was negatively correlated with tissue-P, tissue-K, tissue-Ca, and tissue-Zn, yet trended positively with PW, tissue-N and tissue-Mn. Similarly, PW was negatively correlated with tissue-P, tissue-Ca, and tissue-Zn, while showing a positive correlation with tissue-Cu and tissue-Mn (Table 55).

Tissue-N was positively correlated with tissue-Mn, while tissue-P was positively correlated with tissue-K, tissue-Zn, and tissue-S. Tissue-K was positively correlated with tissue-Mg, tissue-Zn, and tissue-S. Tissue-Ca was positively correlated with tissue-Mg, while the variable tissue-Mg trended positively with tissue-Zn, tissue-Fe, and tissue-S. Tissue-Zn and tissue-Cu both showed positive correlations with tissue-S, while tissue-Fe was positively correlated with tissue-Cu, tissue-Mn, and tissue-S (Table 56).

Table 55 Correlation coefficients among 18 visual, physiological, and plant-tissue nutrient variables used for eight kiwifruit cultivars in the assessment of response to soil pH.

	PCC	SPAD-P	CI	PS	gs	E	LW	PW	Tissue-N	Tissue-P	Tissue-K	Tissue-Ca	Tissue-Mg	Tissue-Zn	Tissue-Fe	Tissue-Cu	Tissue-Mn	Tissue-S
PCC	1.00	**-.62*** **	**.94*** **	.04	**.39***	.25	**-.76*** **	**-.74*** **	**-.58*** **	**.37***	.30	**.48****	.32	.30	-.20	**-.40***	**-.49****	.19
SPAD-P		1.00	**-.68*** **	-.23	**-.51****	**-.42***	**.48****	**.61*** **	.16	**-.38***	**-.36***	-.36	-.30	**-.42***	.07	.28	.28	-.29
CI			1.00	.16	**.42***	.36	**-.73*** **	**-.72*** **	**-.48****	**.38***	.23	**.51****	.36	.30	-.16	**-.37***	**-.39***	.27
PS				1.00	**.76*** **	**.85*** **	-.12	-.07	.27	-.06	-.15	.17	**-.44****	-.28	-.34	**-.39***	**-.41***	**-.40***
g_s					1.00	**.80*** **	**-.52****	**-.42***	-.01	.11	-.01	.33	-.22	-.01	**-.47****	**-.36***	**-.61*** **	-.22
E						1.00	-.25	-.21	.25	-.02	-.11	.13	**-.51****	-.28	**-.50****	**-.64*** **	**-.44***	**-.46****
LW							1.00	**.72*** **	**.50****	**-.62*** **	**-.39***	**-.56****	-.30	**-.37***	.23	.26	**.60*** **	-.34
PW								1.00	.36	**-.54****	-.34	**-.42***	-.30	**-.40***	.14	**.43***	**.39***	-.30
Tissue-N									1.00	-.05	-.10	-.28	-.20	-.07	.18	.14	**.54****	-.02
Tissue-P										1.00	**.58*** **	.31	**.53****	**.64*** **	.14	.23	-.06	**.67*** **
Tissue-K											1.00	.05	**.37***	**.46***	.05	.08	-.20	**.50****
Tissue-Ca												1.00	**.37***	.24	.01	-.07	-.34	.24
Tissue-Mg													1.00	**.53****	**.54****	.34	.28	**.74*** **
Tissue-Zn														1.00	.22	.31	.00	**.57*** **
Tissue-Fe															1.00	**.40***	**.63*** **	**.54****
Tissue-Cu																1.00	.35	**.45***
Tissue-Mn																	1.00	.27
Tissue-S																		1.00

Non-significant (no asterisk) (P≥0.05) *Significant at P<0.05 **Significant at P<0.01 ***Significant at P<0.001.
All correlations estimated using pair-wise method.

Table 56 Correlation coefficients among 33 visual, physiological, plant-tissue nutrient, and soil-related variables used for eight kiwifruit cultivars in the assessment of response to soil pH.

	Soil-pH	Soil-Cond.	Soil-Nitrate	Soil-P	Soil-K	Soil-Ca	Soil-Mg	Soil-S	Soil-Na	Soil-Fe	Soil-Zn	Soil-Mn	Soil-Cu	Soil-B	Soil-OM
PCC	**.41***	-.30	**-.56***	.20	.30	.27	.30	-.16	-.07	-.12	-.19	-.15	**.40***	.08	.19
SPAD-P	-.32	.35	**.57****	-.28	-.29	-.26	-.29	.25	.25	.12	.06	.11	**-.42***	.00	-.17
CI	.35	**-.37***	**-.60*****	.20	.26	.23	.26	-.24	-.19	-.07	-.12	-.11	**.41***	.01	.14
PS	**.67*****	**.45***	.10	**.81*****	**.79*****	**.76*****	**.80*****	**.50****	.34	**-.77*****	**-.42***	**-.64*****	.24	**.71*****	**.78*****
gs	**.69*****	.25	-.18	**.75*****	**.76*****	**.74*****	**.79*****	.35	.26	**-.64*****	**-.41***	**-.58*****	.33	**.57*****	**.66*****
E	**.72*****	**.37***	-.02	**.86*****	**.80*****	**.79*****	**.74*****	**.40***	.30	**-.83*****	**-.57*****	**-.73*****	.18	**.60*****	**.76*****
LW	**-.47****	**.37***	**.71*****	-.34	**-.42***	-.36	**-.46***	.17	.13	.15	.15	.18	**-.47****	-.13	-.25
PW	**-.43***	**.44***	**.71*****	-.29	-.30	-.30	-.30	.32	.26	.13	.15	.17	**-.37***	.03	-.14
Tissue-N	-.28	.05	.24	.11	-.09	-.11	-.09	-.04	-.20	-.01	.18	.06	-.20	-.11	-.06
Tissue-P	.03	**-.68*****	**-.75*****	.05	-.02	-.06	.03	**-.59*****	**-.59*****	.25	.21	.18	.28	-.34	-.23
Tissue-K	.18	-.30	**-.42***	.06	.10	.08	.13	-.21	-.17	.05	-.02	-.02	.19	-.04	.00
Tissue-Ca	.29	-.23	**-.41***	.21	.26	.23	.30	-.14	-.14	-.11	.00	-.08	.31	.07	.18
Tissue-Mg	**-.44***	**-.68*****	**-.47****	**-.53****	**-.51****	**-.55****	**-.42***	**-.65*****	**-.58*****	**.69*****	**.57*****	**.66*****	.26	**-.62*****	**-.60*****
Tissue-Zn	-.07	**-.51****	**-.52****	-.09	-.11	-.15	-.03	**-.44***	**-.43***	.31	.31	.25	.29	-.30	-.25
Tissue-Fe	**-.65*****	**-.45***	-.10	**-.59*****	**-.63*****	**-.66*****	**-.58*****	**-.51****	**-.48****	**.65*****	**.56****	**.71*****	.00	**-.63*****	**-.63*****
Tissue-Cu	**-.52****	-.18	.11	**-.51****	**-.50****	**-.51****	**-.40***	-.23	-.24	**.55****	**.53****	**.53****	-.05	**-.37***	**-.50****
Tissue-Mn	**-.82*****	-.34	.15	**-.60*****	**-.77*****	**-.76*****	**-.77*****	**-.49****	**-.52****	**.65*****	**.54****	**.65*****	-.30	**-.69*****	**-.72*****
Tissue-S	**-.34*****	**-.73*****	**-.59*****	**-.36***	**-.40***	**-.44***	-.32	**-.69*****	**-.64*****	**.59*****	**.50****	**.55****	.23	**-.59*****	**-.54****

Non-significant (no asterisk) (P≥0.05) *Significant at P<0.05 **Significant at P<0.01 ***Significant at P<0.001
All correlations estimated using pair-wise method.

Table 57 Correlation coefficients among 15 soil-related variables used for eight kiwifruit cultivars in the assessment of response to soil pH.

	Soil-pH	Soil-Cond.	Soil-Nitrate	Soil-P	Soil-K	Soil-Ca	Soil-Mg	Soil-S	Soil-Na	Soil-Fe	Soil-Zn	Soil-Mn	Soil-Cu	Soil-B	Soil-OM
Soil-pH	1.00	**.42***	-.14	**.85*****	**.93*****	**.96*****	**.87*****	**.51****	**.50****	**-.89*****	**-.71*****	**-.89*****	.27	**.80*****	**.88*****
Soil-Cond.		1.00	**.80*****	**.36***	**.49****	**.52****	**.46***	**.93*****	**.88*****	**-.65*****	**-.45***	**-.57****	-.15	**.79*****	**.64*****
Soil-Nitrate			1.00	-.15	-.07	-.04	-.07	**.60*****	**.56****	-.15	-.02	-.09	**-.36***	.34	.14
Soil-P				1.00	**.94*****	**.92*****	**.90*****	**.47****	.35	**-.90*****	**-.61*****	**-.83*****	.27	**.72*****	**.85*****
Soil-K					1.00	**.98*****	**.97*****	**.62*****	**.54****	**-.93*****	**-.67*****	**-.86*****	.31	**.86*****	**.95*****
Soil-Ca						1.00	**.92*****	**.62*****	**.55****	**-.95*****	**-.71*****	**-.91*****	.25	**.86*****	**.95*****
Soil-Mg							1.00	**.60*****	**.51****	**-.85*****	**-.54****	**-.76*****	**.37***	**.83*****	**.90*****
Soil-S								1.00	**.95*****	**-.70*****	**-.58*****	**-.63*****	-.04	**.88*****	**.74*****
Soil-Na									1.00	**-.63*****	**-.63*****	**-.59*****	-.08	**.79*****	**.66*****
Soil-Fe										1.00	**.76*****	**.92*****	-.08	**-.86*****	**-.94*****
Soil-Zn											1.00	**.82*****	.13	**-.64*****	**-.65*****
Soil-Mn												1.00	.01	**-.81*****	**-.86*****
Soil-Cu													1.00	.14	.23
Soil-B														1.00	**.93*****
Soil-OM															1.00

Non-significant (no asterisk) (P≥0.05) *Significant at P<0.05 **Significant at P<0.01 ***Significant at P<0.001
All correlations estimated using pair-wise method.

PCC and was also negatively correlated with soil-nitrate and positively correlated with soil-Cu. CI exhibited the same trends, although was also significantly and negatively correlated with soil-conductivity. Conversely, SPA-P was positively correlated with soil-nitrate, but negatively correlated with soil-Cu. *PS*, g_s, and *E* all exhibited positive correlations with soil-pH, soil-conductivity (except for g_s), and soil-concentrations of the elements P, K, Ca, Mg, B, and OM. However, negative correlations were observed between these physiological variables and soil-Fe, Zn, and Mn. LW was negatively correlated with soil-pH, and soil-K, Mg, and Cu, yet positively correlated with soil-conductivity and soil-nitrate. Similarly, PW as negatively correlated with soil-pH and soil-Cu, while showing positive correlations with soil-conductivity and soil-nitrate (Table 57).

Tissue-P was negatively correlated with soil-conductivity, nitrate, S, and Na, while tissue-K and tissue-Ca were also negatively correlated with soil-nitrate. Tissue-Mg exhibited negative correlations with soil parameters: pH, conductivity, nitrate, P, Ca, Mg, S, Na, B, and OM, yet was positively correlated with soil-Fe, soil-Zn, and soil-Mn. Similarly, tissue-Fe, tissue-Cu, tissue-Mn, and tissue-S were all negatively correlated with soil parameters pH, P, K, Ca, Mg (except for tissue-S), S (except for tissue-Cu), Na (except for tissue-Cu), B, and OM. Tissue-Fe and tissue-S were also negatively correlated with soil-conductivity, while tissue-S and soil-nitrate showed a negative trend. All three previously mentioned tissue-cations were positively correlated with soil-Fe, soil-Zn, and soil-Mn. Tissue-Zn trended similarly, having negative correlations with soil-conductivity, soil-S, soil-Na, but also soil-nitrate (Table 56).

Soil-pH and soil-conductivity were positively correlated with soil parameters conductivity, nitrate (soil-conductivity only), P, K, Ca, Mg, S, Na, B, and OM, while negatively correlated with soil-Fe, soil-Zn, and soil-Mn. Soil-nitrate trended positively with soil-S and soil-Na, but negatively with soil-Cu. Soil nutrients P, K, Ca, and Mg were all positively correlated with soil-S, soil-Na, soil-B, and soil-OM, but showed negative correlations with soil cations Fe, Zn, and Mn. Soil-P, soil-Ca, and soil-Mg were all also positively correlated with one another. In a similar manner, soil-S and soil-Na trended positively with each other, soil-B, and soil-OM, yet was negatively correlated with soil-Fe, soil-Zn, and Soil-Mn. Soil-Fe and soil-Zn were positively correlated with soil-Mn and each other, but negatively correlated with soil-B and soil-OM. Finally, Soil-Mn was negatively correlated with soil-B, while both soil-Mn and soil-B were negatively correlated with soil-OM (Table 57).

Discussion

Comparison of Visual Responses

Percent Canopy Chlorosis, SPAD Percentage, and Chlorosis Index by Site

SPAD-P was consistently and significantly higher at NAC in relation to CS across years, representing a generally higher average SPAD value for chlorotic in relation to healthy tissue. Both PCC and CI exhibited opposite trends across years, with both variables averaging higher values at NAC in 2018 and at CS in 2019.

The higher (not significantly) average PCC and CI at NAC in the first year are likely due to the rapid onset of chlorosis that appeared to result from a problem with

macronutrient adjustment (fertigation) in June. Symptoms appeared to be consistent with "fertilizer burn", exhibiting bright interveinal chlorosis (particularly in older leaves) and some marginal and interveinal necrosis later on that was visually consistent with magnesium deficiency. Analysis of leaf tissue (two weeks after symptom appearance) and soil conductivity did not reveal major problems. Nevertheless, these are strongly implicated with some form of osmotic and possibly nutritional stress.

Both PCC and CI were significantly and dramatically higher on average at CS during the second year, with appearance of chlorosis more isolated often isolated to specific cultivars at NAC. This trend of more prevalent and intense chlorosis, resulting in a higher average comprehensive index (CI) value observed at the more alkaline site that was more representative of expectations.

Visual Responses by Cultivar

Percent Canopy Chlorosis

PCC was employed as an assessment of the prevalence or special coverage of chlorosis on plant foliage. There was considerable and significant variation for PCC among cultivars at both sites during both years. At CS in 2018, DRAGON exhibited the highest percent of canopy chlorosis, whereas AUTH had the least with approximately one-third as DRAGON. HAYWARD and GOLD were significantly different from the two extremes. There was less variation among cultivars (as evident by the narrower range) at NAC in 2018. However, there was sufficient variation to produce two cultivars with significantly different means: BRUNO and AUTH. As mentioned earlier, PCC at

this site in 2018 appeared to be positively skewed by an unusual nutritional or osmotic stress that was associated with correction of macronutrient deficiencies. This anomaly may have also masked some of the inter-cultivar variation that was seen during the second year at this site.

The range of cultivar averages observed at CS in 2019 revealed slightly less variation in 2019. While DRAGON was outranked by CK-3 for the highest ranking cultivar, these (along with SUN) were statistically not different. As in 2018, AUTH produced the lowest value (again approximately one-third that of the highest), whereas, FITZ, HAYWARD, and BRUNO were intermediate between these two. Notably, three cultivars that ranked highest for PCC were of the *A. chinensis* species. As previously mentioned, there was a much smaller range for PCC observed at NAC in 2019. DRAGON, and AUTH were statistically different, while none of the intermediate cultivars were different from these two.

The year x cultivar and site x cultivar interactions for PCC resulted from several changes in rank over sight and year, particularly in the intermediate rankings. However, AUTH consistently exhibited the lowest value across all four environments, whereas DRAGON was in the highest statistical group in three environments, including both years at CS. These two cultivars also ranked among the highest and lowest (respectively) for tissue-N, tissue-Fe, and tissue-Mn.

SPAD Percentage by Cultivar

SPAD-P represented the relative intensity of chlorosis symptoms by quantifying the percentage of chlorotic R-SPAD value relative to healthy value. SPAD-P was significantly different among cultivars across both sites and both years. At CS in 2018 AUTH and DRAGON represented the statistical extremes, with FITZ and SUN being statistically different as intermediate cultivars. CK-3 exhibited the highest average SPAD-P value at NAC in 2018, whereas BRUNO the lowest. All other intermediate cultivars were not statistically different from these extremes. Notably, the four of the five highest ranks were occupied by *A. chinensis* cultivars in this environment.

Similar to the first year, AUTH represented the highest and HAYWARD / DRAGON the lowest-ranking cultivars for SPAD-P at CS in 2019. In fact, relative rankings varied little from year to year at this site. At NAC in 2019, AUTH exhibited the highest average value, whereas BRUNO and HAYWARD the lowest. Of the intermediate cultivars, only DRAGON was significantly different from these two extremes. Similar to the first year, three of the top four ranks consisted of *A. chinensis* cultivars. Specifically, the differing response to site of golden kiwifruit cultivars DRAGON and SUN in relation to green cultivars can be seen in their change from the intermediate rankings at NAC to or near the lowest at CS. This is in contrast to AUTH, which appeared at or the near the top consistently in all four environments.

Chlorosis Index by Cultivar

CI was considered the most comprehensive assessment of chlorosis as it accounted for prevalence (PCC), relative intensity (SPAD-P), and R-SPAD value deviation from 'reference' plants. DRAGON, consistently ranked highest among cultivars for CI in three of the four environments, including and notably both years at CS. In addition to widespread and intense chlorosis, foliage of DRAGON also took on a mottled and stunted appearance similar to that of virus-infected plants during the spring at CS both years. SUN followed closely behind, ranking second and statistically the same as DRAGON in all environments, including NAC in 2018 where it ranked highest. CK-3 ranked third (statistically different from DRAGON/SUN) both years at CS, yet toward the middle among cultivar rankings both years at NAC. FITZ, HAYWARD, and GOLD generally appeared in the lower half in all environments, with rankings varying between year and site. Perhaps most noteworthy was the constant ranking of AUTH in last place with the lowest average index. In fact, individual plants of this cultivar were without a single leaf with any sign of chlorosis.

The relative difference in CI among individual cultivars was striking, particularly as observed at CS. Difference among cultivars, in addition to reports such as Viti et al. (1990) suggest that there is sufficient genetic variation to develop genotypes with greatly improved tolerance to soil alkalinity. However, it remains to be determined whether or not this observed tolerance in cultivars such as AUTH is transferrable into a scion shoot system.

Visual Responses by Species

Comparison of visual responses by species indicated that there was a differential response to site by species for all three variables (PCC, SPAD-P, and CI). Average SPAD-P was higher in *A. chinensis* at NAC over years. However, by individual year, a higher average SPAD-P was observed in *A. deliciosa* at CS, whereas *A. chinensis* was higher at NAC in 2018. Observation of a greater degree of light green appearance associated with the young foliage of most *A. deliciosa* plants may have also resulted in the overestimation of PCC values for cultivars of this species. Species responded differently by year for both PCC and CI and site. Average PCC for A. *chinensis* was significantly higher than *A. deliciosa* at NAC during both years, along with CI. This, along with a higher average CI for both years at CS and the second year at NAC with differential SPAD-P response suggest that golden kiwifruit, as a species, is more likely to develop symptoms of chlorosis, particularly on alkaline soils. While this implication is limited to the number of cultivars observed, such results have not been reported in literature to date.

Visual Responses by Propagation Method

PCC showed a significant response only at CS in 2019. However, the prevalence of chlorosis was generally higher among clonal cultivars, especially at the alkaline CS site. Conversely, SPAD-P, representing the relative intensity of chlorosis, was less favorable for seedlings, though only significantly at NAC. Finally, CI was consistently higher among clonally propagated plants in all four environments-particularly the CS, even if

the difference was only significant at CS in the second year. This trend might also explain the response of GOLD's differing response to all three visual measures of alkalinity in relation to *A. chinensis,* as a species. On the contrary, AUTH consistently exhibited the best performance (visually) at the alkaline site, in spite of being clonally propagated. Indeed, when comparisons were made by both species and propagation method at CS, significant interactions were observed between treatments for PCC, SPAD-P, and CI (data not shown).

Kiwifruit (both species) are known for their relatively spreading root system with many fine roots and lack of dominant taproot (Hughes et al., 1991; Lemon and Considine, 1993; McAneney and Judd, 1983), with architecture differing little between young clonal and sexual plants. However, Piccotino et al. (1991) observed greater soil exploration (per m^3) among grafted seedlings and tissue culture (TC) plants compared to cutting-grown, while Xiloyannis et al. (1997) found that seedlings and TC plants produced a greater root:canopy ratio on average. It would seem therefore that this more expansive and denser root system provides seedlings with an advantage in the uptake of nutrients, particularly when limited by concentration or availability.

Comparison of Physiological Responses

As previously discussed, gas exchange measurements were conducted only on the two cultivars, AUTH and DRAGON, representing the relative extremes of previously observed visual chlorosis symptoms. Gas exchange data was complicated by many interactions that masked most treatment effects. *PS* and *E* responded differentially

to year over sites, while g_s and *E* responded differentially to cultivar over site. *E* also showed inconsistent response to site over year over cultivar.

Average *PS* was significantly higher at CS as compared to NAC for both individual years. This was surprising, considering the lower average tissue-Fe and tissue-Mn. There was a significant (P = 0.002) positive relationship between *PS* and tissue-N in 2018 (data not shown), as average tissue-N was significantly higher at CS. However, average tissue-N was significantly lower at CS during the second year where *PS* remained significantly greater with no such relationship. Specific leaf area and leaf thickness were not measured in this study. However, leaves at CS displayed noticeably greater thickness, likely due to generally windier and harsher conditions. A greater number of palisade parenchyma cells (per cm^2) at this site may have allowed for greater capture of solar energy—a response not necessarily resulting from the higher soil pH.

There was no significant difference between cultivars at either site during either year with respect to average g_s and *E*. The exception was at CS during the second year where average g_s was significantly higher (approximately 78% higher) in DRAGON than AUTH. The highly-chlorotic leaves of DRAGON may have attempted to compensate with a greater stomatal aperture (as evident by the higher *E* rate) in order to maintain a level of *PS* that was comparable to the healthier AUTH leaves. However, this was the only example in which the more chlorotic plant exhibited relatively more physiological stress than the non-chlorotic plant at the alkaline site.

Comparison of Plant Growth Responses

Leaf Weight and Pruning Weight by Site

LW was considered a measure of general plant health and vigor, to some degree. Unlike leaf area measurements, weight measurement was more consistent, particularly between sites with differing climate and sunlight. Unfortunately, treatment effects were masked by interactions, as LW did not respond consistently to site by cultivar or year. Additionally, LW was inconsistent by cultivar over site over years. Some of this interaction was likely due to the large variability within cultivars-even on a single plant. Regardless, two patterns were discernable, even if not statistically: average LW increased with year (plant age) and was consistently greater among cultivars at NAC (by as much as 2X) than CS.

Average pruning weight (dry weight) per plant was believed to be a more reliable index for vigor than other parameters such as trunk diameter. Although average PW was not consistent over site and year, site averages were significantly higher at NAC compared to CS during both years. Additionally, all cultivars were consistently heavier at NAC than at CS (by a factor similar to that for LW) across years. While this difference was not surprising, other factors such as higher humidity, lower summer temperatures, less wind, and greater soil porosity were likely at play other than simply soil pH.

Leaf Weight by Cultivar

While site and plant age strongly influenced leaf size, differences among cultivar were also highly evident. Interestingly, relative ranking in each given environment

changed relatively little across sites and especially between years. However, as discussed earlier, weight was significantly and noticeably different between the two sites. LW also generally trended (inversely) with severity of chlorosis. GOLD, closely followed by HAYWARD and BRUNO, consistently produced the heaviest leaves at CS. A similar pattern was observed at NAC both years, with the exception of the second year in which GOLD dropped into the lower half of cultivars by weight. DRAGON and CK-3, which are known for having relatively small leaves, were consistently last in all four environments, with SUN only slightly larger. FITZ and AUTH mostly ranked intermediately in size in all four environments.

Pruning Weight by Cultivar

Plant vigor or yield (in this case biomass) is generally considered the most important measure of plant performance and adaptation. Like LW, PW tended to be inversely related to chlorosis severity. Relative PW ranking among cultivars was unchanged between years at CS and relatively stable at NAC. GOLD and AUTH were consistently the largest and second largest (statistically not different) cultivars across all four environments. BRUNO, and in the second year HAYWARD, were not statistically smaller than GOLD and AUTH at CS, whereas it ranked toward the lower end both years at NAC. FITZ and CK-3 typically ranked in the middle or toward the lower half, except at NAC in 2019 in which CK-3 was similar in size to AUTH. SUN and DRAGON were consistently the ranked 7^{th} and 8^{th} in size at CS. SUN consistently produced the smallest plant at NAC, whereas DRAGON responded very differently

between CS and NAC where it ranked toward the middle and attained a weight similar to AUTH there in the second year.

The inconsistency of response to differing sites was most noticeable in AUTH, DRAGON, and GOLD. Whereas GOLD and AUTH consistently produced large plants with little chlorosis at both sites, DRAGON exhibited medium vigor with moderate chlorosis at NAC and severe chlorosis and very low vigor at CS. Comparison of PW between species and propagation method yielded inconsistent results. Cutting-grown plants are reportedly generally smaller and less vigorous (Loreti et al., 1991; Piccotino et al., 1991; Xiloyannis et al., 1997), with clonal propagation used in order to reduce scion size (Clearwater, et al., 2004).

Little information has been published regarding comparison of plant size between *A. chinensis* and *A. deliciosa,* although the former is generally considered to be more vigorous in the industry. Seedling-treatments such as BRUNO, HAYWARD, and certainly GOLD generally produced larger plants than clonal cultivars such as CK-3, DRAGON, and SUN. However, cutting-grown AUTH was consistently the second-largest cultivar in all four environments. While there did not appear to be any clear pattern to PW with respect to species at NAC, the upper half of cultivar rankings at CS consisted of *A. deliciosa* cultivars-the exception being GOLD. While *A. chinensis'* candidacy as an understock has been heavily discounted, the apparent tolerance to soil alkalinity and high vigor of GOLD suggests its potential for challenging sites, particularly where obtaining sufficient vigor is a serious limitation.

Comparison of Topsoil Parameters

Soil-pH (upper 30-cm) between CS and NAC was significantly different throughout this study—the former being reportedly unsuitable or at least challenging and the latter being slightly below or at ideal pH. pH appeared to gradually increase with soil depth at CS, whereas the opposite trend was observed at NAC. By the second year, soil-pH had dropped significantly at CS and noticeably at NAC. This change was observed in all three horizons at NAC, but not in the upper horizon at CS. Average conductivity was lower as compared to CS and below the recommended range (for fruit crops) at NAC during the first year. Average EC was much higher during the second year, particularly at CS, though still well below the high threshold for kiwifruit. OM was relatively high for the region, particularly at CS, despite the same soil preparation measures. This higher percentage might be attributable to the relatively coarse soil texture, higher humidity, and higher rainfall at NAC. The significant, near five-fold higher concentration of calcium at CS was one of the most notable features of soil composition between sites, along with the high OM likely resulting in a well-buffered substrate. In spite of this buffering capacity, decomposition of the composted pine bark material (as evident by the increase in OM between years) was apparently sufficient to drop the topsoil pH by 0.15 units in less than a year at this site. Nevertheless, the respective soil conditions at CS and NAC presented contrasting environments for this study.

Nitrate-N, which was comparable between site, was much lower in 2018, likely because of the much later sampling date, which was well after fertigation had ended. Potassium and magnesium at NAC were nearly one-quarter and one-half (respectively)

that of CS, with soil-K concentration appearing inadequate at NAC during the second year. Sulfur was also significantly lower at NAC, particularly in 2019.

Concentrations of zinc and copper were comparably low, but certainly adequate across site and year. Boron levels were above optimal at CS, particularly during the second year rose significantly at both sites by the second year, most likely due to accumulation from the load in the ground water that was used for irrigation there for much of 2019. This was also likely the case for sodium, which was more concentrated in the irrigation water at CS where levels were becoming excessive at CS by the second year. Both iron and manganese were much lower at CS across years. While concentrations at CS were adequate (relative to recommended levels), this reported sufficiency was likely deceiving, considering the reduced availability associate with the calcareous conditions. This may have also been the case for soil-Cu and soil-Zn, which also exhibit reduced availability with increasing soil pH (Broadley et al., 2012). Conductivity was consistently higher at CS across years, and more than twice as high (on average) by the second year.

Comparison of Plant Tissue Nutrition

Plant Tissue Nutrition by Site and Year

Tissue-N was inconsistent over site by year. Average concentrations were higher at CS as compared to NAC in 2018, where they were slightly above the optimal range and satisfactory at NAC. Conversely, the average at CS was optimal, whereas average tissue-N was much higher and well above the optimal range at NAC during the second

year. The higher concentration at CS during the first year was not unexpected due to early termination of N fertigation at NAC in response to a problem with application. However, the higher tissue concentration at NAC in 2018 occurred despite nearly identical average soil-N between sites.

Tissue-P did not respond consistently to site by cultivar, over year, or across site, year, and cultivar-making comparisons difficult. Average tissue concentrations were comparable and slightly above optimal during the first year and nearly identical and toward the upper range of optimal during the second year at each site. Tissue-K responded inconsistently to site over years, with a slightly higher average at NAC in 2018 and significantly higher average concentration at CS in 2019. 2018-concentrations were above optimal at both sites and optimal while slightly above optimal at NAC and CS (respectively) during the second year.

Tissue-Ca responded inconsistently to site over years and to cultivar over years. Average tissue-Ca concentrations were somewhat higher at CS in 2018 and 2019. However, most importantly, tissue-Ca was undoubtedly deficient across year and site for this experiment. With respect to plant uptake and tissue concentration, K, Mg, and Ca are known for their antagonistic relationship with one another. Therefore, it stands to reason that the above-optimal (but not excessive) concentrations of tissue-K (present in all four environments) were influential, even with sufficient soil-Ca levels. This was surprising, considering that soil-K at NAC in 2019 appeared inadequate.

Tissue-Mg was also inconsistent over site and year as well as over cultivar and year. Average concentrations were higher at NAC during both years and especially

during the first year in relation to CS. However, unlike tissue-Ca, this nutrient was not within the optimal range in all four environments, except for at CS in 2019 where the average concentration was slightly below, but certainly not in the deficiency range. Tissue-S also did not behave consistently over site and year, but was higher on average at NAC compared to CS both years. Average concentrations were optimal at both sites during the first year, but were lower during the second year—even below optimal at CS, although not deficient.

As with most elements, tissue-Na did not respond consistently over year. Average tissue concentration at CS was more than double that of the value at NAC during the first year. However, average concentrations were more comparable during the second year, with CS showing higher value. None of these concentrations were near the toxic threshold. Tissue-Zn was inconsistent over site and cultivar and year and cultivar. However, average concentrations were higher at NAC both years, although both sites exhibited lower average tissue-Zn levels during the second year. Relative to recommended levels, concentrations were within the optimal range at both sites (slightly above at NAC in 2018), although considerably lower during the second year. This was noteworthy particularly at CS, given the higher soil pH.

Tissue-Fe was the only element that behaved consistently over year for both site and cultivar. The average concentration was approximately 37% higher over years at NAC as compared to CS, with no significant difference between years. Average tissue-Fe was well below optimal at NAC and below the deficiency threshold at CS both years. The low levels were particularly surprising at NAC, given the adequate soil

concentration and relatively low soil-pH. Tissue-Cu did not respond consistently to site over year and also exhibited a differential response to site over year over cultivar. However, average concentrations were higher at NAC in 2018 and 2019 and slightly below optimal at CS (but not deficient) in 2018.

Tissue-Mn did not respond consistently to site over year, although average concentration was much higher at NAC in 2018 (44% higher) and particularly in 2019 (310% higher) as compared to CS. While these values were optimal both years at NAC, the average concentration of 37 mg/kg in 2018 was well below optimal and the concentration of 27 mg/kg in 2019 was below the deficiency threshold at CS. Tissue-B responded inconsistently to site over year, site over cultivar, and cultivar over year. Average concentrations were nearly identical between sites in 2018, noticeably higher at CS in 2019, but still within the optimal range for all four environments.

As a note, generally lower average tissue concentrations were observed for many nutrients (at both sites) during the second year in comparison to 2018 for elements such as P, K, Ca, Mg, S, and Zn. These lower concentrations may be attributable some dilution effect resulting from the plants being much larger (as evident by average LW and PW) with more rapid growth as well as different timing. As previously discussed plant tissue collection in 2018 took place during autumn, whereas samples were taken during mid-summer during 2019.

Plant Tissue Nutrition by Cultivar

Plant Tissue Nitrogen

Plants were generally well-supplied with N at CS in 2018, where all but two cultivars (CK-3 and DRAGON) were found to exceed the optimal threshold for tissue-N. At NAC during the same year, all cultivars at (with the slight exception of DRAGON) were within optimal range. Average tissue-N was only significantly different among cultivars at CS in 2019. In this environment tissue-N for BRUNO was slightly above optimal, CK-3 was slightly below, whereas the remaining cultivars were within the optimal range. Tissue-N for all cultivars was above the optimal range at NAC in 2019. Across years, SUN/AUTH and DRAGON/CK-3 tended to have the relative highest and lowest average concentrations of tissue-N.

Plant Tissue Phosphorus

As previously mentioned, plants were well-supplied with P at both sites during the first year. All but two cultivars (SUN and DRAGON) at CS and all cultivars at NAC exhibited average tissue-P concentrations that were above optimal range in 2018. At CS, GOLD and SUN/DRAGON were significantly different with respect to concentration. Concentrations were generally lower during the second year, with all but one cultivar (GOLD) falling within the optimal range at CS and DRAGON and FITZ reporting below optimal, but not deficient. Across year and site, there was no clear pattern among individual cultivars. However, BRUNO/AUTH generally and DRAGON generally exhibited the relatively highest and lowest concentrations.

Plant Tissue Potassium

The vast majority of cultivars exhibited well above-optimal average tissue-K concentrations at both sites in 2018, with only CK-3 and DRAGON appearing within the optimal range. The number of cultivars with above-normal concentrations was lower during the second year, with FITZ, BRUNO, HAYWARD, and SUN at CS and only HAYWARD at NAC exhibiting concentrations outside the optimal range. Species appeared to play a role, as HAHYWARD, BRUNO, and FITZ-all *A. deliciosa*- were the top accumulators of K across site and year, whereas CK-3, DRAGON, SUN were consistently ranked near the lower end.

Plant Tissue Calcium

As discussed earlier, Ca was one of the few elements that was found to be deficient in this study. All cultivars at CS and at NAC both years were deficient, while there was significant variation among cultivars in all four environments. A. chinensis cultivars CK-3 and SUN were consistently the greatest Ca-accumulators. High concentrations of carbonates and bicarbonates-generally associated with high soil concentrations of Ca-have been heavily implicated in development of iron chlorosis through inactivation of the ferric reductase enzyme at the root level as well as inactivation of Fe in the shoot tissue (Tagliavini and Robolà, 2001; Tagliavini et al.,

1995). While tissue-bicarbonate concentration was not measured, it does not appear that inactivation of shoot tissue-Fe was prevalent, but rather prevention of root uptake.

Plant Tissue Sulfur

Significant variation was observed among cultivars at both sites during both years with respect to tissue-Mg. Average concentrations were generally higher, but still optimal both years at NAC. The majority of the cultivars at CS during the second year reported concentrations below normal. However, these levels, which were well above deficiency threshold along with the absence of visual symptoms associated with Mg deficiency do not suggest inadequacy was a problem. BRUNO and SUN generally exhibited the highest relative tissue-Mg concentrations over site and year.

Average tissue-S was generally found to be within the normal range during the first year, with the exception of CK-3 at CS. However, all cultivars at CS during 2019 (except for GOLD) exhibited average concentrations below normal, with CK-3 falling slightly below the deficiency threshold. Likewise, FITZ, DRAGON, and CK-3 reported below normal range at NAC during the second year. SUN / GOLD and DRAGON/CK-3 were generally found to have the relative highest and lowest tissue-S over site and year.

Plant Tissue Sodium

Excessive sodium is known for contributing to or exacerbating plant nutritional problems and resulting chlorosis (Asher et al., 1987). Kiwifruit have a relatively low tolerance to Na in the soil solution (Norton, 1994; Sale and Lyford 1990). However,

none of the tissue concentrations observed in this study at either site during either year would be expected to be problematic. That being said, Smith et al. (1987) reported that kiwifruit are able to exclude Na from the shoot system, suggesting that potential problems associated with excessive concentrations in the roots might have gone undetected. The uniform values reported for all cultivars at NAC in 2019 were apparently the result of a different analytical protocol with a minimum threshold of 79.98 mg/kg used during the second year. Tissue-Na values in this environment were therefore likely overestimated.

Plant Tissue Zinc

Average tissue-Zn varied significantly by cultivar in all environments except for CS during 2018. Average concentrations were above the lower threshold of normal for all cultivars at each site during both years, with several cultivars being well above the upper threshold for normal, primarily during the first year at NAC. There were also no visual symptoms observed that were consistent with zinc deficiency in this study. Over site and years, BRUNO showed the highest average concentration SUN generally had the lowest average levels.

Plant Tissue Iron

As discussed earlier, tissue-Fe was consistently lower at NAC as compared to CS both years. Surprisingly, all of the cultivars in all four environments reported average concentrations well below the normal range-including at NAC. All cultivars at CS and

approximately half of the cultivars at NAC (particularly GOLD, DRAGON, and CK-3) fell into the deficient range during both years. With the exception of a few, relative cultivar rank at each site over year was relatively consistent. AUTH, and to a lesser degree, BRUNO, generally exhibited the highest average tissue-Fe, whereas DRAGON, and to a lesser degree, CK-3 and FITZ the relative lowest. GOLD, HAYWARD, and SUN were consistently found in the intermediate ranks. While these intermediate ranks included members of both species, it is noteworthy that the two highest and two lowest were generally occupied by *A. deliciosa* and *A. chinensis*, respectively. Visual symptoms associated with iron deficiency were observed for all cultivars (except AUTH) and were most notable in CK-3 and DRAGON. Over both sites during both years, the observed deficiency of iron appeared to be the result of insufficient tissue concentrations, rather than inactivation in spite of adequate levels, as reported by Tagliavini and Rombolà (2001).

Plant Tissue Manganese

Average tissue-Mn was significantly different among cultivars at both sites in 2019. Average concentrations were below normal range for all cultivars at CS during both years, along with two (DRAGON and CK-3) at NAC during the first year. Of these, all but two cultivars (AUTH and BRUNO) reported in deficient range at CS during the second year. The “intercoastal” (“herringbone”) type chlorosis observed at mid-shoot was widespread at CS, especially in the second year. BRUNO and particularly AUTH generally exhibited the relative highest average concentrations, whereas CK-3 and

especially DRAGON the lowest. The trend in favor of the fuzzy kiwifruit species that was observed for tissue-Fe appeared to be consistent with tissue-Mn as well. As with tissue-Fe, deficiency of this nutrient appeared to be from inadequate tissue levels.

Plant Tissue Copper and Boron

There was significant variation among cultivars for tissue-Cu at all four environments. All but two (GOLD and BRUNO) cultivars at CS in 2018 and three at CS in 2019 (GOLD, AUTH, and BRUNO) were below normal range based on average tissue-Cu. Only two cultivars (DRAGON and FITZ) reported below average concentrations during the second year at NAC. Of these, none of were considered to be below the deficiency threshold. Over site and years, GOLD and BRUNO were generally the highest Cu-accumulators, whereas SUN and DRAGON were largely the lowest.

As a phloem immobile nutrient, copper-deficiency in kiwifruit appears on young leaves as a uniform discoloration that progresses into interveinal chlorosis symptoms similar to, but generally less intense than that that of iron deficiency (Asher et al., 1987). However, the same author also reported that deficiency of this nutrient mostly occurs on sandy-acid sites. Nevertheless, the observed tissue-Cu inadequacy in addition to reported decreased availability with soil alkalinity implicates this element as a potential candidate in contributing to observed chlorosis.

Like Na, kiwifruit have a lower tolerance to soil boron (Norton, 1994), with toxicity resulting in chlorosis and interveinal necrosis (Smith et al., 1987). All of the concentrations observed in this study were well within the normal range for this element.

Principle Component Analysis

Three Principle Component Analyses were able to account for a combined 74.3% of the total variance. Partial contribution of explained variance was well distributed among 33 significant factors that were included in the model. However, 16 factors collectively accounted for 83% of the contribution to PCA 1, which explained 43.2% of the total variance. These included (in order of decreasing contribution): soil-Ca, soil-Fe, soil-OM, soil-K, soil-pH, soil-B, soil-Mn, soil-Mg, soil-P, *E*, *PS*, tissue-Mn, soil-Zn, soil-S, g_s, and tissue-Fe.

Trends between cultivar scores and factors were not clearly discernable. However, site scores for CS were most notably associated with soil-pH, soil-Mg, soil-P, soil-K, soil-Ca, soil-OM, and soil-B, and PCC. Conversely, scores for NAC were most notably associated with soil-Fe, soil-Mn, soil-Zn, tissue-Mn, tissue-Fe, LW, PW, tissue-Cu, SPAD-P. Cultivars BRUNO and AUTH and to a lesser degree, the species *A. deliciosa*, appeared to be somewhat associated with tissue-N, tissue-Mn, and tissue-Fe. These trends generally agreed with previously discussed differences.

Correlations

Chlorosis Development

The value of the significant correlation estimates previously mentioned were limited by their attempt to explain a complicated dataset from two highly contrasting sites. Nevertheless, some of the trends that were identified were enlightening. For CI, the strongly positive and negative correlations with PCC and SPAD-P suggest that it was an accurate indicator of both prevalence and severity of the visual chlorosis. All three visual parameters also coincided respectively with both LW and PW as measures of vigor.

For both PCC and CI, the negative correlation with tissue-Cu implies that the observed inadequacy of this element in some cases may have contributed to the appearance of chlorosis. While tissue-Cu was generally low (particularly at CS), it's positive correlations with all three visual variables suggest that it may have played a roll, especially if it was related to the uptake of bicarbonates. It is also suggested that tissue-N, while never in a state of deficiency, also reduced chlorosis. While tissue-Fe and visual chlorosis were not significantly correlated, their effect remains clear based on its low concentration in the most severe cultivars. The significant and negative correlations with tissue-Mn, along with visual observations among cultivars relative to concentration, suggest that this element may have been more involved in chlorosis development than iron, particularly at CS where pH and soil-OM were also higher.

Physiological Responses

The generally inverse relationship indicated between gas exchange and several essential plant nutrients (particularly Mg, Fe, and Mn) is not biologically reasonable. Rather, this trend most likely resulted from the coincidentally lower gas exchange at NAC, where concentrations of these elements were higher. Indeed, this trend was reversed when sites were analyzed individually (data not shown). While there was no evidence of reduced photosynthetic ability in chlorotic leaves, the reduced yield (LW and PW) associated with the alkaline CS site and most symptomatic plants is telling.

Soil and Plant Tissue Nutrients

The strongly negative correlations observed between soil pH and tissue concentrations of Fe and Mn were not surprising, considering their reduced solubility in alkaline soils. However, the relative strength of these correlations may also at least partly be the result of lower soil concentrations, resulting in lower tissue concentrations of these elements at NAC. Reduced availability of Fe and particularly Zn have been reportedly associated with only adequate levels of soil P in alkaline soils (George, 2012). Both soil-Fe and soil-Mn (but not soil-Zn) were negatively correlated with soil-P in this experiment. However, soil-P levels were relatively low at both sites. Additionally, these correlations were positive when only data from CS was considered.

The strongly negative correlations associated with soil-Ca and tissue concentrations of Fe, Mn, and to a lesser degree, Cu appeared to confirm that the

presence of calcium (likely with associated carbonate and bicarbonates ions) resulted in impaired uptake of these nutrients.

Apparently antagonistic relationships were observed among several tissue-nutrients and soil parameters. However, many of these proved to be heavily influenced by trends at specific sites, such as the negative correlations between soil-OM and tissue concentrations of Fe and Mn resulting from the relatively high OM and correspondingly low soil and tissue concentrations of Fe and Mn at CS.

Conclusion

The objective of this experiment was to evaluate several *Actinidia chinensis* and *A. deliciosa* cultivars' response to contrasting soil pH and identify possible physiological and nutritional responses to soil alkalinity. Sites strongly differed with respect to soil pH as well as soil concentrations of K, Mg, Na, Fe, and Mn (among others). Plant tissue analysis revealed numerous differences between site, year, and among cultivars for the majority of plant nutrients assessed. Major differences were observed between sites and among cultivars for all three visual assessments of chlorosis, with higher values observed at the more alkaline College Station, TX site. *A. chinensis,* as a species, appeared to be more prone to exhibiting chlorosis along with clonally-propagated plants as compared to *A. deliciosa* and seedlings, although exceptions were evident.

Among cultivars, 'AU Authur' and 'AU Golden Dragon' exhibited respectively the most and least severe symptoms. However, differences in physiological response such as photosynthesis were not observed between these two cultivars. Ultimately,

increased chlorosis symptom intensity resulted in reduced plant yield, both in terms of site and cultivar. Based on this study, there appears to be sufficient genetic variation to develop rootstocks conferring improved tolerance to alkaline soil conditions into kiwifruit, assuming these observed responses are transferrable into the scion.

Trends identified by multivariate analysis suggest that detrimental response to soil alkalinity was most closely related with tissue concentrations of Fe and Mn, as previously suspected. However, deficiency of these nutrients appeared to result from inadequate tissue-concentrations, rather than inactivation in the shoot tissue. Copper, which was also widely deficient in plant tissue, was also implicated as a contributing factor. Nitrogen, which was never observed to be deficient, was negatively associated with chlorosis.

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CHAPTER V

CONCLUSIONS

The objective of this project was to explore the feasibility commercial kiwifruit (*Actinidia chinensis* and *A. deliciosa*) production in Texas. Three key areas were identified as perceived limitations to the adaptation of this crop: cold tolerance, winter chilling requirement, and soil pH. Each of these factors served as the focus of applied studies.

The first study aimed to document frost damage to young field-grown plants following an unusually early and hard frost event. Results identified major differences in apparent tolerance between species and cultivar. The fuzzy kiwifruit species (*A. deliciosa*), particularly cultivars 'AU Authur' and 'AU Fitzgerald' were more susceptible, whereas golden kiwifruit (*A. chinensis*), and most notably Zespri Gold™ seedlings sustained less damage, in spite of the latter species exhibiting greater vigor. Such differences in cold tolerance between these species have not been reported elsewhere, to date. *A. deliciosa* plants specifically showed a greater propensity for basal damage and basal cracking—a factor that could potentially limit this species' suitability for rootstock use. Cold tolerance was not significantly different between clonally-propagated plants and seedlings in this experiment. It is important to bear in mind that these results are unique to the cultivars observed and the timing of the frost event. Frost injury, particularly to young plants with little cold acclimation, likely presents the single-greatest challenge to establishment of commercial kiwifruit plantings in Texas.

However, it has been widely reported that plants' tolerance improves greatly with age. Successful establishment will likely require frost protection of young plants during the first three to four years or selection of a location with milder winter temperatures, assuming winter chilling requirements can be satisfied.

The second study was conducted to compare the effects of continuously-supplied simulated winter chilling against simulated chilling with intermittent warm temperature interruption in two cultivars over two years in order to assess the potential for negation of chilling accumulation in two cultivars. Chilling was supplied as one-week levels (base through five weeks chilling). Reduced floral activity in *A. deliciosa* 'AU Fitzgerald' was observed with warm temperature interruption at the five weeks chilling level during the second year and at the second-highest level in both years, suggesting that this cultivar is susceptible to negation by warm temperature, as representative of winter temperature patterns in southeastern Texas. *A. chinensis* 'AU Golden Dragon', in contrast, exhibited increased floral activity with warm temperature interruption at the four and five weeks chilling during the first year and no difference between chilling type during the second year or over both years, suggesting that chilling accumulation in this golden kiwifruit cultivar is not subject to negation by the same warm temperatures. Future studies with additional cultivars of both species are needed to determine if results observed in this experiment are representative of each species. Chilling type had no effect on vegetative budbreak or shoot development in either cultivar. Chilling requirements (under continuous chilling conditions) for these cultivars were comparable to those previously reported. While relatively high winter chilling requirements for both cultivars limit the

geographic range of successful fruit production to more northern latitudes, ‘AU Golden Dragon’ offers the advantage of not being additionally limited by negation or effective loss of chilling.

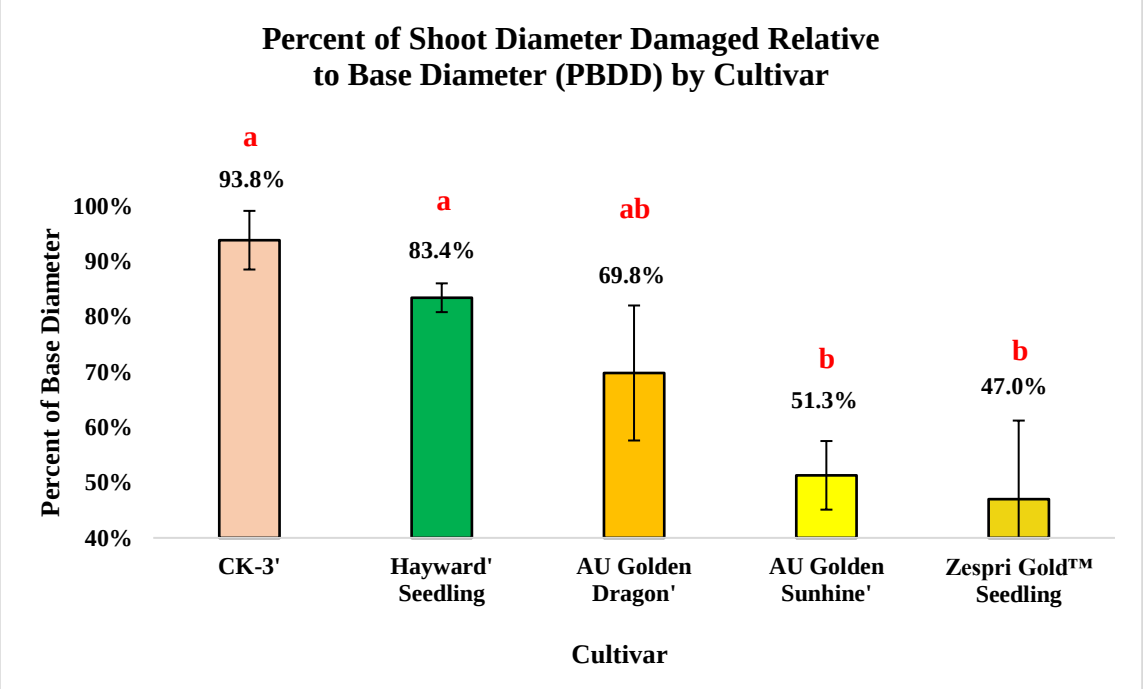
The final study was conducted to explore the response of kiwifruit plants to alkaline soil conditions and to identify differences between species and among cultivars. The two sites used in this experiment provided a unique testing scenario, with contrasting soil pH and soil concentrations of plant nutrients, notably calcium, iron, and manganese. More severe visual symptoms of chlorosis were observed at the alkaline College Station site, as compared to the Nacogdoches site with acid soil. While plants at the former site exhibited lower vigor, physiological responses such as photosynthesis were not inhibited. Clonally-propagated plants and those of the *A. chinensis* species were generally more susceptible to developing chlorosis symptoms as compared to seedling plants and *A. deliciosa* plants, respectively. Striking variability was observed among individual cultivars, with ‘AU Authur’ and ‘AU Golden Dragon’ exhibiting the least- and most-severe chlorosis. Development of these symptoms was strongly associated with inadequate leaf tissue concentrations of iron, manganese, and copper, with no evidence of shoot tissue inactivation of iron, as previously reported. High soil pH would be expected to limit commercial production of this crop in many, primarily more western, portions of Texas. However, differential responses by species and cultivar observed in this experiment suggest that sufficient genetic variation may exist to allow for the selection of more alkaline-tolerant rootstocks. Further research is needed to

determine whether or not the apparent improved tolerances in specific cultivars observed would be transferable into the scion shoot system, once grafted.

All three factors studied in this project appear to present major limitations to the successful commercial production of kiwifruit in Texas. However, through careful site selection and shrewd management, the adoption of certain provisions such as frost protection during young plant establishment, and the availability and selection of plant material with improved adaptation, small-scale production could be.

APPENDIX A

CHAPTER TWO APPENDICES



A. 1 Percent of shoot system damaged relative to base diameter (PBDD) by cultivar ('AU Authur' and 'AU Fitzgerald' removed) assessed in the response of young field-grown kiwifruit plant to fall frost.

A. 2 Correlation coefficients between six variables assessed in the response of young field-grown kiwifruit plants to fall frost (only seedling cultivars considered).

	BD	MDD	PBDD	CB	DB	PSD
Base Diameter	1.00****	-0.73ns[a]	-0.88*	-0.79ns[a]	-0.81*	-0.84*
Maximum Diameter Damaged	-0.73ns[a]	1.00****	0.96**	0.93**	0.94**	0.83*
Percent of Base Diameter Damaged	-0.88*	0.96**	1.00****	0.94**	0.95**	0.91*
Base Cracking	-0.79ns[a]	0.93**	0.94**	1.00****	0.99****	0.91*
Base Damage	-0.81*	0.94**	0.95**	0.99****	1.00****	0.89*
Percent Shoot Damaged	-0.84*	0.83*	0.91*	0.91*	0.89*	1.00****

[a] Non-significant (P≥0.05) *Significant at P<0.05 **Significant at P<0.01 ***Significant at P<0.001 **** Significant at P<0.0001

A. 3 Mean tissue nitrogen concentration by cultivar from adjacent plot at College Station, TX October 3, 2018 in the assessment of response of young field-grown kiwifruit plants to fall frost.

Cultivar	Average Percent Nitrogen	Standard Deviation
'AU Authur'	3.05	0.191
'AU Fitzgerald'	2.88	0.096
'AU Golden Dragon'	2.65	0.129
'AU Golden Sunshine'	2.90	0.432
'Hayward' Seedling	2.83	0.250
'CK-3'	2.68	0.222
Zespri Gold™ Seedling	3.00	0.283
Whole-leaf samples collected from adjacent planting approximately five weeks prior to frost (November 13-14, 2018		

APPENDIX B

CHAPTER THREE APPENDICES

B. 1 Comparison of continuous chilling (C.C.) and warm temperature interruption (W.T.) effect on mean vegetative budbreak number per cane in 'AU Golden Dragon' kiwifruit for 2017-2018.

Chilling Level	Consistent Chilling				Warm Temperature Interruption				Significance
	Days Warm Temp.	Mean	Standard Deviation	Standard Error	Days Warm Temp.	Mean	Standard Deviation	Standard Error	
Base	0	3.34	0.583	0.412	3	4.09	0.583	0.412	ns
1 week	0	3.67	0.319	0.225	6	3.17	0.319	0.319	ns
2 weeks	0	3.83	0.492	0.348	9	4.50	0.492	0.348	ns
3 weeks	0	4.42	0.886	0.627	12	5.33	0.886	0.627	ns
4 weeks	0	5.34	0.903	0.403	15	5.84	0.695	0.403	ns
5 weeks	0	5.25	0.549	0.388	18	6.17	0.549	0.388	ns

Budbreak based on Brundell, 1975.
Pair-wise comparison at each level of chilling ($\alpha = 0.05$).

B. 2 Comparison of continuous chilling (C.C.) and warm temperature interruption (W.T.) effect on mean vegetative budbreak number per cane in 'AU Golden Dragon' kiwifruit for 2018-2019.

Chilling Level	Consistent Chilling				Warm Temperature Interruption				Significance
	Days Warm Temp.	Mean	Standard Deviation	Standard Error	Days Warm Temp.	Mean	Standard Deviation	Standard Error	
Base	0	3.84	0.439	0.310	3	4.09	0.439	0.310	ns
1 week	0	4.38	0.655	0.463	6	4.58	0.655	0.463	ns
2 weeks	0	4.58	0.912	0.645	9	5.25	0.912	0.645	ns
3 weeks	0	5.34	0.787	0.556	12	5.08	0.787	0.556	ns
4 weeks	0	4.67	0.821	0.580	15	5.92	0.821	0.580	ns
5 weeks	0	4.42	0.614	0.434	18	5.25	0.614	0.434	ns

Budbreak based on Brundell, 1975.
Pair-wise comparison at each level of chilling ($\alpha = 0.05$).

B. 3 Comparison of continuous Chilling (C.C.) and warm temperature interruption (W.T.) effect on mean vegetative budbreak number per cane in 'AU Fitzgerald' kiwifruit for 2017-2018.

Chilling Level	Consistent Chilling				Warm Temperature Interruption				Significance
	Days Warm Temp.	Mean	Standard Deviation	Standard Error	Days Warm Temp.	Mean	Standard Deviation	Standard Error	
Base	0	2.50	1.127	0.797	3	2.88	1.127	0.797	ns
1 week	0	2.17	0.499	0.353	6	2.75	0.499	0.353	ns
2 weeks	0	3.00	0.629	0.445	9	3.42	0.629	0.445	ns
3 weeks	0	3.08	0.996	0.704	12	3.92	0.996	0.704	ns
4 weeks	0	2.33	0.712	0.503	15	3.25	0.712	0.503	ns
5 weeks	0	2.58	0.651	0.460	18	3.46	0.651	0.460	ns

Budbreak based on Brundell, 1975.
Pair-wise comparison at each level of chilling ($\alpha = 0.05$).

B. 4 Comparison of continuous chilling (C.C.) and warm temperature interruption (W.T.) effect on mean vegetative budbreak number per cane in 'AU Fitzgerald' kiwifruit for 2018-2019.

Chilling Level	Consistent Chilling				Warm Temperature Interruption				Significance
	Days Warm Temp.	Mean	Standard Deviation	Standard Error	Days Warm Temp.	Mean	Standard Deviation	Standard Error	
Base	0	2.33	0.614	0.434	3	3.17	0.614	0.434	ns
1 week	0	2.25	0.545	0.385	6	1.92	0.545	0.385	ns
2 weeks	0	2.59	0.438	0.310	9	2.67	0.438	0.310	ns
3 weeks	0	3.17	0.440	0.311	12	2.92	0.440	0.311	ns
4 weeks	0	3.50	0.437	0.309	15	2.75	0.437	0.309	ns
5 weeks	0	3.17	0.210	0.149	18	2.75	0.210	0.149	ns

Budbreak based on Brundell, 1975.
Pair-wise comparison at each level of chilling ($\alpha = 0.05$).

APPENDIX C

CHAPTER FOUR APPENDICES

C. 1 Chemical analysis of irrigation water (river) used at College Station, TX site.

Parameter	Average Value	Range	Units	Analysis Method
Calcium (Ca)	28	28 – 28	mg/kg	ICP
Magnesium (Mg)	12.5	12 – 13	mg/kg	ICP
Sodium (Na)	69.5	64 – 75	mg/kg	ICP
Potassium (K)	6	6 – 6	mg/kg	ICP
Boron (B)	0.15	0.13 – 0.17	mg/kg	ICP
Carbonate (CO_3)	0	0	mg/kg	Titration
Bicarbonate (HCO_3)	131	126 – 136	mg/kg	Titration
Sulfate (SO_4^-)	56.5	49 – 64	mg/kg	ICP
Chloride (Cl^-)	78	73 – 83	mg/kg	Titration
Nitrate ($NO3^-$)	0.05	0.01 – 0.08	mg/kg	Cadmium Reduction
Phosphorus (P)	0.04	0.02 – 0.05	mg/kg	ICP
pH	7.39	6.63 – 8.15		Ion Selective Electrode
Conductivity	0.338	0.091 – 0.585	dS/m	Conductivity
Hardness	7	7 – 7	Grains $CaCO_3$/gallon	Calculated
Hardness	121	119 – 123	mg/kg $CaCO_3$	Calculated
Alkalinity	107	103 – 111	mg/kg $CaCO_3$	Calculated
Total Dissolved Solids (TDS)	382	368 – 396	mg/kg	Calculated
Sodium Absorption Ratio (SAR)	2.75	2.6 – 2.9		Calculated
Charge Balance (cation/anion *100)	100.5	100 – 101		Calculated

Data based on average of two sampling dates
Results generated by Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory, 2478 TAMU College Station, TX 77843

C. 2 Chemical analysis of irrigation water (well) used at College Station, TX site.

Parameter	Average Value	Range	Units	Analysis Method
Calcium (Ca)	133.5	124 – 143	mg/kg	ICP
Magnesium (Mg)	36.5	35 – 38	mg/kg	ICP
Sodium (Na)	112	108 – 116	mg/kg	ICP
Potassium (K)	4	4 – 4	mg/kg	ICP
Boron (B)	0.78	0.67 – 0.89	mg/kg	ICP
Carbonate (CO_3)	0	0	mg/kg	Titration
Bicarbonate (HCO_3)	714.5	710 – 719	mg/kg	Titration
Sulfate (SO_4^-)	36	35 – 37	mg/kg	ICP
Chloride (Cl^-)	48.5	36 – 61	mg/kg	Titration
Nitrate ($NO3^-$)	0.35	0.01 – 0.68	mg/kg	Cadmium Reduction
Phosphorus (P)	0.08	0.07 – 0.08	mg/kg	ICP
pH	6.82	6.67 – 6.97		Ion Selective Electrode
Conductivity	1.288	1.1271 – 1.304	dS/m	Conductivity
Hardness	28	27 – 29	Grains $CaCO_3$/gallon	Calculated
Hardness	483.5	467 – 500	mg/kg $CaCO_3$	Calculated
Alkalinity	585.5	582 – 589	mg/kg $CaCO_3$	Calculated
Total Dissolved Solids (TDS)	1,086	1,074 – 1,098	mg/kg	Calculated
Sodium Absorption Ratio (SAR)	2.2	2.1 – 2.3		Calculated
Charge Balance (cation/anion *100)	105	104 – 106		Calculated

Data based on average of two sampling dates
Results generated by Texas A&M AgriLife Extension Soil, Water, and Forage Testing Laboratory, 2478 TAMU College Station, TX 77843