



Seasonal changes in leaf nutrient concentration of male and female hardy kiwifruit grown in Oregon

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Summary

Leaf nutrient concentration of hardy kiwifruit [*Actinidia arguta* var. *arguta* (Siebold and Zucc.) Planch. ex Miq.] was evaluated throughout two growing seasons (2008–2009) for determinate and indeterminate shoots of female ('Ananasnaya') and male vines. While research has been conducted on the more commonly grown fuzzy kiwifruit (*A. chinensis* var. *deliciosa* C.F.Liang & A.R.Ferguson), little is known about the nutrient requirements, leaf tissue sufficiency levels, or optimum time and method of leaf sampling in hardy kiwifruit. The objectives of this study were to determine whether leaf sampling should be conducted based on phenology or calendar date, and to identify best practices for sampling. Leaves were sampled from determinate and indeterminate shoot types separately from male and female vines every 2 weeks from mid-May to late October, starting when flower buds reached 6 mm in diameter and finishing after fruit harvest. Leaf nutrient concentration varied by year, plant gender, and shoot type for many nutrients and sampling dates. Key plant developmental stages were 2 weeks earlier in 2009 than 2008 despite fewer cumulative growing degree days (GDD) at each phenological stage in 2009, except at harvest when there were more GDD in 2009. Patterns of change in nutrient concentration were similar between years, and did not always match the 2-week shift in phenology. Female vines had higher concentrations of N, K, S, Cu, and Zn early in the season, but were similar to males later in the season. For other nutrients, including P, Mg, Ca, Fe, and Mn, females had consistently higher leaf concentrations across the whole season. Shoot type affected the concentration of many nutrients in both female and male vines for many sampling dates, but generally followed the same pattern of change through the season. The currently recommended leaf sampling time for kiwifruit vines in the northern hemisphere is August. While there were phenological differences between years, calendar-based sampling in mid- to late-August provided a better window of relative stability for most nutrients than phenology-based sampling for female vines. When compared to existing available standards for fuzzy kiwifruit, the hardy kiwifruit in this study often fell outside of the recommended ranges and, for B, into the excessive range, suggesting revisions to nutrient standards for hardy kiwifruit in Oregon may be appropriate.

Significance of this study

What is already known on this subject?

- No recommendations for leaf nutrient sampling specific to *A. arguta* currently exist, so the methods and sufficiency standards for *A. deliciosa* are used instead.

What are the new findings?

- Leaves from determinate and indeterminate shoots of female vines should be sampled for leaf nutrients separately from males in mid- to late-August.

What is the expected impact on horticulture?

- Following this new sampling procedure will lead to more useful tissue analysis and accuracy of plant fertility recommendations in *A. arguta* females.

Keywords

Actinidia arguta var. *arguta*, kiwiberry, baby kiwi, tissue sampling, nutrient standards, sampling time

Introduction

Hardy kiwifruit [*Actinidia arguta* var. *arguta* (Siebold and Zucc.) Planch. ex Miq.] is the only important *Actinidia* species grown commercially in Oregon. While they are the most minor berry crop in Oregon, with 60–80 ha planted of 9,300 ha total, Oregon is the leading production region in North America and hardy kiwifruit is economically important as a high yielding, high value crop. Fruit is grown for fresh market local sales in Oregon, as well as for export. 'Ananasnaya' is the most widely grown cultivar (Strik and Hummer, 2006). Plants are polygamodioecious, requiring male plants to pollinate the females at a recommended rate of one male for every eight females, interplanted in the field (Strik and Hummer, 2006; Strik, 2005). Production methods for hardy kiwifruit largely follow successful practices of the more widely grown "fuzzy" kiwifruit 'Hayward' (*Actinidia chinensis* var. *deliciosa* C.F.Liang & A.R.Ferguson), including fertilization and nutrient management (Hemelrick and Powell, 1998; Strik, 2005). Fertilization recommendations are based on the requirements of female vines despite differences in management of male vines, especially summer pruning as compared to winter or dormant pruning.

Nutrient sufficiency standards for fuzzy kiwifruit have been recommended by Clark et al. (New Zealand, 1986), Cresswell (Australia, 1989), and Velemis et al. (Greece,

1995). However, it is unknown whether these standards can be applied to hardy kiwifruit while maintaining fruit production and quality at a commercially acceptable level. Hardy kiwifruit had higher concentrations of P, Ca, Zn, and Fe and lower levels of K and Cu in the fruit than fuzzy kiwifruit (Latocha et al., 2015), possibly resulting in differing leaf nutrient concentration and fertilizer needs. In a preliminary study of hardy kiwifruit, leaf concentration of many macronutrients followed a similar pattern as fuzzy kiwifruit over the season, but differed for several micronutrients, and some nutrients had much higher concentrations overall in hardy kiwifruit (Decorte et al., 2015).

Existing *A. deliciosa* nutrient sampling recommendations also lack sufficient detail or are contradictory for which leaves should be sampled. Clark et al. (1986) and Warrington and Weston (1990) suggested that the most recent fully expanded leaves should be sampled at the same phenological stage [prior to fruit set (four to six weeks after leaf emergence)], rather than by calendar date to best diagnose nutrient disorders. However, they also provided optimum nutrient standards for samples taken in February (August in the northern hemisphere) for leaves collected from the second position past the final fruiting cluster. Cresswell (1989) also recommended sampling during February (August, northern hemisphere) because of a lower rate of change in nutrient composition during that time, and used the first leaf past the fruiting clusters. Velemis et al. (1995) developed nutrient guidelines for *A. deliciosa* using leaves from the middle of current season shoots two months after the end of flowering (mid-July to mid-August, northern hemisphere), and developed sufficiency levels that differed somewhat from those given by Clark et al. (1986). Lalatta et al. (1990) collected the fifth and sixth leaves from the base of moderately vigorous shoots of *A. deliciosa* at the end of July and the end of August (northern hemisphere) and suggested that sampling in late July to early August minimized seasonal fluctuation in leaf nutrient concentration. Sampling the most recent fully expanded leaves in five cultivars of *A. arguta* grown in Belgium, *A. arguta* showed similar patterns of nutrient changes as *A. deliciosa* (Decorte et al., 2015). It is not indicated in the aforementioned studies whether leaves were sampled from indeterminate shoots (that continue to grow throughout the season) or determinate shoots (that cease growth earlier in the season), or a combination. Shoot type could potentially impact leaf nutrient concentration due to differing leaf ages when sampled on the same calendar date. These discrepancies in sampling method and known differences in leaf nutrient concentration (for some nutrients) from varying nodes on a shoot (Cresswell, 1989) make it difficult to compare studies and provide advice to growers. A standard sampling method and time period is important for comparison across years and for providing sufficiency standards.

The fertilizer nutrient requirements and leaf nutrient concentrations of male vines have rarely been studied, though having sufficient nutrient levels is critical to the abundant growth and flowering required for pollination of

female vines. As these vines do not bear fruit and are typically pruned during the summer, after bloom, rather than while dormant like the females, they may differ in nutrient status. Lalatta et al. (1990) found that male *A. deliciosa* plants had higher leaf P and Ca than 'Hayward' vines, which may have been due to the heavy crop of the fruiting female vines. If nutrient status differs between male and female hardy kiwifruit vines, growers would need to ensure that they are only sampling females when collecting tissue.

The objectives of this study were to determine whether leaf nutrient sampling in *A. arguta* is best conducted at a certain phenological stage or if a range of calendar dates when nutrient concentration is stable can be used. In addition, differences in the leaf nutrient status of female and male *A. arguta* from indeterminate and determinate shoots will be determined to further identify optimum sampling methodology.

Materials and methods

Study site

The study was conducted in 2008 and 2009 in a mature field planting at Oregon State University's North Willamette Research and Extension Center, Aurora, OR [lat. 45°16'47"N, long. 122°45'23"W; USDA hardiness zone 8b (U.S. Department of Agricultural Research Service, 2014)]. The climate is temperate and it is unusual to receive rainfall between mid-June and late September. Weather data for this site are available from an AgriMet weather station (U.S. Dept. Interior, 2017). The soil is mapped as a Willamette silt loam, classified as a fine-silty, mixed, superactive mesic Pachic Ultic Argixeroll. Soil properties are shown in Supplemental Table S1.

Planting establishment and management

Two-year-old plants (4-L pots; One Green World Nursery, Molalla, OR) were arranged in a completely randomized design with three one-plant replicates per gender. Planting was done on April 13, 1990 except for one male that was planted on May 12, 1998. Plants were spaced 5.5 m within row and 4.6 m between rows (395 plants ha⁻¹) and trained to a T-bar trellis with 1.5-m-long cross arms and 3 supporting wires at 1.8 m height. Vines were drip irrigated with four 3.8 L hr⁻¹ emitters per plant. Sprinklers were used for frost protection in late winter to early spring and were activated when temperatures dropped below 0.5°C after early bud break (green tip stage), unless temperatures were predicted to stay below freezing for an extended amount of time. A weed-free strip was maintained with herbicides beneath the trellis and a permanent grass alley was maintained between rows and mowed as necessary. The study was conducted in 2008–2009 when the plants were mature. No fungicides, including those that may contain micronutrients, were applied during, or prior to, the study period. A split application of fertilizer was applied each year, with 34 kg ha⁻¹ N (8N–17P–17K: 3% ammoniacal-N, 5% urea-N, P from phosphate, K from potassium chloride) and 45 kg ha⁻¹ N (40N–0P–0K–6S: 35% urea-N, 5%

SUPPLEMENTAL TABLE S1. Soil nutrient concentration measured in March 2009 at Oregon State University's North Willamette Research and Extension Center (Aurora, OR), related to Tables 2–4.

pH	OM ^z (%)	N Release (kg ha ⁻¹)	ppm										
			P	K	Ca	Mg	S	B	Fe	Mn	Cu	Zn	Al
5.7	2.68	82.9	176	347	1253	270	21	0.55	272	20	2.52	2.43	1341

^zOrganic matter.

ammonium-N) applied on April 15 and May 20, respectively. Females were pruned annually in the dormant season while males were pruned shortly after flowering according to standard practice (Beutel, 1990; Strik, 2005). Males received two additional applications of fertilizer (11 kg ha⁻¹ N each on June 29 and July 13, 2009) just prior to summer pruning (which occurred on July 8, 2008 and July 22, 2009) to stimulate new shoot growth. Male vines did not receive any additional fertilizer N in 2008.

Leaf sampling

Leaves were sampled biweekly starting when female plants had flower buds measuring 6 mm in diameter and continuing through fruit harvest. Most recent fully expanded leaves were sampled from indeterminate shoots (similar to grower practice in Oregon and Decorte et al., 2015, though shoot type was not specified) while leaves in the first through fourth node positions past the fruiting zone were sampled from determinate shoots (similar to Clarke et al., 1986; Cresswell, 1989; and Warrington and Weston, 1990). Approximately six leaves were collected per plant and shoot type, and samples were shipped to Brookside Labs (New Bremen, OH) for analysis of macro- and micronutrients. Leaves were not washed prior to shipping (Hart et al., 2006). Leaf N was determined using a combustion analyser with an induction furnace and a thermal conductivity detector (Gavlak et al., 1994). Other nutrients, including P, K, Ca, Mg, B, Cu,

Mn, Zn, and Al were determined using an inductively coupled plasma (ICP) spectrophotometer after wet ashing the samples in nitric/perchloric acid (Gavlak et al., 1994).

Data analysis

Leaf nutrient concentrations were analyzed for a split-split plot design using PROC MIXED (SAS version 9.3) with year as the main effect ($n=2$), gender (male or female) as the subplot effect ($n=2$), and shoot type (determinate or indeterminate) as the sub-subplot effect ($n=2$) with a Satterthwaite approximation used, as needed, for main effect comparisons. Since year had a significant effect on many nutrients and sampling dates, data were analyzed separately by year for gender and shoot type. Changes in leaf macro- and micronutrient concentration from female and male indeterminate shoots in 2008 and 2009 are presented in Figures 1 and 2. Nutrient concentrations in indeterminate and determinate shoots for female and male vines are compared in Figures 3 and 4. As no samples from male determinate shoots were taken in the three sampling dates after summer pruning due to limited available leaf tissue in 2008, data from 2009 are presented in Figures 3 and 4.

In order to determine if leaf sampling should be conducted based on a phenological stage or a calendar date range in female vines, and whether or not shoot type is an important consideration, an analysis of year ($n=2$), sampling date ($n=3$), and shoot type ($n=2$) was conducted. For the pheno-

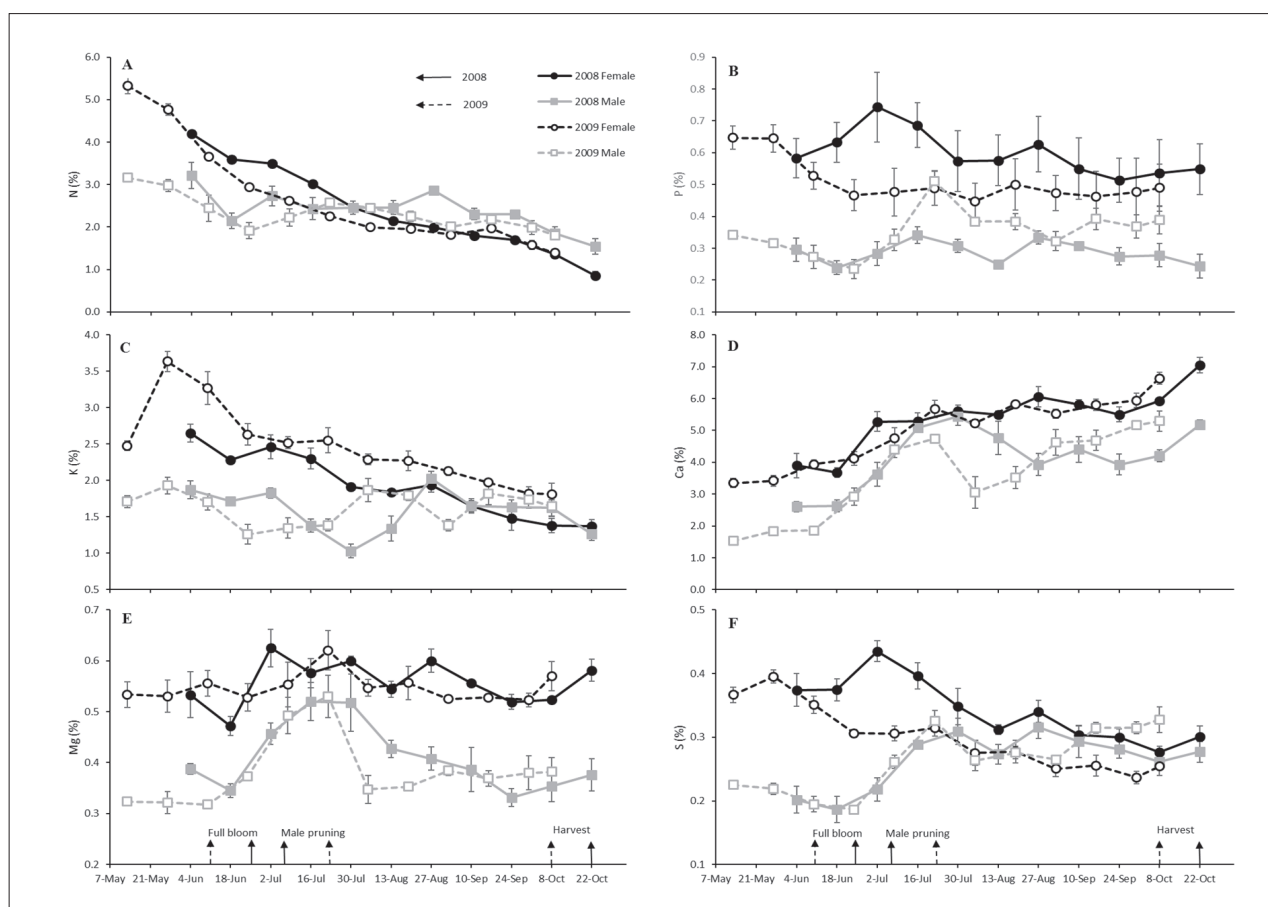


FIGURE 1. Effect of year, sample date, and gender on the concentration of macronutrients in indeterminate leaves of male and female hardy kiwifruit (*Actinidia arguta* ‘Ananasnaya’) over the growing season at the North Willamette Research and Extension Center, Aurora, Ore., 2008–2009. A = nitrogen; B = phosphorus; C = potassium; D = calcium; E = magnesium; F = sulfur. Bars indicate standard error for each gender and shoot type combination ($n=3$ on each date). Key phenological and management stages for each year are labeled with an arrow as indicated in the legend.

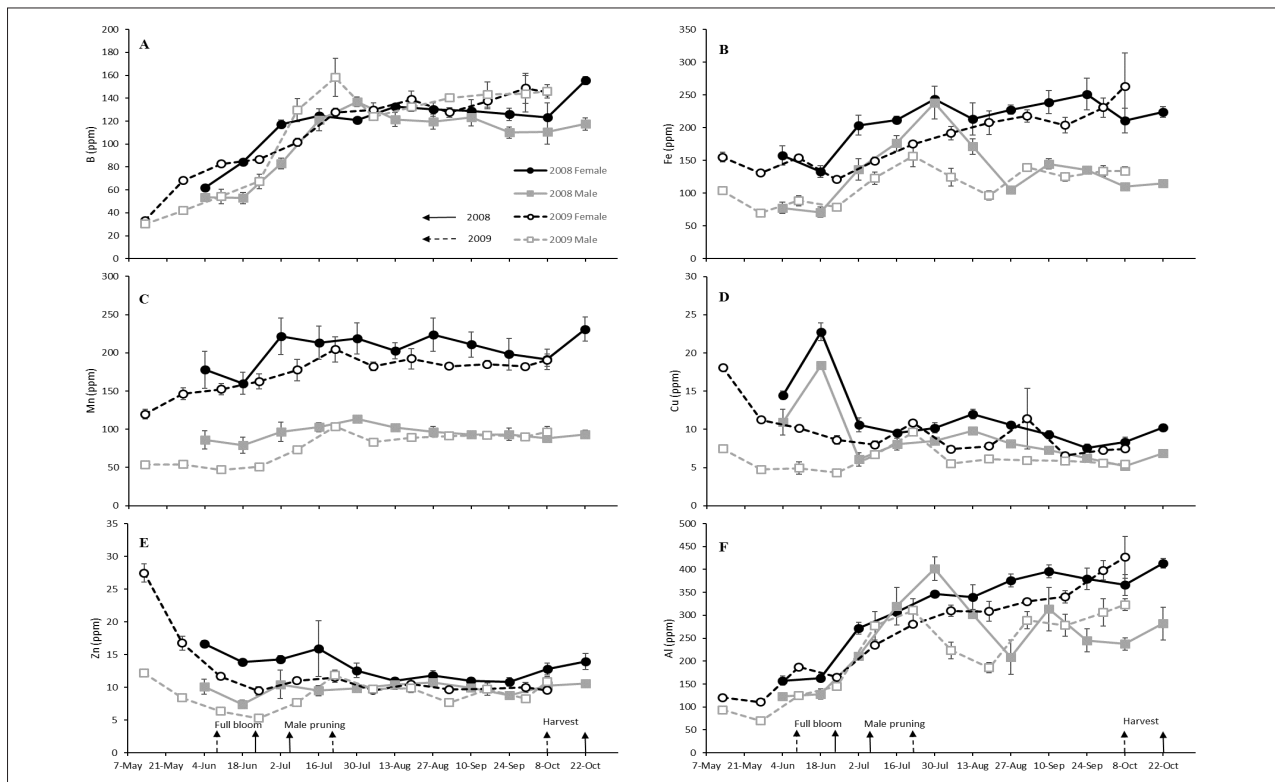


FIGURE 2. Effect of year, sample date, and gender on the concentration of micronutrients in indeterminate leaves of male and female hardy kiwifruit (*Actinidia arguta* ‘Ananasnaya’) over the growing season at the North Willamette Research and Extension Center, Aurora, Ore., 2008–2009. A = boron; B = iron; C = manganese; D = copper; E = zinc; F = aluminum. Bars indicate standard error for each gender and shoot type combination (*n* = 3 on each date). Key phenological and management stages for each year are labeled with an arrow as indicated in the legend.

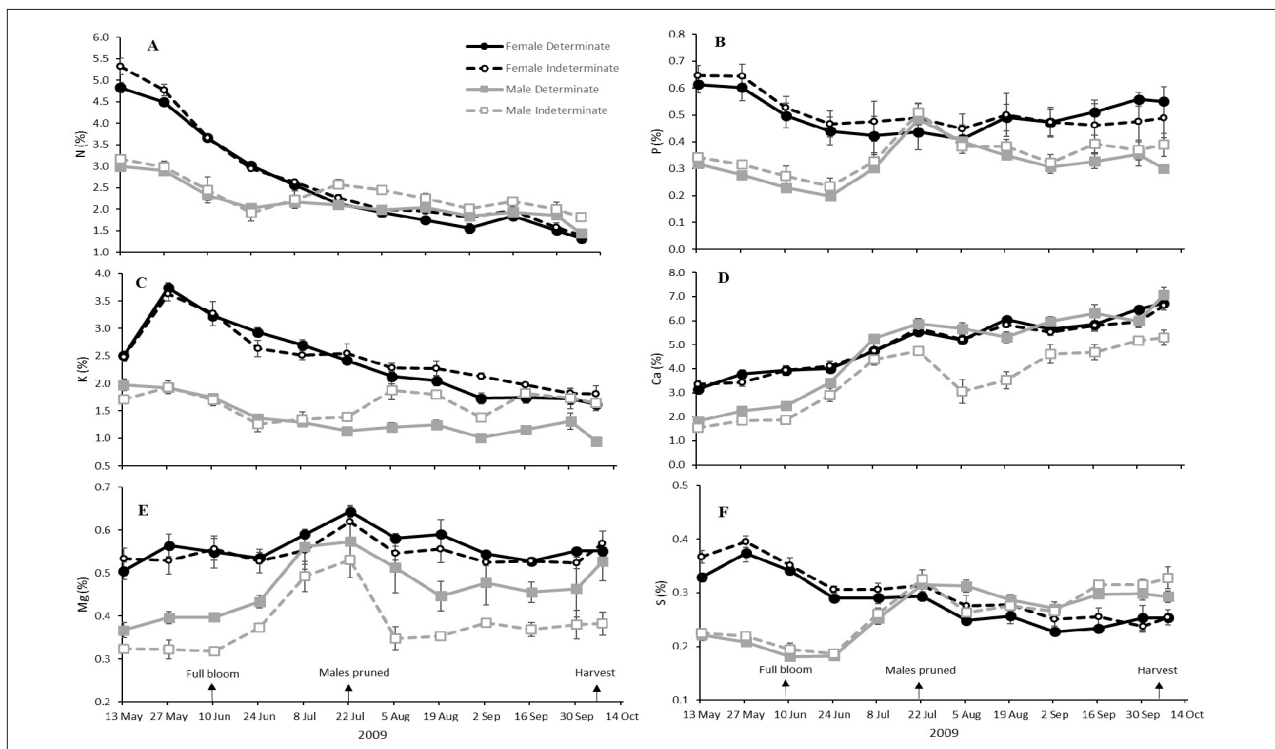


FIGURE 3. Effect of sample date, gender, and shoot type on the concentration of macronutrients in indeterminate and determinate shoot leaves of male and female hardy kiwifruit (*Actinidia arguta* ‘Ananasnaya’) over the growing season at the North Willamette Research and Extension Center, Aurora, Ore., 2009. A = nitrogen; B = phosphorus; C = potassium; D = calcium; E = magnesium; F = sulfur. Bars indicate standard error for each gender and shoot type combination (*n* = 3 on each date). Key phenological and management stages are labeled with an arrow.

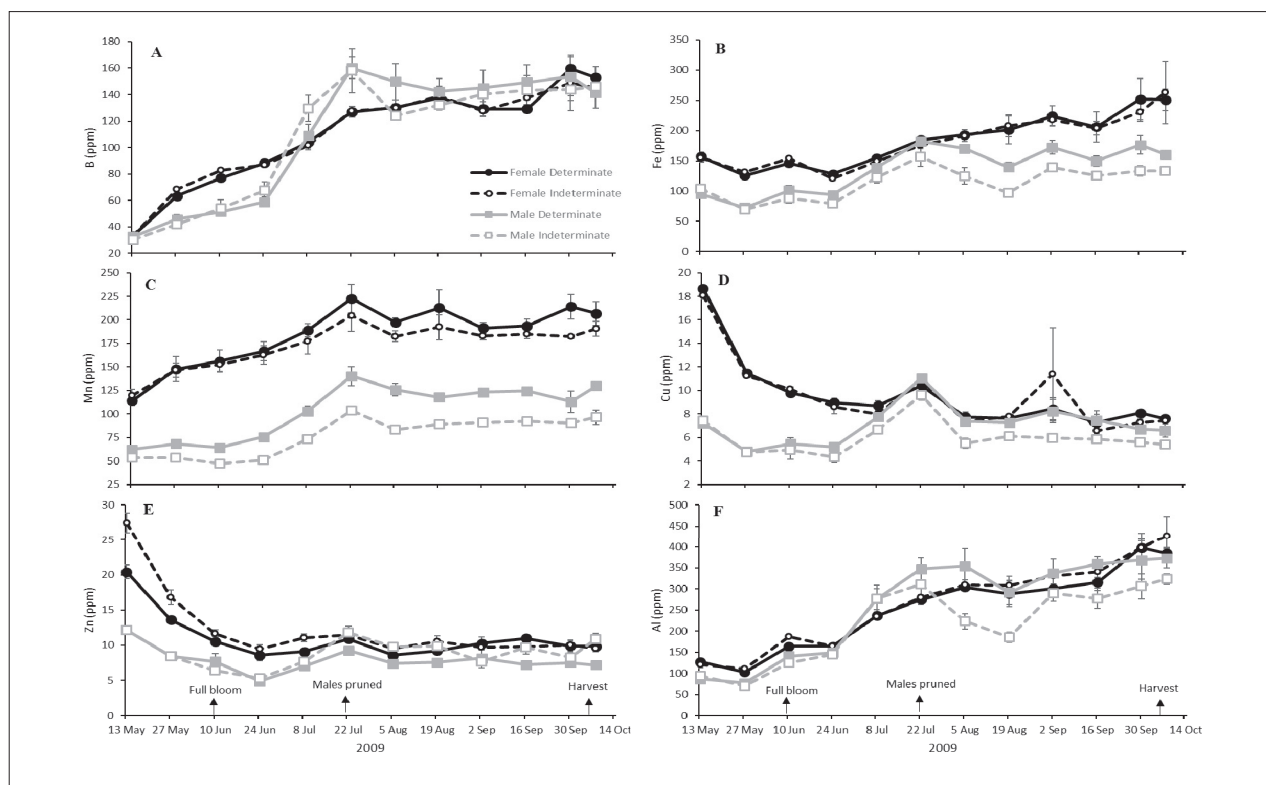


FIGURE 4. Effect of sample date, gender, and shoot type on the concentration of micronutrients in leaves of male and female (hardy kiwifruit (*Actinidia arguta* 'Ananasnaya') over the growing season at the North Willamette Research and Extension Center, Aurora, Ore., 2009. A = boron; B = iron; C = manganese; D = copper; E = zinc; F = aluminum. Bars indicate standard error for each gender and shoot type combination ($n=3$ on each date). Key phenological and management stages are labeled with an arrow.

logical comparison, data from samples when fruit averaged 18, 20, and 23 mm in diameter for each year were compared. These phenological stages were reached at different calendar dates in 2008 and 2009, as described in the results. Growth stages were chosen based on previous recommendations of sampling approximately 8 weeks past bloom, which corresponded to the 23 mm fruit diameter growth stage in this study. Calendar dates were chosen for early-, mid-, and late August based on the previous recommendations for August sampling. Mean comparison was conducted using LSMEANS and the PROC PLM procedure.

PROC UNIVARIATE and the Shapiro-Wilk procedure were used to assess normality of the data for all the aforementioned analyses. As the tissue concentration of many nutrients was not normal, a log transformation was used to improve homogeneity of variance and to assess proportional effects. Data were back transformed for presentation.

Results and discussion

Year effect

In 2009, specific growth stages or vine phenology were 2 to 3 weeks advanced compared to 2008 with higher growing degree day (GDD) accumulation on any given calendar date after early April, despite fewer GDD accumulated at each phenological stage throughout the season except for at harvest (key stages shown in Table 1). Degree day modeling in perennial crops is difficult and models, including date or phenological stage from which GDD accumulation starts, as well as upper and lower threshold temperatures, must be adapted to individual species and cultivars in order to be accurate (Day et al., 2008; Kirk and Isaacs, 2012; Snyder et

al., 1999). Phenological development can also be impacted by other factors such as soil moisture (Miller et al., 2001). Hall et al. (1996) proposed that temperature has less impact on mid- to late-season kiwifruit development compared to early season growth (bloom to mid-season). Due to these differences in growth and weather, sampling dates were analyzed by year rather than for a year effect. However, we present the seasonal patterns of change in nutrients for indeterminate leaves of female and male vines in both years (Figures 1 and 2). With advanced phenology in 2009, it was expected that there would be a shift in the pattern of change for each nutrient reflecting that 2 to 3 week difference, and this was seen in males and females for K, in females for P, S, and Cu, and in males for Al. However, this shift was not seen in many nutrients, including N, Ca, Mg, B, Fe, Mn, or Zn, where similar patterns and timings of nutrient shifts were seen within males and females across both years. Nutrient concentrations were generally lower in 2009 than 2008 for some portion of the season in both males and females, except for K (higher in 2009 females), Mg, and B (similar levels in both years) (Figures 1 and 2).

Gender influence

Female and male vines had significantly different leaf concentrations of most nutrients for some or all of the season (Figures 1–4). Female vines generally had higher concentrations of leaf nutrients than males, particularly in the early to mid-season. For N, K, Ca, S, Cu, and Zn, these differences were most pronounced from May (the beginning of leaf sampling) to late June/early July, when concentrations became more similar between genders. However, for P, Mg, and Fe there was an effect of gender in the early- and late season,

TABLE 1. Dates on which leaf tissue samples were collected as related to stage of plant development (phenology) for key stages of female hardy kiwifruit grown at Oregon State University's North Willamette Research and Extension Center (Aurora, OR). Males were sampled on the same dates as females.

Stage of development	Sample dates		Weeks relative to 6 mm flower bud	GDD ² from January 1	
	2008	2009		2008	2009
6 mm flower bud	4 June	13 May	0	518	340
Full bloom	25 June	10 June	+ 3	730	724
Green fruit 15 mm	16 July	24 June	+ 6	1138	913
Green fruit 18 mm	30 July	8 July	+ 8	1353	1133
Green fruit 20 mm	13 Aug	22 July	+ 10	1599	1385
Green fruit 23 mm	27 Aug	5 Aug	+ 12	1851	1699
50–75% red fruit, 5% yellow leaf	8 Oct	16 Sep	+ 18	2421	2386
Harvest	22 Oct	8 Oct	+ 20	2499	2614

²Cumulative growing degree days (GDD) using base 50°F (10°C) and a maximum of 86°F (30°C).

while males and females had similar levels during the month of July. Leaf Mn concentration was consistently higher in female vines, while B and Al were similar between genders throughout the season. Seasonal changes for each nutrient are described in more detail below.

Shoot type

Differences between shoot types predominated in male vines. While some significant differences between determinate and indeterminate shoots were found in female vines (for example, in mid- to late-season K and Mn), for most nutrients there were few instances throughout either season where shoot type had an impact on leaf nutrient concentration (Figures 3 and 4). When there was a gender × shoot type interaction, it was often influenced by the differences in male shoot types rather than in females (data not shown). In male vines, differences in shoot types occurred particularly after summer pruning, when nutrient concentrations of indeterminate shoots dropped rapidly for Ca, Mg, B, Cu, and Al, and somewhat for P, S, and Fe. These reductions in nutrient concentration were likely from dilution as rapid shoot re-growth after pruning was competing for less mobile nutrients. As leaves aged, concentrations of many of these nutrients, but especially Ca, increased, similar to findings in grape (*Vitis vinifera* L.; Christensen, 1969; Pradubsuk and Davenport, 2010) and apple (*Malus domestica* Borkh.; Kucukyumuk and Erdal, 2011; Nachtigall and Dechen, 2006).

In 2008, male vines were pruned more severely than in 2009 such that insufficient determinate leaves were present on 3 subsequent sampling dates and the drop in nutrient concentration on indeterminate leaves was about 2 weeks later than in 2009. New growth may have been slower to emerge after the particularly severe prune, thus older leaves on determinate shoots were being sampled in 2008. In addition, supplemental fertilizer was applied to male vines in the weeks prior to summer pruning in 2009 only, which may have increased the rate of growth of new shoots in that year. Notably, leaf K concentration of male indeterminate shoots increased at this time in 2009 (Figure 3), and followed a similar delayed pattern in 2008 (data not shown).

Seasonal nutrient patterns

In order to determine a suitable leaf sampling time, patterns of nutrient change across the growing season were examined. The most rapid period of leaf area accumulation is in the first 8 weeks after leaf emergence (Smith et al., 1987). In this study, sampling began after this period, when small

flower buds were formed, and continued until harvest was completed.

Leaf N gradually declined throughout the season in female vines (Figure 1), with indeterminate and determinate shoot leaves following similar patterns in both years (2009 shown in Figure 3). The most rapid decline in female vines occurred in the early season when shoots are still growing quickly and N is moving to the fruit at the highest rate (Clark and Smith, 1988). The rate of change decreased starting in early August. In 2008, leaves from different shoot types did not differ on any dates (data not shown), whereas in 2009 there were more differences, particularly in male vines (Figure 3). In male vines, leaves showed a decline in N concentration in the early season, then an increase around the time of pruning and another gradual decline throughout the rest of the season (Figure 3). Differences between male and female vines may be attributed not only to summer pruning in males, but also the movement of N from leaves to fruit in female vines (Smith et al., 1987).

Leaf P fluctuated somewhat throughout the season, but leaf concentration was similar at the beginning and end of the season. Plant gender had the most significant effect on leaf P, with female vines (indeterminate and determinate shoots) having higher concentrations on many sample dates in 2008 and 2009 than males, particularly in early season (Figures 1 and 3). This is contrary to Lalatta et al. (1990) who found that males had higher leaf P than females, though it is not clear in their study how male vines were pruned, which may have impacted leaf P. In the present study, males showed a decline in leaf P for both shoot types after pruning. The decrease in leaf P in late spring/early summer in female vines may be related to translocation to the flowers, which are a sink for P (Hongwen, 2016). Shoot type had a significant effect on leaf P concentration in the early season of 2009, but differences were minimal the rest of the season (Figure 3).

Leaf K concentration declined through the season in both shoot types of female vines, but leaves on determinate shoots were either higher (most of 2008 and early 2009) or lower (mid- to late-season, 2009) than on indeterminate shoots (Figure 3). More fluctuation in leaf K was seen in male vines, where leaf K declined in early season for both shoot types, then increased in mid-season in indeterminate and determinate leaves in 2008, or remained steady in leaves on determinate shoots in 2009 (Figure 3). Leaf K concentration was higher in female vines than males, particularly in early season (Figures 1 and 3). As K is present in notably high concentrations in the fruit compared to other fruit crops (along

with Ca; Ferguson, 1980) it follows that female vines may have higher leaf K to support their crop.

Leaf Ca increased overall in both genders and shoot types in 2008–2009, similar to what was reported in *A. deliciosa* (Clark et al., 1987). Female vines had a higher leaf Ca concentration than male vines on most dates (Figures 1 and 3), except for determinate shoots in males in 2009 (Figure 3). Transpiration from the fruit of female vines until 6 to 8 weeks after fruit set may, in part, be responsible for higher leaf Ca in female vines, as it requires the plant to draw more Ca from the soil (Xiloyannis et al., 2001) and the fruit becomes a sink for Ca until the end of cell division (Ferguson, 1980), estimated as early August in Oregon (Tiyayon and Strik, 2004). In contrast, Lalatta et al. (1990) found that *A. deliciosa* males had higher leaf Ca than females in shoots that were likely indeterminate. Similar to leaf P, the differing results could be due to time or severity of male summer pruning. Leaf Ca concentration in determinate and indeterminate shoots in male vines increased to mid-season, then declined (most likely due to summer pruning as previously described) before increasing again into the late season (Figure 3). Differences between shoot types were much more pronounced in males than females, especially after pruning when indeterminate leaf Ca decreased rapidly, a pattern that has also been seen in summer-pruned peaches (*Prunus persica* L. Batsch; Huett et al., 1997).

Leaf Mg was relatively stable in female vines, with little effect of shoot type (Figure 3). Larger differences in shoot types were seen in male vines, especially after the decline related to summer pruning measured in both determinate and indeterminate shoots. Female vines had higher leaf Mg concentration overall compared to male vines (Figures 1 and 3).

Leaf S concentration decreased slightly over the season in female vines and increased in male vines, with the largest difference between genders in the early season. Shoot type rarely impacted leaf S. Concentrations were quite similar between years in males, while leaf S in female indeterminate shoots was higher in 2008 for much of the season (Figures 1 and 3).

Male and female vines showed similar patterns in leaf B concentration: a steady increase in leaf B that stabilized mid- to late-season. Female vines had higher leaf B in early season, but there were few significant effects of gender or shoot type later in the season (Figure 4). Boron deficiency is a common concern in Oregon (Hart et al., 2006) due to alluvial soils that are naturally low in B where kiwifruit and other berries are typically grown. Deficiency can lead to decreased seed number and berry size (Sotomayor et al., 2010). Growers typically apply supplemental B on an annual basis to achieve sufficient leaf tissue concentrations in other crops such as blueberry (*Vaccinium corymbosum*, Hart et al., 2006), however, kiwifruit tissue levels in this study were in the excessive range indicated by Warrington and Weston (1990) despite having low to moderate soil B (Table S1; Horneck et al., 2011) and no foliar B applications. No toxicity symptoms were seen, and Decorte et al. (2015) also found one cultivar ('Issai') of hardy kiwifruit that had similarly high leaf concentrations of B.

Leaf Mn concentration either had little change (2008) or increased slightly through the season (2009) for both male and female vines (Figure 2). Female vines had higher leaf Mn concentration than males across both seasons and leaf Mn was higher in the early season of 2008 than 2009 (Figures 2 and 4). Shoot type had a significant effect on leaf Mn mainly in male vines in 2009 (Figure 4).

Leaf Zn and Cu concentrations showed similar patterns of a steep (female vines) or moderate (male vines) decline between the first 2 to 3 sampling dates, then remained mainly stable for the rest of the season (Figures 2 and 4). In 2009, sampling started earlier, enhancing the steep decline, whereas in 2008, less of a dramatic decline was observed (Figure 2). A peak in leaf Cu was present for both male and female vines in mid-June. For both nutrients, female vines had higher leaf concentrations in early season, then were similar to males while shoot types did not have a consistent effect (Figure 4).

Leaf Fe and Al showed similar patterns of increased concentration from the beginning to the end of the season, but different patterns were observed in male and female vines (Figures 2 and 4). In female vines, leaf Fe and Al generally increased steadily through the season, leveling out toward the end of 2008 while increasing until the end of the 2009 season (Figures 2 and 4). In males, however, leaf Fe and Al declined after summer pruning, before gradually increasing and leveling out until the end of the season. The largest effect of shoot type was seen in male vines (Figure 4), likely because of the effect of pruning off significant amounts of growth during the season and subsequent dilution due to rapid shoot growth. While there are no sufficiency standards for Al, likely due to high amounts of soil Al usually present, leaf Al could be an indicator of soil conditions in need of modification. Soil pH lower than desirable for kiwifruit (5.5 to 6; Strik, 2005) can lead to increased Al uptake and toxicity symptoms due to competitive uptake with other cations.

Leaf sampling time

Since leaf concentrations of several nutrients were significantly different between male and female vines, sampling should either be restricted to only female vines or conducted separately if the nutrition of male vines is of concern. We analyzed for optimal sampling time for females only.

Contrary to some past recommendations (Clark et al., 1986), early season leaf sampling is not ideal for hardy kiwifruit in our region due to rapidly changing levels of nutrients including N, K, Ca, B, Cu, Zn, and Al (Figures 1 and 2). In addition, sampling based on phenology at 8, 10, or 12 weeks post 6 mm flower buds (similar to sampling methods suggested by Velemis et al., 1995) resulted in a year \times phenological stage interaction for nearly all nutrients (Table 2), indicating that testing results could not clearly be interpreted from year to year even when sampled at the same stage. It is also beneficial to growers to have a longer window of time in which nutrient concentrations are stable and sampling can be conducted. However, all nutrients were significantly different in at least one year between the phenological stages presented here (Table 2), limiting the sampling window to a very short period of time when vines are at the exact same phenology each year.

Alternatively, comparing nutrient concentrations based on calendar date showed that nutrient concentrations were quite stable from mid- to late-August (Table 3). According to Tiyayon and Strik (2004), this corresponds closely to the lag phase of fruit development when seeds are maturing but cell division and expansion slows. Similarly, in blueberry and most blackberry (*Rubus* sp.) types grown in Oregon, leaf nutrient sampling was best conducted during a certain range of calendar dates rather than based on phenology, despite large differences in growth types and fruiting seasons (Strik and Vance, 2015, 2017). Sampling in early August was not ideal for Ca, Mg, Mn, or Zn in at least one year because concentra-

TABLE 2. Effect of year, phenological stage of development, and shoot type on female hardy kiwifruit (*Actinidia arguta* 'Ananasnaya') at the North Willamette Research and Extension Center, Aurora, Ore., 2008–2009.

	%										ppm					
	N ^z	P	K	Ca	Mg	S	B	Fe	Mn	Cu	Zn	Al				
Tissue standards ^y	2.2–2.8	0.18–0.22	1.8–2.5	3.0–3.5	0.3–0.4	0.25–0.45	40–50	80–200	50–100	10–15	15–30	n/a				
Year (Yr)																
2008	2.25	0.59	1.99	5.71	0.59	0.34	129	237a ^x	226	10.8a	12.0a	355a				
2009	2.25	0.45	2.43	5.19	0.59	0.29	120	175b	195	8.9b	10.1b	273b				
Stage of development																
18 mm fruit	2.54a	0.50	1.99b	5.44bcd	0.60abc	0.57bcd	124a	243a	183b	10.0b	8.3c	340b				
20 mm fruit	2.22b	0.52	2.01b	5.60bc	0.56d	0.63a	135a	180b	218a	11.9a	10.7b	278d				
23 mm fruit	1.98c	0.53	1.98b	6.09a	0.61ab	0.56cd	128a	234a	190b	10.4b	11.3b	307c				
Shoot type (St)																
2008	2.30a	0.50b	2.09b	5.43	0.60	0.34a	125	212	220a	9.8	10.8	314				
2009	2.20b	0.53a	1.89c	5.47	0.58	0.33a	124	200	202b	9.8	11.3	315				
Determinate																
Indeterminate																
Significance ^w																
Yr	NS	NS	NS	NS	NS	NS	NS	0.0074	NS	0.0144	0.0192	0.0103				
Stage	<0.0001	NS	0.0077	0.0022	NS	NS	0.0038	NS	NS	<0.0001	0.0038	0.0002				
Yr x Stage	NS	NS	0.0105	0.0119	0.0005	0.0285	0.0424	0.0083	0.0028	0.0439	0.0023	0.0469				
St	NS	0.0218	NS	NS	NS	NS	NS	NS	<0.0001	NS	NS	NS				
Yr x St	0.0257	NS	0.0266	NS	NS	0.0419	NS	NS	NS	NS	NS	NS				
Stage x St	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
Yr x Stage x St	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				

^z N = nitrogen; P = phosphorus; Mg = magnesium; K = potassium; Ca = calcium; S = sulfur; B = boron; Fe = iron; Mn = manganese; Cu = copper; Zn = zinc; Al = aluminum.

^y Recommended sufficiency range for fuzzy kiwifruit (*Actinidia deliciosa* 'Hayward') when sampled in August (Clark et al., 1986); no sufficiency levels are available for aluminum (n/a).

^x Means followed by the same letter within treatment or the interaction are not significantly different (LSMeans) ($P > 0.05$).

^w Non-significant ("NS") or actual P value provided when significant by analysis of variance.

TABLE 3. Effect of year, sampling date, and shoot type on female hardy kiwifruit (*Actinidia arguta* 'Ananasnaya') during the recommended sampling time at the North Willamette Research and Extension Center, Aurora, Ore., 2008–2009.

	%											ppm				
	N ^z	P	K	Ca	Mg	S	B	Fe	Mn	Cu	Zn	Al				
Tissue standard ^y	2.2–2.8	0.18–0.22	1.8–2.5	3.0–3.5	0.3–0.4	0.25–0.45	40–50	80–200	50–100	10–15	15–30	n/a				
Year (Yr)																
2008	2.25a ^x	0.59	1.99	5.71	0.59a	0.34	129	237a	226	10.8	12.0a	355a				
2009	1.83b	0.47	2.10	5.58	0.56b	0.26	132	206b	193	8.4	9.6b	307b				
Sampling date																
Early August	2.22a	0.49b	2.10	5.44bc	0.60ab	0.56cd	127	218	228a	8.8	13.4a	323				
Mid August	2.05b	0.54a	2.08	5.60abc	0.56cd	0.57bc	136	219	218ab	9.8	11.2b	326				
Late August	1.84c	0.55a	1.96	6.09a	0.61a	0.53d	128	227	234a	10.1	11.3bc	344				
Shoot type (St)																
Determinate	2.30a	1.74c	2.09ab	5.66	0.58	0.29	131	226	219a	9.3	10.7	327				
Indeterminate	2.20a	1.92b	1.89c	5.62	0.56	0.30	130	217	200b	9.9	10.9	335				
Significance ^w																
Year (Yr)	0.0093	NS	NS	NS	0.0243	NS	NS	0.0039	NS	NS	0.0336	0.0039				
Date	<0.0001	0.0243	NS	0.0043	NS	NS	NS	NS	NS	NS	NS	NS				
Yr x Date	NS	NS	NS	0.0192	0.0084	NS	NS	NS	0.0397	NS	0.0034	NS				
Shoot type (St)	NS	NS	NS	NS	NS	NS	NS	NS	<0.0001	NS	NS	NS				
Year x St	0.0027	NS	0.0001	NS	NS	NS	NS	NS	NS	NS	NS	NS				
Date x St	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS				
Yr x Date x St	0.0113	NS	0.0012	NS	NS	NS	NS	NS	NS	NS	NS	NS				

^z N = nitrogen; P = phosphorus; Mg = magnesium; K = potassium; Ca = calcium; S = sulfur; B = boron; Fe = iron; Mn = manganese; Cu = copper; Zn = zinc; Al = aluminum.

^y Recommended sufficiency range for fuzzy kiwifruit (*Actinidia deliciosa* 'Hayward') when sampled in August (Clark et al., 1986); no sufficiency levels are available for aluminum (n/a).

^x Means followed by the same letter within treatment or the interaction are not significantly different (LSMeans) ($P > 0.05$).

^w Non-significant ("NS") or actual P value provided when significant by analysis of variance.

tions were significantly different from the mid- and late-August dates, whereas these nutrients along with P, K, S, B, Fe, Cu, and Al were all stable between the mid- and late-August dates. Neither leaf N nor Mg concentration were stable using either the phenological or calendar-based sampling methods. Based on a visual assessment of these nutrients (Figures 1 and 3), it appeared that September may be a period where these nutrients were stable, but further statistical analysis showed this was not the case for N, and that Mg was only stable in both years from mid- to late-September (Table 4). Unfortunately, it is likely not practical to sample at a different time of year for one nutrient, unless there is a specific concern. Leaf N concentration was in continuous decline throughout the season, so leaf analysis results from samples taken in August should be carefully combined with visual assessments of the plants, yield, and fruit quality to determine whether any changes in N fertilization are required.

Sampling in the mid- to late-August time period largely eliminated the effect of shoot type on leaf nutrient concentration, with the exception of Mn where determinate shoots had significantly higher levels than indeterminate shoots. In addition, there were interactions for year \times sampling date and year \times sampling date \times shoot type for leaf N and K resulting from indeterminate shoots having higher concentrations than determinate shoots in 2009, but lower in 2008 (Table 3). When shoot types do not differ in leaf nutrient concentration, there is no need to differentiate between shoot types while sampling, making the process easier and yielding more consistent results.

Male vine nutrition was noticeably impacted by summer pruning and may be better suited to phenology-based sampling rather than calendar-based sampling, similar to primocane fruiting blackberries which are routinely tipped in Oregon to encourage branching and increased yield (Strik, 2015).

Comparison to *A. deliciosa* standards

The patterns of change observed for leaf nutrients in our study are similar to the findings of Decorte et al. (2015) for *A. arguta* and also follow similar trends reported in *A. deliciosa* (Clark et al., 1987; Cresswell, 1989; Smith et al., 1987). Actual concentrations of nutrients found by Decorte et al. (2015) varied by cultivar (and did not include 'Ananasnaya') but were generally lower than those found in the present study for leaf Ca, Mg, S, B, Fe, and Mn concentration. Leaf P, K, and Cu concentrations were similar between our studies, while leaf N and Zn were slightly lower in our study.

In our study, only leaf S concentration was within the sufficiency range suggested by Clark et al. (1986) for the August sampling time across all years, genders, and shoot types (Tables 2 and 3). When only female vines are considered, leaf K and S were considered sufficient, N and Cu were sufficient in some cases but were otherwise below the range, P, Ca, Mg, B, Fe, and Mn were above the sufficiency range (with B in the "excess" category), and Zn was considered deficient despite no apparent symptoms of deficiency or toxicity on the plants. There is no current sufficiency standard for leaf Al.

Velemis et al. (1995) suggested revised sufficiency standards for *A. deliciosa* in Greece, providing ranges more similar to those found in our study for P, Mg and Mn, but not fully encompassing the levels of N and K in our study. Leaf B and Zn concentration would still be considered excessive (B) or deficient (Zn) using these revised standards. Comparing our results to those for *A. arguta* in Belgium (Decorte et

TABLE 4. Effect of year, sampling date, and shoot type on leaf nitrogen (N) and magnesium (Mg) in female hardy kiwifruit (*Actinidia arguta* 'Ananasnaya') in September at the North Willamette Research and Extension Center, Aurora, Ore., 2008–2009.

	%			
	N ^z		Mg	
<i>Year</i>				
2008	1.86		0.56	
2009	1.71		0.53	
<i>Sampling date</i>	2008	2009	2008	2009
Early September	2.00a ^y	1.69d	0.61a	0.53b
Mid September	1.84bc	1.90ab	0.54b	0.53b
Late September	1.75cd	1.54e	0.52b	0.54b
<i>Shoot type (St)</i>	<i>Early</i>	<i>Mid</i>	<i>Late</i>	
Determinate	1.78a	1.86a	1.65b	0.55
Indeterminate	1.91a	1.88a	1.64b	0.54
<i>Significance^x</i>				
Year (Yr)	NS		NS	
Date	0.0001		0.0021	
Yr \times Date	0.0005		0.0023	
St	NS		NS	
Yr \times St	NS		NS	
Date \times St	0.0057		NS	
Yr \times Date \times St	NS		NS	

^zN = nitrogen; Mg = magnesium.

^yMeans followed by the same letter within treatment or the interaction are not significantly different (LSMeans) ($P > 0.05$).

^xNon-significant ("NS") or actual P value provided when significant by analysis of variance.

al., 2015) and *A. deliciosa* (Clark et al., 1986; Velemis et al., 1995) suggests that levels of most nutrients differ by cultivar, which has also been seen in other crops including apple (Kucukyumuk and Erdal, 2011), blueberry (Strik and Vance, 2015), and blackberry (Fernandez-Salvador et al., 2015; Strik, 2015), as well as growing conditions and that region specific nutrient standards may be beneficial.

While the concentration of nutrients in the fruit was not analyzed in this study, Latocha et al. (2015) found that *A. arguta* had higher fruit P, Ca, and Fe than *A. deliciosa*. This might suggest that leaf P, Ca, and Fe would be lower in *A. arguta* due to competition for nutrients, but the opposite was found. In addition, fruit K and Cu were lower in *A. arguta* (Latocha et al., 2015), and the leaf nutrient concentration of K and Cu were sufficient or close to sufficient (Clark et al., 1986) in our study. These results suggest that there may be different relationships between vegetative and fruit nutrient requirements in *A. arguta* compared to *A. deliciosa*, and highlights that existing standards for *A. deliciosa* do not fit the typical range of leaf nutrient concentrations for *A. arguta* in Oregon.

Conclusion

Despite the 2 to 3 week shift of plant phenology between years, many nutrients did not show the same shift in leaf concentration throughout the season. Instead, changes in nutrient levels may be related to environmental factors such as day length. Because the majority of leaf nutrients did not rapidly change in concentration during mid- to late-August,

and since shoot types did not differ in leaf nutrient concentration in many cases during this sampling window, we suggest that this is the most appropriate time for leaf sampling female vines.

Sampling female vines separately from male vines is critical given the large differences in nutrient concentrations we found in August and across the growing season. Fortunately, this sampling time makes distinguishing between males and females easy as fruit has not yet been harvested from female vines and males bear no fruit. Unless there are visual or physical cues that the nutrient status of male vines is deficient or in excess, it may not be cost effective or critical to sample male vines, but care should be taken to avoid these vines when sampling females.

The results reported here for *A. arguta* grown in Oregon did not match the sufficiency standards currently in use for *A. deliciosa* except for leaf K and S in females during the mid-to late-August period. Until further research is conducted to determine leaf nutrient sufficiency standards for cultivars and growing conditions in this region, the best recommendation is to maintain detailed records of leaf tissue sample results and yield annually as well as visual observations of shoot growth and leaf color (symptoms) for determination of best nutrient management practices.

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