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# Interactive effects of water and nitrogen supply on growth, biomass partitioning, and water-use efficiency of young apple trees

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**Drought and low concentration of soil nitrogen (N) limit crop growth and production in arid and semi-arid regions. Improving the efficient use of these limited resources is an important challenge. We tested one-year-old trees of three apple cultivars (*Malus domestica* cv. 'Golden Delicious', 'Naganofuji No. 2', and 'Pink Lady') grafted on *M. hupehensis* to determine how water and N supplies influence growth, biomass production, and water-use efficiency (WUE). Two watering regimes (80 and 50% field water capacity) and three N supplies (N0: 0, NL: 75, and NH: 150 mg N kg<sup>-1</sup> soil) were used. Drought dramatically diminished plant height (PH), basal diameter (BD), biomass production, total leaf area (LA), specific leaf area (SLA), leaf relative water content (RWC), photosynthetic rate ( $P_n$ ), stomatal conductance ( $g_s$ ), and WUE, but increased root/shoot ratio. NL significantly increased PH, BD, LA, SLA, RWC,  $P_n$ ,  $g_s$ , and WUE, but NH reduced or had little influence on these parameters.  $P_n$ ,  $g_s$ , LA, and root biomass significantly and positively correlated with WUE. Our results suggest that water and N are correlated, and NL enhance drought tolerance and WUE through increased photosynthetic capacity and water uptake. Thus, an appropriately low N supply would be recommended under dry condition, but excess N supply should be avoided.**

**Key words:** Biomass production, drought, growth, *Malus domestica*, nitrogen, water-use efficiency.

## INTRODUCTION

Arid and semi-arid regions generally have diminished primary crop productivity due to the combination of low, unpredictable water supply and reduced soil-nitrogen concentrations (Hooper and Johnson, 1999; Monclus et al., 2006). Ecosystem managers are challenged to improve the efficient use of finite resources and alleviate drought-induced injury in order to benefit vegetative growth (Wu et al., 2008). James et al. (2005) suggested that modifications in nutrient availability would have a greater impact on improving community and ecosystem properties than changes in water availability or efficiency

of water utilization. Fertilization could increase the availability of limited nutrients, thereby altering system properties. Such efforts might be a practical way to stimulate plant growth, enhance stress tolerance, and raise the efficiency of finite resources in infertile and dry environments (Patterson et al., 1997; Singh et al., 2005; Wu et al., 2008).

Generally, plants adapt to dry conditions by developing a strong below-ground system for gathering limited soil resources, showing higher resource-use efficiencies and greater biomass allocation to root (Patterson et al., 1997; Chaves et al., 2002). An appropriate N supply could stimulate plant growth, improve water-use efficiency (WUE), and alleviate the effects of drought stress (Wu et al., 2008; Brueck et al., 2010). However, excess N applications can reduce biomass allocation to root (Patterson et al., 1997), increase leaf sensitivity to stress

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**Table 1.** Physical and chemical characteristics of substrate soil.

pH	Bulk density (g cm <sup>-3</sup> )	Gravel content (%)	Water field capacity (%)	Organic matter (g kg <sup>-1</sup> )	Available N (mg kg <sup>-1</sup> )	Available P (mg kg <sup>-1</sup> )	Available K (mg kg <sup>-1</sup> )
8.31	0.84	14.28	18.27	0.95	50.33	30.97	50.65

(Tan and Hogan, 1997), and lead to depressed plant growth (Pharis and Kramer, 1964) under drought conditions. Adequate N supply could enhance plant drought tolerance (Chapin, 1991; Arora et al., 2001), whereas additional amounts of N do not always play a positive role in counteracting the adverse effects of drought (Ashraf et al., 2001; Wu et al., 2008; Song et al., 2010).

In their frequent co-relation, a lack of water not only directly constrains plant growth and survival, but also indirectly influences plant responses to nutrients (Wu et al., 2009; Song et al., 2010). There is abundant evidence that drought stress reduces nutrient uptake and their availability for growth (Misra and Tyler, 2000; Wu et al., 2009; Song et al., 2010). Likewise, increased levels of soil water positively affect nutrients input, decomposition, mineralization, and physical transport, all of which can increase their availability to the plant (Zak et al., 1994; Burke et al., 1997). This interaction between soil nutrients and water suggests a trade-off between their respective efficiencies under dry and infertile conditions. Therefore, it is important to understand how soil water and nutrients act together to regulate plant growth in adverse environments. Such knowledge enables managers to improve resource-use efficiency, increase biomass accumulation, and alleviate drought-induced injury under water- and nutrient-limited conditions.

Apple (*Malus domestica* Borkh.) is one of the most economically important fruits worldwide. It is mainly cultivated in arid and semi-arid regions,

e.g. the Northwest Loess Plateau in China. Scarce water and low soil-N concentrations in that area are major limitations to fruit development. Glenn (2010) has reported that drought reduced rates of photosynthesis in 'Empire' apple trees, affected their WUE, while Yang et al. (2011) have demonstrated that drip irrigation and fertilization methods can influence growth, physiology, and WUE of young apple plants. Furthermore, different genotypes of *Malus* rootstocks show various responses to drought stress, as manifested by their biomass accumulations, allocations, and WUE (Ma et al., 2010). However, it is unclear whether there is a correlation between soil water and N supply for those parameters.

In the present study, we examined the interactive effects of soil water and N supply on growth, biomass production, and WUE of three apple cultivars. Our objective was to obtain information that can be used to improve fruit production and alleviate drought-induced injury when trees are grown under arid or semi-arid conditions. It was hypothesized that appropriate N supply could improve the adaptability of apple plants under dry condition.

## MATERIALS AND METHODS

### Plant material and experimental design

One-year-old apple trees of three cultivars (*M. domestica* cv. 'Golden Delicious', 'Naganofuji No. 2', and 'Pink Lady') grafted on *M. hupehensis* rootstocks were used in present study. All plants were greenhouse-grown in plastic pots (38

cm × 23 cm; 15 L) filled with a local topsoil:sand:grass peat mix (5:1:1, v:v:v) at Northwest A&F University, Yangling (34° 20' N, 108° 24' E), Shaanxi, China. Soil characteristics are presented in Table 1. Prior to the start of our experiments, all trees were irrigated daily and supplied weekly with 100% Hoagland's solution (pH = 6.5 ± 0.1).

After two months of growth under those well-watered conditions, we instituted different levels of watering and nitrogen supplies on 19 May 2010. Experiments were arranged in a completely random design, with six replicates (three plants per replicate) for two watering regimes (control, 80% field capacity) and drought (50% of FC) and three nitrogen treatments (control, N0; low N, NL; high N, NH). Soil field capacity was determined with a digital moisture recorder (ZTS-II; Zhejiang, China). A 10-mL solution containing 0.0, 1.5, and 3.0 g urea (46% N) (corresponding to 0, 75, and 150 mg N kg<sup>-1</sup> soil) was applied to the three different N treatments, respectively. To avoid N rapid loss, the solution was applied at 5 cm beneath the soil surface.

Surface evaporation was minimized by covering the pots with a 3-cm layer of sieved (2 mm) sand. As a control, four pots without plants per treatment were used to determine evaporative water loss from the soil surface throughout the experimental period. Transpiration water loss was evaluated gravimetrically by weighing all pots and re-watering on alternate days at 18:00 h. The amount of water added to each pot was defined as the difference between the weight of a re-watered pot and its weight 48 h later. To avoid edge effects, all pots were rotated weekly. These experiments were terminated on 19 July 2010.

### Measurements

At the end of the experimental period, plant height (PH) and basal diameter (BD) were recorded. Afterward, six plants were harvested from each treatment and divided into leaf, stem, and root portions to determine values for

**Table 2.** *F*-values and probability levels from univariate ANOVA for morphological and physiological variables of 3 apple cultivars under different watering and nitrogen regimes.

Variable	PH	BD	AB	RB	TB	R/S	LA	SLA	RWC	WUE	P <sub>n</sub>	g <sub>s</sub>
<i>F<sub>W</sub></i>	148.22***	94.50***	91.71***	89.17***	101.03***	112.13***	118.63***	78.49***	201.49***	95.59***	64.33***	32.56***
<i>F<sub>N</sub></i>	25.47***	16.61***	22.38***	19.37**	19.34***	12.26**	14.13***	52.16***	21.11***	31.31***	38.32***	36.25***
<i>F<sub>C</sub></i>	47.81***	21.18***	90.47***	63.63***	27.73***	5.17*	23.99***	67.77***	61.09***	55.69***	70.27***	38.59***
<i>F<sub>W × N</sub></i>	10.25**	8.58**	16.01**	5.63*	11.33**	4.03*	6.12**	10.11***	5.66*	31.30***	9.35**	10.05**
<i>F<sub>W × C</sub></i>	11.82**	9.22**	8.95*	1.46 <sup>ns</sup>	4.61*	2.20 <sup>ns</sup>	13.91***	2.54 <sup>ns</sup>	32.13***	5.69*	3.29*	9.53***
<i>F<sub>N × C</sub></i>	7.94*	3.28 <sup>ns</sup>	5.84*	10.36**	0.25 <sup>ns</sup>	0.44 <sup>ns</sup>	2.67*	9.31***	4.20*	3.56*	13.08***	1.36 <sup>ns</sup>
<i>F<sub>W × N × C</sub></i>	1.22 <sup>ns</sup>	1.79 <sup>ns</sup>	2.98 <sup>ns</sup>	1.19 <sup>ns</sup>	2.46 <sup>ns</sup>	0.73 <sup>ns</sup>	1.72 <sup>ns</sup>	1.46 <sup>ns</sup>	2.56 <sup>ns</sup>	3.40*	3.99**	1.21 <sup>ns</sup>

PH: Plant height; BD: basal diameter; AB: above-ground biomass; RB: root biomass; TB: total biomass; R/S: root shoot ratio; LA: total leaf area; SLA: specific leaf area; RWC: leaf relative water content; WUE: water-use efficiency; P<sub>n</sub>: photosynthetic rate; g<sub>s</sub>: stomatal conductance. *F<sub>W</sub>*: Watering regime effect; *F<sub>N</sub>*: nitrogen supply effect; *F<sub>C</sub>*: cultivar effect; *F<sub>W × N</sub>*: watering regime × nitrogen supply interactive effect; *F<sub>W × C</sub>*: watering regime × cultivar interactive effect; *F<sub>N × C</sub>*: nitrogen supply × cultivar interactive effect; *F<sub>W × N × C</sub>*: watering regime × nitrogen supply × cultivar interactive effect. Level of significance: ns,  $P > 0.05$ , \*,  $P \leq 0.05$ , \*\*,  $P \leq 0.01$ , \*\*\*,  $P \leq 0.001$ .

above-ground biomass (AB), root biomass (RB), total biomass (TB), and the root/shoot ratio (R/S, root biomass divided by shoot biomass). Materials were oven-dried at 70°C to a constant weight before measuring total dry weight for each tissue type. Total leaf area (LA) was obtained with an AM-100 Area Meter (Analytical Development Company, Hertsfordshire, UK). Specific leaf area (SLA) was calculated as LA/leaf dry weight.

WUE was defined as the ratio of dry biomass production to total water transpired during the experimental period. The initial average dry mass of plants was subtracted from their final biomass for WUE calculation. While calculating the amount of water transpired over time, evaporative loss from the pot was taken into account by subtracting the average amount of water loss from the control pots.

Leaf relative water content (RWC) and photosynthetic parameters were measured at 10, 20, 30, 40, 50, and 60th day of the treatment. Six fully expanded leaves were collected pre-dawn from the mid-canopy position of treated plants, and RWC was determined as described by Wu et al. (2008). Photosynthetic parameters were measured on the eighth leaf from the shoot apex, using a Li-Cor 6400 portable photosynthesis system (Li-Cor Inc., Lincoln, NE, USA). Photosynthesis rate (P<sub>n</sub>) and stomatal conductance (g<sub>s</sub>) were obtained from six plants per cultivar and treatment. Measurements were made on sunny days (9:00 to 11:00 h) at 1500 μmol m<sup>-2</sup> s<sup>-1</sup> PPFD, as provided by a Q-Beam (blue and red diode) light source. Leaf temperature and ambient water vapor pressure were

maintained at 28.7 ± 1.0°C and 1.30 ± 0.15 kPa, respectively. Mean values for RWC, P<sub>n</sub>, and g<sub>s</sub> recorded at the six time points were used to demonstrate the effect of soil water and N supply over the experimental period.

#### Statistical analysis

Analyses of variance (ANOVA) were used to evaluate treatment differences. Relationships among variables were determined using the Pearson's correlation coefficients test at 0.05 levels. All statistical analyses were performed using SPSS statistical software package (Standard released version 11.5 for Windows; SPSS Inc., IL, USA). Results were presented as mean ± standard deviation (SD) ( $n = 6$ ).

## RESULTS

### Interactive effect of cultivar, water, and N supply on single variables

Multivariate comparisons (Table 2) revealed the main and interactive effects of cultivar, soil water, and N supply on individual variables. All parameters were significantly impacted by the main effect of cultivar, water, and N. The W × N interaction significantly affected all parameters,

while the W × C interaction was significant for all except RB, R/S, and SLA. The N × C interaction significantly influenced all parameters except BD, TB, R/S, and g<sub>s</sub>, while the W × N × C terms were notable for WUE and P<sub>n</sub>.

### Growth and biomass partitioning

Drought stress, N supply, and their interaction significantly influenced PH, BD, AB, RB, TB, R/S, LA, SLA, and RWC at the end of these experiments (Table 2). Regardless of N supply, drought stress greatly diminished PH, BD, AB, RB, TB, LA, SLA, and RWC, but increased the value for R/S (Table 3). Under both watering regimes, NL significantly ( $P \leq 0.05$ ) enhanced PH, BD, AB, RB, TB, LA, SLA, and RWC for all cultivars. NH slightly decreased or had little effect on PH, BD, AB, TB, SLA and RWC, but caused significant ( $P \leq 0.05$ ) reductions in RB and LA under drought conditions. Under well-watered condition, the value of R/S was significantly ( $P \leq 0.05$ ) increased with the increase of nitrogen concentration. Under drought condition, NL

**Table 3.** Influence of N supply on plant height (PH, cm), basal diameter (BD, mm), above-ground biomass (AB, g), root biomass (RB, g), total biomass (TB, g), total leaf area (LA, 10<sup>3</sup> cm<sup>2</sup>), specific leaf area (SLA, cm<sup>2</sup> g<sup>-1</sup>), root/shoot ratio (RSR), and leaf relative water content (RWC, %) for 3 apple cultivars under well-watered (control) or drought condition.

Variable		'Golden delicious'		'Naganofuji No.2'		'Pink lady'	
		Control	Drought	Control	Drought	Control	Drought
PH (cm)	N0	60.13 ± 1.71	53.33 ± 2.31	57.62 ± 1.28	49.17 ± 2.46	55.21 ± 2.31	45.17 ± 1.66
	NL	70.16 ± 2.22*	65.92 ± 2.30*	68.35 ± 2.15*	62.64 ± 1.37*	67.08 ± 1.65*	59.02 ± 2.25*
	NH	61.71 ± 1.91	52.97 ± 2.17	56.29 ± 2.27	47.08 ± 2.32	53.11 ± 2.38	40.75 ± 2.54
BD (mm)	N0	7.25 ± 0.68	6.31 ± 0.51	6.75 ± 0.27	6.17 ± 0.55	6.43 ± 0.53	5.88 ± 0.61
	NL	9.12 ± 0.45*	7.46 ± 0.38*	8.30 ± 0.23*	7.21 ± 0.31*	7.76 ± 0.36*	7.05 ± 0.22*
	NH	7.49 ± 0.39	6.75 ± 0.42	6.71 ± 0.77	6.33 ± 0.58	6.52 ± 0.53	6.08 ± 0.38
AB (g)	N0	123.10 ± 7.04	75.32 ± 8.06	108.38 ± 12.2	66.54 ± 5.51	97.24 ± 10.2	66.59 ± 7.12
	NL	163.41 ± 5.59*	94.45 ± 6.82*	136.41 ± 4.61*	86.36 ± 6.10*	118.93 ± 6.19*	85.53 ± 4.28*
	NH	141.75 ± 5.63	73.52 ± 6.45	122.84 ± 5.28	71.49 ± 3.92	105.27 ± 5.89	60.71 ± 4.04
RB (g)	N0	54.41 ± 2.24	49.90 ± 1.52	49.86 ± 2.48	43.28 ± 2.24	51.40 ± 2.49	40.18 ± 2.75
	NL	70.05 ± 3.34*	58.67 ± 2.56*	61.73 ± 2.91*	54.48 ± 3.15*	60.15 ± 2.59*	50.82 ± 3.42*
	NH	58.16 ± 1.93	40.34 ± 3.27*	51.18 ± 1.39	31.92 ± 4.44*	55.18 ± 1.82	31.02 ± 3.62*
TB (g)	N0	178.21 ± 12.61	125.26 ± 8.06	158.26 ± 15.18	109.73 ± 12.12	148.51 ± 15.17	107.25 ± 12.20
	NL	232.06 ± 9.12*	153.37 ± 6.71*	197.73 ± 9.63*	141.35 ± 9.97*	179.36 ± 9.21*	136.33 ± 8.32*
	NH	200.41 ± 6.41	104.36 ± 10.97	174.62 ± 10.4	103.42 ± 6.28	160.42 ± 9.69	91.67 ± 7.78
LA (10 <sup>3</sup> cm <sup>2</sup> )	N0	1.80 ± 0.06	1.34 ± 0.08	1.59 ± 0.05	1.10 ± 0.06	1.28 ± 0.06	0.72 ± 0.04
	NL	2.03 ± 0.05*	1.59 ± 0.04*	1.77 ± 0.08*	1.26 ± 0.05*	1.42 ± 0.02*	0.87 ± 0.08*
	NH	1.71 ± 0.04	1.02 ± 0.03*	1.49 ± 0.08	0.71 ± 0.10*	1.17 ± 0.06	0.58 ± 0.04*
SLA (cm <sup>2</sup> g <sup>-1</sup> )	N0	105.27 ± 3.81	86.22 ± 4.19	98.39 ± 2.91	78.94 ± 3.44	94.02 ± 6.34	68.33 ± 3.15
	NL	132.35 ± 2.87*	108.65 ± 7.74*	129.31 ± 0.73*	100.28 ± 4.54*	112.05 ± 2.34*	92.75 ± 5.89*
	NH	101.42 ± 4.73	75.43 ± 6.62	90.06 ± 5.93	61.26 ± 5.13	84.71 ± 2.55	57.49 ± 1.83
RSR	N0	0.54 ± 0.02	0.66 ± 0.03	0.56 ± 0.02	0.65 ± 0.03	0.63 ± 0.03	0.60 ± 0.02
	NL	0.43 ± 0.02*	0.52 ± 0.02*	0.45 ± 0.02*	0.53 ± 0.04*	0.51 ± 0.02*	0.49 ± 0.04*
	NH	0.41 ± 0.03*	0.58 ± 0.03	0.41 ± 0.01*	0.59 ± 0.02	0.48 ± 0.05*	0.56 ± 0.03
RWC (%)	N0	70.72 ± 0.76	67.59 ± 0.62	67.32 ± 0.90	63.85 ± 0.85	66.32 ± 0.60	61.52 ± 0.68
	NL	73.64 ± 1.37*	69.82 ± 0.40*	69.28 ± 0.59*	67.03 ± 0.75*	68.71 ± 1.21*	65.07 ± 1.44*
	NH	71.12 ± 1.53	64.57 ± 0.54	67.52 ± 0.59	61.82 ± 0.58	65.47 ± 0.56	59.46 ± 0.69

Control, 80% soil field capacity; drought, 50% soil field capacity. N0, NL, and NH: zero, low-, and high-N supply. Values are means of six replicates ± standard deviation. Asterisks show statistically significant differences among N treatments under the same water conditions ( $P \leq 0.05$ ).

significantly ( $P \leq 0.05$ ) decreased the root/shoot ratio for all cultivars whereas no significant difference in R/S was observed between N0 and NH.

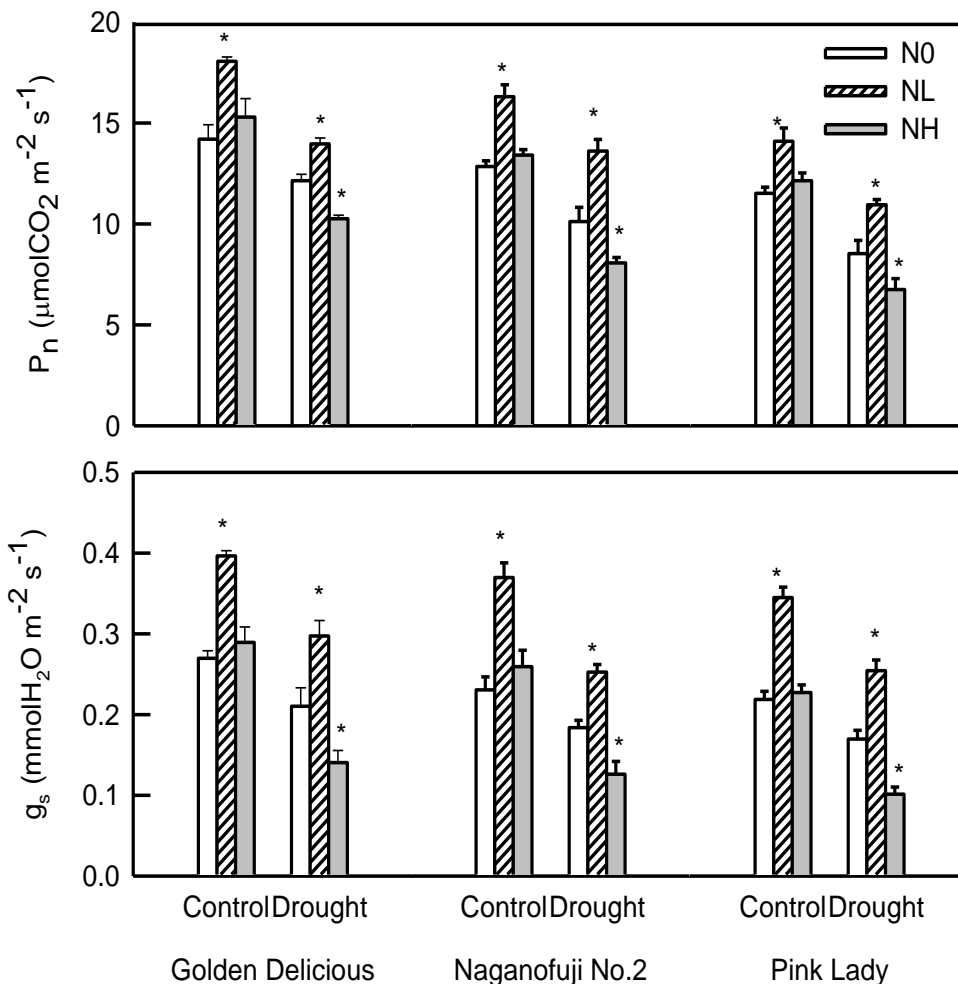
### Gas exchange

Both  $P_n$  and  $g_s$  were significantly influenced by soil water ( $P \leq 0.001$ ), N supply ( $P \leq 0.001$ ), and their interaction ( $P \leq 0.01$ ) (Table 2). Those parameters were greatly decreased by drought stress (Figure 1). NL significantly

( $P \leq 0.05$ ) increased  $P_n$  and  $g_s$  for all cultivars under both water conditions.  $P_n$  and  $g_s$  for all cultivars were significantly ( $P \leq 0.05$ ) decreased by NH under drought condition, but no significant influence of NH on these two parameters were observed when plants were well-watered.

### Water-use efficiency

WUE of our apple trees was highly and significantly



**Figure 1.** Influence of N supply on photosynthesis rate ( $P_n$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) and stomatal conductance ( $g_s$ ,  $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) for 3 apple cultivars under well-watered (control, 80% soil field capacity) or drought (50% soil field capacity) condition. NO, NL, and NH: zero, low-, and high-N supply. Values are means of six replicates  $\pm$  standard deviation. Asterisks show statistically significant differences among N treatments under same watering regime ( $P \leq 0.05$ ).

( $P \leq 0.001$ ) influenced by drought stress, N supply, and their interaction (Table 2) whereas, for all cultivars, this efficiency was diminished by drought (Figure 2). NL significantly ( $P \leq 0.05$ ) increased WUE for all cultivars under both watering regimes. By contrast, under those same irrigation scenarios, NH significantly ( $P \leq 0.05$ ) decreased WUE for 'Pink Lady' while no significant difference was observed in 'Golden Delicious' and 'Naganofuji No.2' when receiving either the NO or NH treatments.

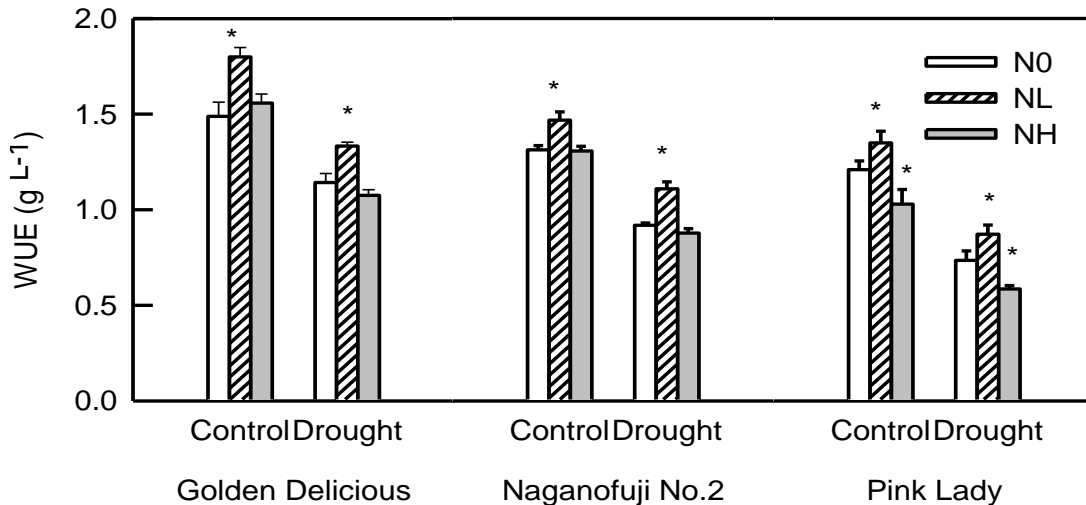
Parameters RB, LA,  $P_n$ , and  $g_s$  were significantly ( $P \leq 0.05$ ) and positively correlated with WUE (Figure 3).

## DISCUSSION

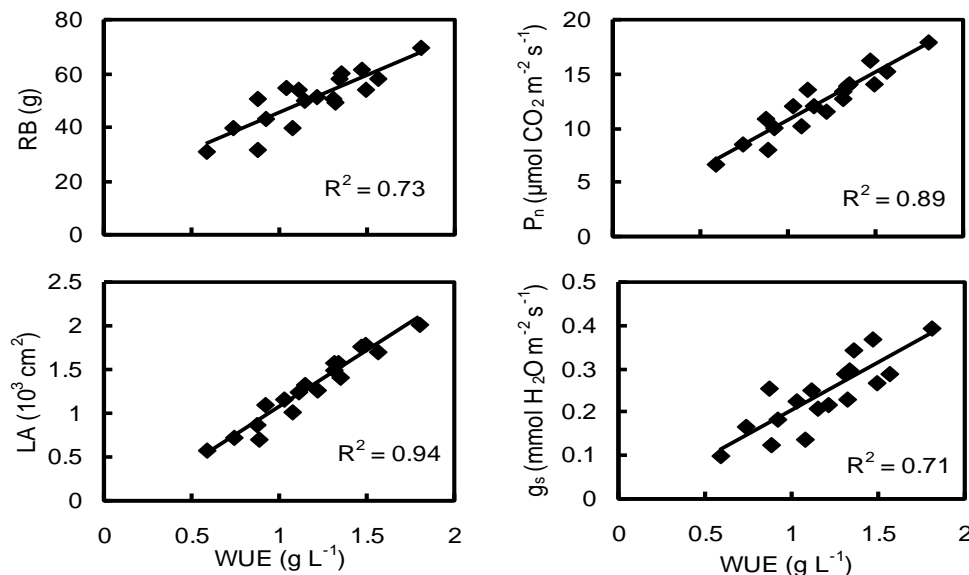
Our results indicate that drought stress and the nitrogen supply have great influences on growth and biomass

production (Tables 2 and 3), a conclusion also reported with other plant species (Brück et al., 2001; Cechin and Fumis, 2004; Wu et al., 2008; Song et al., 2010). Regardless of the concentration of N applied, reduced irrigation dramatically decreased both growth and the accumulation of biomass (Table 3). Parameters such as height, basal diameter, biomass production, and total leaf area showed positive responses to NL under both watering regimes (Table 3), but a higher level of N applied under drought treatment could not alter the negative effects of withholding irrigation. These findings imply that drought is the primary limiting factor while nitrogen has only a secondary role. The same observations have been reported with plants grown in both relatively wet and relatively dry regions (Seagle and McNaughton, 1993; Wu et al., 2008).

Patterson et al. (1997) have suggested that adaptations in morphology may be a primary mechanism by which



**Figure 2.** Influence of N supply on water-use efficiency (WUE,  $\text{g L}^{-1}$ ) (mean  $\pm$  SD,  $n = 6$ ) for 3 apple cultivars under well-watered (control, 80% soil field capacity) or drought (50% soil field capacity) condition. N0, NL, and NH: zero, low-, and high-N supply. Values are means of six replicates  $\pm$  standard deviation. Asterisks show statistically significant differences among N treatments under same watering regime ( $P \leq 0.05$ ).



**Figure 3.** Linear correlations (Pearson's coefficients) among water-use efficiency (WUE), total leaf area (LA), root biomass (RB), photosynthetic rate ( $P_n$ ), and stomatal conductance ( $g_s$ ) for 3 apple cultivars under different watering and N regimes.

plants cope with the environmental characteristics of their respective habitats. Biomass allocation may be a key sensitive predictor of functional responses to stress (James et al., 2005). Partitioning more biomass to roots, thereby maintaining a higher R/S, may be advantageous for drought-adapted plants at the onset of drought stress because greater water absorptive component can sustain

more water and nutrient uptake for transpiration and respiration (Yin et al., 2005; Villagra and Cavagnaro, 2006). Our results are in accord with this theory. Reducing their LA and SLA is another effective strategy for drought tolerance that decreases plant transpiration rates (Liu and Stützel, 2004; Villagra and Cavagnaro, 2006).

In our experiments, plants exhibited inconsistent morphological responses to different N supplies under various watering conditions (Table 3). Plants receiving NL were taller and had greater basal diameters, biomass production, and total leaf area. In contrast, those treated with NH showed either the opposite tendency or were unchanged in those parameters compared with the control. Under either watering regime, R/S was reduced when the N supply was increased. Monclus et al. (2006) have suggested that such nitrogen-induced responses can be attributed to the balance between absorption and utilization of water and nutrients, that is, plants adjust their developmental patterns to facilitate the acquisition and distribution of growth-limiting resources. Although drought stress led to diminished RWC values, we noted that NL treatment increased RWC under both irrigation conditions (Table 3). This is in agreement with observations by Uprety and Mahalaxmi (2000), who reported that adequate nitrogen enhances the relative water content in leaves. By contrast, NH decreased RWC (Table 3), which may attribute to the more severe moisture stress by soluble fertilizer as reported in *Populus* (van den Driessche et al., 2003).

The photosynthetic rate reflects the degree of plants drought tolerance and their capacity to recover to a favorable water status (Singh and Singh, 2003). Our results indicated that drought stress led to lower  $P_n$  values (Figure 1), similar to findings reported previously (Chartzoulakis et al., 2002; Bacelar et al., 2007; Ahmed et al., 2009). This response was mainly due to stomata closure, as manifested by reduced  $g_s$ . NL treatment induced increases in  $P_n$  and  $g_s$  under both water conditions (Figure 1), implying that a lower application of nitrogen might improve drought tolerability in apple trees similar to that found with other plant species (Uprety and Mahalaxmi, 2000; Toth et al., 2002; Cechin and Fumis, 2004). However, NH treatment decreased (under drought conditions) or had little effect (under well-watered conditions) on both parameters (Figure 1), possibly because of either a reduction in Rubisco quantity within the chloroplasts (Nakaji and Izuta, 2001), or additional moisture stress related to the use of soluble fertilizer (van den Driessche et al., 2003). Therefore, this demonstrates that an appropriate supply of water and nitrogen may improve drought tolerance and contribute to higher carbon assimilation. As proposed by Broadley et al. (2000), plants often use N primarily for the production and maintenance of leaves in order to maximize carbon fixation for optimal growth.

WUE, the functional indicator strongly related to plant development and health under a moisture deficit, is dependent upon the amount of water used for growth and biomass production (Liu and Stützel, 2004; Monclus et al., 2006; Zhang et al., 2008). Although WUE can be improved when water is limited (Liu et al., 2005; Ma et al., 2010), the opposite has also been shown (Wu et al., 2008; Song et al., 2010). Here, WUE in our three

cultivars was significantly decreased by drought stress (Figure 2), perhaps attributing to lower biomass production. Patterson et al. (1997) have suggested that the increased availability of one finite resource might enhance the efficient use of other finite resources in multi-resource-limited systems. Although NL treatment improved WUE in all of our apple cultivars, results were inconsistent in their response to NH (Figure 2). The same observations have been reported by Brueck et al. (2010) and Wu et al. (2008), and may be related to differences among cultivars in their production of biomass.

We found that several morphological and physiological variables were strongly correlated with WUE, based on Pearson's coefficients (Figure 3). Leaf area, photosynthesis rate, and stomatal conductance play dominant roles in determining WUE because they are sensitive to biomass production, which is a direct factor that influences WUE (Monclus et al., 2006). Root biomass also strongly impacts WUE because a larger water-absorptive component (that is, the roots) can improve water uptake and sustain more water supplies for transpiration (Donovan and Ehleringer, 1994). Therefore, our results suggest at least two mechanisms by which the N supply affects plants drought tolerance and WUE. First, the increased availability of soil nitrogen might lead to higher values for leaf area, photosynthesis rate, and biomass production, and, hence, improved drought tolerance and WUE. Second, an adequate N supply may lead to a greater root biomass, which can enhance water uptake and then result in a higher drought tolerance and WUE. Singh et al. (2005) have suggested that a balance exists between the N supply and WUE or drought tolerance. Our results also allow us to conclude that sufficient nitrogen is associated with greater adaptability of apple trees to drought conditions, which is manifested by better growth, increased biomass production, and improved WUE.

In summary, our hypothesis that N supply could improve adaptability of plants under dry condition was only partly evidenced as discussed above in this study. Water, nitrogen, cultivar and their interaction greatly influenced plant development, biomass production, and WUE in our potted young apple trees. Drought stress dramatically decreased growth and biomass accumulation; increased biomass allocations to the roots; and diminished values for SLA, RWC,  $P_n$ ,  $g_s$ , and WUE. Our NL treatment enhanced plant adaptability to drought while the NH trial led to the reverse. A lower application of nitrogen was associated with higher WUE, mainly through increased photosynthetic capacity and water uptake. Therefore, we recommend that an appropriately low supply of N is optimal under drought conditions, and managers should avoid utilizing a higher concentration. Nevertheless, the present study was implemented only on potted young apple trees and resource requirements fluctuate with plants developmental stages. More researches are required to implement in field conditions

and to elucidate the drought-adaptive mechanism of *M. domestica* in detail.

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