The Uptake and Partitioning of Nitrogen in *Phalaenopsis* Sogo Yukidian ‘V3’ as Shown by ^{15}N as a Tracer

Hadi Susilo¹ and Ying-Chun Peng¹

Department of Horticulture and Landscape Architecture, National Taiwan University, 1 Roosevelt Road Sec. 4, Taipei 10617, Taiwan

Shui-Cheng Lee

Institute of Nuclear Energy Research, Atomic Energy Council, Taoyuan County 32546, Taiwan

Yu-Chun Chen and Yao-Chien Alex Chang²

Department of Horticulture and Landscape Architecture, National Taiwan University, 1 Roosevelt Road Sec. 4, Taipei 10617, Taiwan

ADDITIONAL INDEX WORDS. moth orchid, nitrogen-15, foliar application, sink-source relationship, nitrogen storage

ABSTRACT. *Phalaenopsis* is currently the world’s number one potted flower crop. It is a slow-growing plant that responds slowly to nitrogen (N) fertilization and is noted for great resilience against N deficiency. Despite the great significance of N during the cultivation of *Phalaenopsis*, little has been studied on the uptake and partitioning of N in this crop. The stable isotope ^{15}N was used as a tracer to investigate the uptake and partitioning of N and the roles of organs in sink and source relationship of N partitioning during different stages in *Phalaenopsis*. Fertilizer labeled with ^{15}N was applied to *Phalaenopsis* Sogo Yukidian ‘V3’ during the vegetative growth stage on different parts of plants. Both leaves and roots were able to take up N. Nitrogen uptake efficiency of young roots was the highest, followed by old roots, whereas that of leaves was lowest. No difference of N uptake efficiency was found between the upper and lower leaf surfaces. Movement of fertilizer N to the leaves occurred as early as 0.5 day after fertilizer application to the roots. The partitioning of N depended on organ sink strength. During the vegetative growth stage, newly grown leaves and newly formed roots were major sinks. Sink strength of leaves decreased with the increase in leaf age. Stalks and flowers were major sinks during the reproductive growth stage. Mature leaves were a major location where N was stored and could serve as a N source during the reproductive growth stage and also for new leaf growth.

Phalaenopsis orchid is currently the world’s most important potted flowering crop (Chang et al., 2013). It is the most highly valued indoor plant in flower auctions in the Netherlands (FloraHolland, 2013), whereas in the United States, orchids, constituted mainly by *Phalaenopsis*, rank first in wholesale value in the potted flowering plant market (U.S. Department of Agriculture, 2012). *Phalaenopsis* is an epiphytic plant with succulent leaves and roots and is noted for its long blooming period and great resilience against stresses, including nutrient deficiency stress (Hou et al., 2010; Hung, 2012; Lei, 2007). Compared with other crops, orchids have similar mineral requirements, but they respond more slowly to fertilizer application (Hew and Yong, 2004). *Phalaenopsis* is a typical orchid in this respect; it is slow-growing and can continue to grow and bloom even under long periods of suboptimal substrate fertility. Experimentally, it is very difficult to induce nutrient deficiency symptoms when starting with healthy *Phalaenopsis* plants, even after several months have passed without additional fertilization (Lei, 2007). We hypothesize that the fleshy leaves of

Phalaenopsis have nutrient storage function, which likely accounts for its resilience against nutrient deficiency. Studies on mineral nutrition in *Phalaenopsis* done by different workers have yielded varying and at times inconsistent results (Lei, 2007; Wang, 2000, 2007; Yoneda et al., 1997; Yu, 2012). We think that the conflicting experimental results may be a result of how much nutrients have been stored in the plants used. Although the ability of some orchids to store nutrients in thickened stems called pseudobulbs has been demonstrated (Ng and Hew, 2000), *Phalaenopsis* lacks such a structure, and the capability of the succulent leaves to store mineral nutrients has not been definitively shown with research.

Nitrogen is one of the essential macronutrients having great influence during the cultivation of *Phalaenopsis*. Nitrogen fertilization regimen significantly affects both the vegetative and reproductive growth of *Phalaenopsis* (Lei, 2007; Yu, 2012) and can be adjusted to manipulate the timing of flowering (Ichihashi et al., 2010). Despite the great importance of N in *Phalaenopsis* production, little has been studied on the absorption and partitioning of N in *Phalaenopsis*. Conventionally, plant N requirements are analyzed by measuring the concentration and content of N in plant structures to determine absorption and use of this element. However, whether the measured N comes from fertilizer or other sources cannot be determined with this method. Nitrogen absorption from fertilizer application is thus often overestimated as a result of the presence of non-fertilizer sources such as previously stored N (Westerman and Kurtz, 1974). The presence of a hypothesized

Received for publication 22 Jan. 2013. Accepted for publication 14 Mar. 2013. This research was funded by a grant from the National Science Council, Executive Yuan, Taiwan (NSC 98-2313-B-002-014-MY3).

This study consists of portions of the theses submitted by Hadi Susilo and Ying-Chun Peng in partial fulfillment of their Master of Science degree requirements. We thank Dr. William B. Miller of Cornell University for his advice on some sections of the manuscript.

¹These authors contributed equally to this work.

²Corresponding author. E-mail: alexchang@ntu.edu.tw.

pool of stored N in the succulent leaves of *Phalaenopsis* would aggravate the aforementioned difficulties encountered when studying use of N fertilizer using traditional methods.

Nitrogen-14 and nitrogen-15 (^{15}N) are the two stable isotopes of N with atmospheric natural abundances of 99.6337% and 0.3663%, respectively. The latter is an important tracer element in chemistry, medicine, and agriculture research and is often used to study the movement of N in plants (Lajtha and Michener, 1994). Using a stable isotope as a tracer, the uptake of N and its absorption efficiency can be determined more accurately compared with conventional analytical methods (Sandrock et al., 2005). Although ^{15}N has been used to trace the fate of N in numerous fruit crops (e.g., Feigenbaum et al., 1987; Munoz et al., 1993; Retamales and Hanson, 1989), it has not been a common research tool used in floriculture crops (e.g., Cabrera et al., 1995; Trepanier et al., 2009). Because *Phalaenopsis* has emerged as the world's number one potted flower crop, and considering its specific characteristics that make nutritional study difficult, investigating N absorption and partitioning in *Phalaenopsis* with ^{15}N is of great relevance.

In this study, we used ^{15}N labeling to compare the absorption and partitioning of fertilizer N applied to *Phalaenopsis* leaves and roots, analyze the fate of fertilizer N after application to the roots, and trace the partitioning of fertilizer N at different growth stages to compare the sink-source relationships between the various organs.

Materials and Methods

PLANT MATERIALS. Unless otherwise stated, vegetatively propagated *Phalaenopsis* Sogo Yukidian 'V3' plants grown in sphagnum moss in 10.5-cm pots (0.75 L) were purchased from a commercial grower (Clone International Biotech, Pingtung, Taiwan). During the experimental period, plants were grown in a Venlo-type greenhouse at National Taiwan University with a pad and fan system with maximum monthly temperature of 27 °C in summer, minimum monthly temperature of 18 °C in winter, and average daily minimum relative humidity of 70%. Light intensity was maintained below a maximum level of 400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ using an adjustable double layer of shade cloth.

EXPT. 1: DIFFERENCES IN FERTILIZER N ABSORPTION AND PARTITIONING THROUGH *PHALAEOPSIS* ROOTS AND LEAVES. The aim of this experiment was to compare the absorption of fertilizer N by *Phalaenopsis* roots and leaves, and how it is subsequently partitioned. Plants were removed from their pots and the sphagnum moss medium was removed. The roots were divided into two halves based on their total length: young roots and old roots. To prevent ^{15}N contamination between these two halves, the young and old roots were fixed onto separate tree fern slabs, as shown in Fig. 1A. Johnson's solution (Johnson et al., 1957) labeled with ^{15}N (22.5 atom% ^{15}N) and supplemented with 0.1% surfactant (Tween-20; Nacalai Tesque, Kyoto, Japan) was applied with a paintbrush to the upper or lower surfaces of all leaves excluding the top leaf, or to the young or old roots once per day for 3 d (consecutive). There were four treatments (i.e., four fertilizer application sites) with six single-plant replications in each treatment. The roots were then wrapped in sphagnum moss and nets after ^{15}N treatment (Fig. 1B) to retain moisture. To prevent ^{15}N contamination between leaves and roots, fertigation was done by spraying regular Johnson's solution (224 $\text{mg}\cdot\text{L}^{-1}$ N) to the sphagnum

Fig. 1. *Phalaenopsis* Sogo Yukidian 'V3' plant fixed onto tree fern slabs. (A) The young roots and old roots were fixed onto the right and the left tree fern slabs, respectively. (B) The roots were wrapped in sphagnum moss and nets after ^{15}N treatment.

moss substrate when it was almost dry. Four weeks after ^{15}N treatment, some organ parts were sampled from newly grown leaves (top leaf, without ^{15}N application), mature leaves (lowest healthy leaf), young roots, and old roots. The leaves were sampled at 2.5-cm length measured from the leaf tip. The roots were sampled as 10-cm-long pieces. Eight weeks after ^{15}N treatment, whole plants were harvested and separated into the following components: newly grown leaves (leaves younger than those fully expanded before ^{15}N treatment), mature leaves (fully expanded leaves before ^{15}N treatment), young roots, old roots, and newly grown roots (roots newly formed on the nodes of the shoot above the sphagnum moss wrapping after ^{15}N treatment). The experimental period was from 24 July to 18 Sept.

EXPT. 2: SHORT-TERM TRANSPORT OF FERTILIZER N TO *PHALAEOPSIS* LEAVES AFTER FERTILIZER APPLICATION TO THE ROOTS. The aim of this experiment was to determine the fate of fertilizer N in *Phalaenopsis* leaves shortly after absorption by the roots. Plants with seven leaves were used, and they were chosen such that the youngest leaves were $\approx 90\%$ to 95% expanded. The plants were removed from the substrate and then repotted into fresh sphagnum moss substrate (rinsed and wrung beforehand to remove soluble nutrients). The ^{15}N -labeling was performed by subirrigating the plants from the bottom of the pots with Johnson's solution enriched with 22.5 atom% ^{15}N . Just before ^{15}N -labeling was performed (0 d), and 0.5, 1, 2, 4, and 8 d after ^{15}N labeling, the first leaf and fourth leaf of each sampled plant were harvested. There were six sampling time points with seven single-plant replications in each sampling time. The samples were then analyzed for total N concentration and ^{15}N concentration. The experimental period was from 20 Nov. to 28 Nov.

EXPT. 3: SINK STRENGTHS OF *PHALAEOPSIS* LEAVES AT VARIOUS AGES. The objective of this experiment was to compare *Phalaenopsis* leaves at various ages for their relative sink strengths for N. Plants were replanted into 10.5-cm pots after purchase. After the plants were transplanted, ^{15}N -labeled Johnson's solution (11.25 atom% ^{15}N) was applied by subirrigation. There were six single-plant replications and leaves were sampled from each replication 8 weeks after ^{15}N treatment and numbered from the first (top) leaf to the sixth (lowest) leaf. Plants were fertigated with regular Johnson's solution (224 $\text{mg}\cdot\text{L}^{-1}$ N) every 2 to 3 weeks after the first fertigation with labeled fertilizer solution. To prevent ^{15}N contamination between leaves resulting from overhead fertigation, the fertilizer solution was applied by subirrigation. The experimental period was from 12 July to 6 Sept.

EXPT. 4: ABSORPTION AND PARTITIONING OF N BY *PHALAEOPSIS* PLANTS DURING VEGETATIVE AND REPRODUCTIVE PHASES. The aim of this experiment was to trace the fate of a single application of fertilizer N over a long cultivation period involving vegetative and reproductive stages. Vegetatively propagated *Phalaenopsis* Sogo Yukidian 'V3' plants, grown in sphagnum moss in 8.5-cm pots, were purchased and transplanted into 10.5-cm pots. After plants were transplanted, ^{15}N -labeled Johnson's solution (11.25 atom% ^{15}N) was applied by subirrigation. Plants were sampled 1, 2, 4, 8, 16, and 38 weeks after ^{15}N treatment. There were six sampling time points with three to four single-plant replications at each sampling time: $n = 4$ at 1, 2, 4, 8, and 16 weeks and $n = 3$ at 38 weeks after ^{15}N treatment. The sampled plants were dissected into newly grown leaves, mature leaves, and roots. Plants began to spike at Week 16 because of natural low temperature, which marked the shift from vegetative growth stage to the reproductive growth stage (when growth was mainly that of reproductive organs). The flowers (including unopened floral buds) and stalks were sampled at Weeks 16 and 38. Fertigation and maintenance of plants were the same as in Expt. 3. The experimental period was from 6 July to 10 May.

ISOTOPIC LABELING. Modified Johnson's solution was used as the fertilizer in all experiments. It contained 16 mM N, 2 mM phosphorus, 6 mM potassium, 4 mM calcium, 1 mM sulfur, 1 mM magnesium, 50 μM chlorine, 25 μM boron, 5 μM magnesium, 4 μM iron, 2 μM zinc, 0.5 μM copper, 0.1 μM molybdenum, and 0.1 μM nickel with ammonium-to-nitrate ratio of 1:7. In Expts. 1 and 2, the potassium nitrate in the Johnson's solution was completely substituted with 60 atom% ^{15}N -labeled potassium nitrate (ISOTEC, Miamisburg, OH). The ^{15}N -labeled solution thus had a ^{15}N concentration of 22.5 atom%. In Expts. 3 and 4, half of the potassium nitrate in the Johnson's solution was substituted with 60 atom% ^{15}N -labeled potassium nitrate. The ^{15}N -labeled solution had a ^{15}N concentration of 11.25 atom%.

SAMPLE ANALYSIS AND DATA CALCULATION. All samples were rinsed with deionized water, dried in a 70- $^{\circ}\text{C}$, forced-air oven for 2 weeks, and ground to fine powders with an electric crusher before analysis. The N concentration (%) and ^{15}N concentration

(atom%) were determined by the Automatic Nitrogen and Carbon Analyzer–Mass spectrometry (MS) system (Europa Scientific, Crewe, UK) comprising a sample preparation unit (autosampler to gas chromatography), a capillary interface, and a MS system. The atom percent ^{15}N excess (atom% excess) was obtained by subtracting natural abundance of ^{15}N (0.366 atom%) from the results of the ^{15}N analyses. The N content = N concentration $\times$ dry weight. The ^{15}N content excess = weight % of excess ^{15}N out of total N $\times$ N content. For each site of fertilizer application in Expt. 1, translocated ^{15}N content excess was calculated by subtracting ^{15}N content excess at the site of application from the whole-plant ^{15}N content excess.

STATISTICAL ANALYSIS. All experiments were single-factor experiments carried out in a completely randomized design. Statistical tests were done using CoStat software (Version 6.101; CoHort Software, Monterey, CA). Data were subjected to one-way analysis of variance to compare differences among treatments. The least significant difference test at $P \leq 0.05$ was used for multiple mean comparisons. The t test was used for comparing difference between two sample means. Graphs were plotted with SigmaPlot software (Version 10.0; Systat Software, San Jose, CA).

Results

EXPT. 1: DIFFERENCES IN FERTILIZER N ABSORPTION AND PARTITIONING THROUGH *PHALAEOPSIS* ROOTS AND LEAVES. Nitrogen concentration at 4 weeks after ^{15}N -labeling treatments was highest in newly grown leaves (2.26% to 2.49%) and lowest in old roots (1.30% to 1.40%), and within the sampled organs, no difference in N concentrations was found among treatments (data not shown). Four weeks after ^{15}N application to the lower or upper surface of mature leaves, the bulk of the absorbed ^{15}N remained on the mature leaves (i.e., the site of application) with much less translocation to the other organs (Table 1). Atom% excess of 0.040% and 0.056% were detected in the mature leaves 4 weeks after ^{15}N was applied to upper and lower leaf surfaces, respectively, whereas much lower atom% excess (0.004% to 0.011%) was found in other organs (Table 1).

Table 1. The ^{15}N concentrations of newly grown leaves, mature leaves, young roots, old roots, and newly grown roots of *Phalaenopsis* Sogo Yukidian 'V3' 4 and 8 weeks after application of isotopic nitrogen (^{15}N) to different organs.

Organs treated with $^{15}\text{N}^z$	^{15}N concn (atom% excess) ^y				
	Newly grown leaves	Mature leaves	Young roots	Old roots	Newly grown roots
4 weeks					
Leaves, upper surface	0.011 ab B ^x	0.040 a A	0.007 b B	0.007 b B	—
Leaves, lower surface	0.004 b B	0.056 a A	0.011 b B	0.007 b B	—
Young roots	0.024 a B	0.014 b B	0.220 a A	0.022 b B	—
Old roots	0.019 a B	0.022 b B	0.028 b B	0.356 a A	—
8 weeks					
Leaves, upper surface	0.023 c C	0.041 a A	0.018 c D	0.011 c E	0.030 c B
Leaves, lower surface	0.016 c B	0.031 b A	0.014 c B	0.008 c C	0.027 c A
Young roots	0.086 a B	0.030 bc C	0.139 a A	0.045 b C	0.094 a B
Old roots	0.062 b B	0.025 c C	0.039 b C	0.158 a A	0.064 b B

^zJohnson's solution (224 mg·L⁻¹ N) enriched with 22.5 atom% ^{15}N was applied with a paintbrush to the upper or lower surfaces of all leaves excluding the top leaf or to the young or old roots once per day for 3 consecutive days.

^yNewly grown leaves were the leaves younger than those fully developed before ^{15}N treatment; mature leaves were the fully developed leaves before ^{15}N treatment; young roots and old roots were defined as the younger half and the older half of the root system before ^{15}N treatment; and newly grown roots were roots newly formed on the nodes of the shoot above the sphagnum moss wrapping after ^{15}N treatment.

^xMean separation was analyzed separately for the 4- and 8-week data. Means followed by different letters within columns (lower case) or rows (upper case) are significantly different at $P \leq 0.05$ by the least significant difference test; $n = 6$.

After application of ^{15}N to old roots and young roots, these sites of application also had the greatest ^{15}N concentrations 4 weeks later; application to old roots resulted in 0.356 atom% excess in old roots, whereas application to young roots resulted in 0.220 atom% excess in young roots (Table 1). The ^{15}N concentrations in other organs were significantly lower (ranging from 0.014% to 0.028% in atom% excess) than in the old or young roots where labeled fertilizer was applied. Four weeks after ^{15}N application, ^{15}N was detectable at low levels in other organs where ^{15}N had not been applied (Table 1).

Whole plants were harvested 8 weeks after treatment. The various treatments produced no difference in the dry weight, N concentration, and N content of various organs (data not shown). Nitrogen concentration was highest in newly grown leaves (2.32% to 2.49%) followed by young roots [1.72% to 1.94% (data not shown)]. Similar to Week 4, application of ^{15}N to either the upper or lower surface of mature leaves or to either old roots or young roots resulted in the highest ^{15}N concentration being found in the respective sites of application (Table 1). Irrespective of the site of application of the labeled fertilizer,

newly grown leaves and newly grown roots had the highest ^{15}N concentrations among the organs that were not sites of fertilizer application (Table 1). Between Weeks 4 and 8, the ^{15}N concentrations in sites of fertilizer application generally decreased, whereas the ^{15}N concentrations in the organs that were not sites of fertilizer application generally increased (Table 1). This shows that greater extent of ^{15}N translocation from the sites of application occurred between Weeks 4 and 8.

Eight weeks after application of ^{15}N -labeled fertilizer, plant ^{15}N content excess varied with the site of application, where it was greatest after application to young roots [82.8 μg (Table 2)]. The second highest plant ^{15}N content excess was found after application to old roots (60.3 μg), whereas plant ^{15}N content excess was lowest after application to either the upper (34.1 μg) or lower surfaces of leaves [25.2 μg (Table 2)].

EXPT. 2: SHORT-TERM TRANSPORT OF FERTILIZER N TO *PHALAENOPSIS* LEAVES AFTER FERTILIZER APPLICATION TO THE ROOTS. Dry weight of the first leaf ranged from 0.91 to 1.23 g, and increased significantly as days after fertilizer application increased (Table 3). Dry weight of the fourth leaf ranged from

Table 2. The ^{15}N content excess of newly grown leaves, mature leaves, young roots, old roots, and newly grown roots of *Phalaenopsis* Sogo Yukidian 'V3' 8 weeks after application of isotopic nitrogen (^{15}N) to different organs.

Organs treated with $^{15}\text{N}^z$	^{15}N content excess (μg) ^y						
	Newly grown leaves	Mature leaves	Young roots	Old roots	Newly grown roots	Total	Translocated ^x
Leaves, upper surface	6.9 b B ^w	19.1 a A	3.5 c C	1.4 c D	3.2 b CD	34.1 c	15.0 b
Leaves, lower surface	4.5 b B	14.1 b A	3.5 c BC	0.7 c D	2.4 b CD	25.2 c	11.2 b
Young roots	24.2 a B	14.5 b C	29.3 a A	5.3 b D	9.5 a D	82.8 a	53.6 a
Old roots	20.1 a A	12.2 b BC	7.0 b D	13.1 a B	7.9 a CD	60.3 b	47.2 a

^zJohnson's solution (224 mg·L⁻¹ N) enriched with 22.5 atom% ^{15}N was applied with a paintbrush to the upper or lower surfaces of all leaves excluding the top leaf or to the young or old roots once per day for 3 d (consecutive).

^yNewly grown leaves were the leaves younger than those fully developed before ^{15}N treatment; mature leaves were the fully developed leaves before ^{15}N treatment; young roots and old roots were defined as the younger half and the older half of the root system before ^{15}N treatment; and newly grown roots were roots newly formed on the nodes of the shoot above the sphagnum moss wrapping after ^{15}N treatment.

^xTranslocated ^{15}N content excess = total ^{15}N content excess – ^{15}N content excess at site of ^{15}N application.

^wMeans followed by different letters within columns (lower case) or rows (upper case) are significantly different at $P \leq 0.05$ by the least significant difference test; n = 6.

Table 3. Changes in dry weight, nitrogen (N) concentration, and N content at different leaf positions of *Phalaenopsis* Sogo Yukidian 'V3' after application of ^{15}N -labeled fertilizer solution at the roots.^z

Leaf position ^y	Time (d)					
	0	0.5	1	2	4	8
Dry wt (g)						
First leaf	0.94 cd ^x	0.91 d	0.95 cd	1.02 bc	1.10 b	1.23 a
Fourth leaf	1.01 b	1.18 a	1.12 ab	1.18 a	1.18 a	1.17 a
Significance ^w	NS	**	*	*	*	NS
N concn (%)						
First leaf	1.93 ab	1.93 ab	1.96 a	1.92 ab	1.94 ab	1.84 b
Fourth leaf	1.23 a	1.14 ab	1.13 ab	1.09 b	1.23 a	1.19 ab
Significance	***	***	***	***	***	***
N content (mg)						
First leaf	18.2 cd	17.5 d	18.7 cd	19.7 bc	21.1 ab	22.5 a
Fourth leaf	12.4 b	13.5 ab	12.7 b	12.9 ab	14.5 a	13.9 ab
Significance	***	**	***	***	***	***

^zJohnson's solution (224 mg·L⁻¹ N) enriched with 22.5 atom% ^{15}N was applied by subirrigating plants freshly repotted into sphagnum medium.

^yFirst leaf refers to the top leaf and fourth leaf refers to the fourth leaf counted from the top.

^wMeans followed by different letters within rows are significantly different at $P \leq 0.05$ by the least significant difference test; n = 7.

^xNS, *, **, *** indicate means within columns are not significantly different or significantly different according to paired *t* test at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; n = 7. Means of first leaf and fourth leaf were compared separately for dry weight, N concentration, and N content data.

1.01 to 1.18 g, but no obvious trend was found as days after fertilizer application increased (Table 3). Nitrogen concentration in the first leaf ranged from 1.84% to 1.96%, which was significantly higher than 1.09% to 1.23% found in the fourth leaf (Table 3). In both the first leaf and the fourth leaf, N concentration did not change with any obvious pattern over the experimental period (Table 3).

Nitrogen content in the first leaf ranged from 17.5 to 22.5 mg, which was significantly higher than the range found in the fourth leaf (Table 3). In the first leaf, N content increased as the number of days after fertilization increased, whereas in the fourth leaf, N content fluctuated between 12.4 and 14.5 mg, but did not seem to be related to the number of days after fertilization (Table 3).

In both the first leaf and the fourth leaf, a significant increase in ^{15}N concentration could be detected as early as 0.5 d after application of ^{15}N -labeled fertilizer at the roots. Between Day 0 and Day 0.5, ^{15}N concentration increased from 0.0006 atom% excess to 0.0016 atom% excess in the first leaf and from 0.0005 atom% excess to 0.0014 atom% excess in the fourth leaf (Fig. 2A). Translocation of fertilizer N to both young and mature leaves 0.5 d after fertilizer application at the roots was also evident as shown by ^{15}N content excess. Between Day 0 and Day 0.5, ^{15}N content excess increased from 0.1 to 0.3 μg in the first leaf, and from 0.1 to 0.2 μg in the fourth leaf (Fig. 2B). Nitrogen-15 concentration and content continued to increase in both the first and fourth leaves over the experimental period, indicating

that fertilizer N continued to accumulate in both young and mature leaves by Day 8 (Fig. 2).

EXPT. 3: SINK STRENGTHS OF *PHALAEOPSIS* LEAVES AT VARIOUS AGES. Dry weight was greatest for second leaves (1.54 g) and decreased successively with lower leaf position with only 0.35 g dry weight present in sixth leaves (Fig. 3A). The first leaves were not fully expanded when sampled and had a lower dry weight than the second and third leaves (Fig. 3A). Nitrogen concentration was highest in the first leaves (2.31%) and decreased successively with lower leaf position, down to 1.16% (Fig. 3B). Nitrogen content was highest in second leaves at 26.7 mg and the first leaves had the second highest at 22.2 mg (Fig. 3C) although first leaves had the highest N concentration. The lower N content was the result of the smaller dry weight of the first leaf compared with the second leaf. Nitrogen content decreased from the third leaf (19.7 mg) to the sixth leaf [4.0 mg (Fig. 3C)].

The concentration of ^{15}N was highest in first leaves (1.082 atom% excess) followed by second leaves [0.803 atom% excess (Fig. 3D)]. Compared with first and second leaves, the third to sixth leaves had lower ^{15}N concentration (0.359 to 0.246 atom% excess), and the differences from third to sixth leaves were small (Fig. 3D). First leaves and second leaves had the highest ^{15}N content excess [257 and 230 μg , respectively (Fig. 3E)]. Third leaves had higher ^{15}N content excess (75 μg) than fourth to sixth leaves [15 to 36 μg (Fig. 3E)].

EXPT. 4: ABSORPTION AND PARTITIONING OF N BY *PHALAEOPSIS* PLANTS DURING VEGETATIVE AND REPRODUCTIVE PHASES. Whole-plant dry weight increased from 10.5 g in Week 1 to 22.4 g in Week 38 (Fig. 4A). The increase in dry weight was mainly the result of the increase in the dry weight of newly grown leaves and the newly grown inflorescence; root dry weight increased only slightly, whereas a slight decrease was observed in the dry weight of mature leaves (Fig. 4A). The plants began to spike in Week 16 with inflorescence dry weight of 0.09 g. By Week 38, the inflorescences were in a stage where half of the blooms were open, and the dry weight of the stalk had increased to 3.9 g while the dry weight of the flower was 1.7 g (Fig. 4A).

Among the various organs, newly grown leaves had the highest N concentration, which increased from 2.37% in Week 1 to 2.71% in Week 16 but decreased after flowering to 2.41% in Week 38 (Fig. 4B). Nitrogen concentration in roots increased from 1.67% in Week 1 to 2.47% in Week 38 (Fig. 4B). In mature leaves, N concentration was lowest, increasing from 1.70% in Week 1 to 2.09% in Week 16. As is the case with newly grown leaves, N concentration in mature leaves decreased after flowering, from 2.09% in Week 16 to 1.84% in Week 38 (Fig. 4B). Nitrogen concentration in newly emerging flower stalk in Week 16 was high at 3.43% but decreased after flowering so that by Week 38, the stalk contained 2.19% N, whereas N concentration was lower in flowers in comparison at only 1.43% (Fig. 4B).

Nitrogen content of mature leaves remained relatively stable from Week 1 to Week 38 [44.2 to 62.2 mg (Fig. 4C)]. As is the case with the dry weight of newly grown leaves, the N content of newly grown leaves increased as the experiment progressed, from 43.3 mg in Week 1 to 161.0 mg in Week 38 (Fig. 4C).

After application of ^{15}N -labeled fertilizer at Week 0, the ^{15}N concentration in roots, mature leaves, and newly grown leaves of the plants increased gradually. By Week 1, the ^{15}N concentration in mature leaves was only 0.08 atom% excess, which

Fig. 2. ^{15}N concentration (atom% excess) and ^{15}N content excess in *Phalaenopsis* Sogo Yukidian 'V3' leaves after application of ^{15}N -labeled fertilizer to the roots. **, *** Significant difference in the first leaf between sampling point and Day 0 by unpaired t test at $P \leq 0.01$ and $P \leq 0.001$, respectively. ##, ### Significant difference in the fourth leaf between sampling point and Day 0 by unpaired t test at $P \leq 0.01$ and $P \leq 0.001$, respectively. Bars indicate SEM; $n = 7$.

Fig. 3. Dry weight, nitrogen (N) concentration, N content, ^{15}N concentration, and ^{15}N content excess of leaves in *Phalaenopsis* Sogo Yukidian 'V3' 8 weeks after application of ^{15}N -labeled fertilizer to the roots. Leaf position 1 (first leaf) is the youngest leaf, and leaf position 6 (sixth leaf) is the oldest leaf of the plant. Columns marked with the same letter are not statistically different at $P \leq 0.05$ by the least significant difference test. Bars indicate SEM; $n = 6$.

was lowest among the organs. The increase in ^{15}N concentration in mature leaves was comparatively small, reaching only 0.65 atom% excess in Week 16 with no further difference by Week 38 at 0.62 atom% excess (Fig. 4D). Changes in ^{15}N concentration in roots and newly grown leaves were similar in the first 8 weeks. In roots, it increased substantially from 0.42 atom%

Fig. 4. Changes in dry weight, nitrogen (N) concentration, N content, ^{15}N concentration, and ^{15}N content excess of newly grown leaves, mature leaves, roots, stalk, and flowers in *Phalaenopsis* Sogo Yukidian 'V3' from the vegetative growth stage through to the reproductive growth stage after a single application of ^{15}N -labeled fertilizer at Week 0. The plants shifted from the vegetative growth stage to the reproductive growth stage at approximately Week 16 when they began to spike. Bars indicate SEM; $n = 4$ for samples collected at Weeks 1 to 16, $n = 3$ for samples collected at Week 38, and $n = 1$ for stalk sample at Week 16.

excess in Week 1 to 1.48 atom% excess in Week 8, but decreased afterward to only 1.24 atom% excess in Week 16 (Fig. 4D). In newly grown leaves, it increased from 0.28 atom% excess to 1.61 atom% excess between Week 1 and Week 16, but decreased to 1.27 atom% excess during flowering at Week 38 (Fig. 4D). The concentration of ^{15}N in newly grown flower stalk at Week 16 was 1.53 atom% excess, which was similar to that of newly grown leaves at 1.61 atom% excess (Fig. 4D). At Week 38, ^{15}N concentration of the flower stalk decreased to 0.92 atom% excess, and a similar ^{15}N concentration was found in the flowers at 0.87 atom% excess (Fig. 4D).

The ^{15}N content excess of the whole plant had an increasing trend throughout the experimental period, but the rate of increase decreased gradually over time (Fig. 4E). The ^{15}N content excess in roots and newly grown leaves increased to a larger extent compared with mature leaves (Fig. 4E).

Discussion

PLANTS OF *PHALAEOPSIS* SOGO YUKIDIAN ‘V3’ CAN ABSORB NUTRIENTS THROUGH BOTH THEIR LEAVES AND ROOTS. Our finding from Expt. 1 shows that foliar absorption of nutrients by *Phalaenopsis* plants is not directly related to stomatal density. Numerous studies have been conducted to elucidate the mechanism of foliar absorption of nutrients in various plant species, which is now known to occur primarily through the cuticular region (Fernandez and Eichert, 2009) facilitated by porous regions called ectodesmata (Haynes and Goh, 1977; Kannan, 1986). *Phalaenopsis* have more stomata on the lower leaf surface (Lee and Lee, 1991). Our observation that lower and upper leaf surfaces of *Phalaenopsis* had similar absorption efficiency of fertilizer N (Tables 1 and 2) despite the difference in stomatal density is in agreement with the hypothesis that foliar absorption is mostly through the cuticular region.

The absorption efficiency of *Phalaenopsis* leaves was less than that of the roots and fertilizer N absorption of the leaves was only approximately half that of the roots (Table 2). We also found that fertilizer N was more efficiently translocated when it was applied to roots than when it was applied to leaves. The ratio of ^{15}N remaining at the site of application to the total ^{15}N absorbed by the plant was much higher when fertilizer N was applied to the leaves {56% [i.e., $(19.1/34.1) \times 100\%$] and 56% [i.e., $(14.1/25.2) \times 100\%$] } than when it was applied to the roots {between 22% [i.e., $(13.1/60.3) \times 100\%$] and 35% [i.e., $(29.3/82.8) \times 100\%$]; calculated from Table 2}. The higher proportion of retained fertilizer N in leaves to the total absorbed N indicates that compared with roots, leaves are less efficient in releasing the absorbed N for translocation to distant organs.

Temporary storage of N likely occurs at the site of fertilizer application. From Expt. 1 we found that 4 and 8 weeks after ^{15}N -labeled fertilizer was applied to the roots or leaves, the sites of fertilizer application consistently had the highest ^{15}N concentrations. One possible explanation to this observation is that the bulk of the absorbed N was stored temporarily in the leaves or roots to which the fertilizer was applied. Another possibility is that labeled fertilizer residue, which could not be removed despite washing during the sampling procedure but had not been absorbed into the living tissue either, accounted for the higher ^{15}N concentration at the sites of application. The first explanation is more probable because the ^{15}N concentration at the sites of fertilizer application decreased from Week 4 to Week 8 (Table 1).

During cultivation, it is not feasible to maintain a film of moisture on leaf surfaces, whereas the roots are by necessity in contact for extended periods with moisture in the substrate. Thus, it is more feasible and logically sound to apply the fertilizer solution to the substrate rather than to the leaves. This is in agreement with the concept that foliar fertilization of crops is generally not sufficient and only supplemental in nature (Fritz, 1978). Wang (2010) reported that foliar fertilization alone was insufficient for obtaining optimal vegetative growth and flowering of *Phalaenopsis*. Based on this, we conclude that fertilizer N should mainly be applied to the roots of potted *Phalaenopsis* plants.

One very useful application of foliar fertilization is when irrigation or fertigation is withheld from potted *Phalaenopsis* plants. Drier conditions at the root system are to be maintained after repotting or before shipping to avoid diseases and are achieved by withholding irrigation or fertigation for a few weeks when using sphagnum moss as the substrate. It is during this period that foliar fertilization should prove useful for supplying the nutrient requirements of the plants.

SIGNIFICANCE OF STORED N POOL IN *PHALAEOPSIS*. By tracing the fate of fertilizer N with ^{15}N -labeling in Expt. 2, we determined the contribution of fertilizer N to the N accumulated in actively growing *Phalaenopsis* leaves. The level of enrichment in the fertilizer solution was 22.5 atom% ^{15}N , corresponding to 23.7% ^{15}N excess by weight out of a total N in the labeled fertilizer, whereas ^{15}N content excess in the first leaf was 117.9 μg on Day 8 (Fig. 2B); therefore, the amount of fertilizer N accumulated in the first leaf by Day 8 was 0.50 mg (i.e., 0.1179 mg/23.7%). Nitrogen content in the first leaf increased from 18.2 mg to 22.5 mg from Day 0 to Day 8 (Table 3), which was an increase of 4.3 mg. Comparing fertilizer N accumulation with the total N accumulated in the first leaf, we found that the contribution of fertilizer N to the increase of N content in the first leaves over the 8-d experimental period was only 12% (i.e., 0.50 mg/4.3 mg). Therefore, despite availability and evident absorption of N at the root zone, a significant amount of N is sourced from elsewhere to meet the requirements of actively growing *Phalaenopsis* leaves, presumably from storage in older tissues.

Leaf N concentration of the plants used in Expt. 2 ranged from 1.09% to 1.96% (Table 3). This range is within a normal range of fertility, because Wang and Konow (2002) reported a N concentration range of 1.10% to 1.60% in the leaves of *Phalaenopsis* plants fertilized with 200 $\text{mg}\cdot\text{L}^{-1}$ N. Therefore, translocation of N still occurs even when *Phalaenopsis* plants are not in a N-deficient stage. In *Phalaenopsis*, not only is N readily transported from roots to the shoot (Expts. 1 and 2), but N is also transported from the leaves to roots (Expt. 1). This highly fluid movement of N agrees with the high mobilization rate of nutrients in epiphytic orchids suggested by Hew and Yong (2004).

THE LEAVES OF *PHALAEOPSIS* HAVE N STORAGE FUNCTION. Nitrogen-15 concentration data in Expts. 2 and 3 show a significant difference. In Expt. 2, first leaves and fourth leaves had very similar ^{15}N atom% excess during the 8-d period after fertilizer application (Fig. 2A). By contrast, in Expt. 3 the fourth leaves had a much lower ^{15}N atom% excess compared with first and second leaves 8 weeks after fertilizer application (Fig. 3D). Hence, accumulation of fertilizer N occurred in the fourth leaves shortly after fertilizer application, but after a longer-term period, the stored N was translocated elsewhere. This observation

suggests that older leaves of *Phalaenopsis* can act as temporary storage for fertilizer N shortly after fertilizer application.

Based on the results of Expt. 4, the concentrations of both N and ^{15}N decreased in *Phalaenopsis* leaves as the plant shifted from vegetative to reproductive stages (Figs. 4B and 4D). In newly grown leaves, this decrease could be the result of a dilution effect as more dry weight was accumulated. However, in mature leaves, the likely cause was the translocation of N out of the mature leaves, because there was no significant change in the dry weight of mature leaves (Fig. 4A).

Our finding thus indicated that the mature leaves of *Phalaenopsis* can store N and translocate it for future use, thus serving the function of N source. The cactus pear (*Opuntia ficus-indica*), a CAM crop that has strong buffering capacity to fertilizer applications, has a high water content and stores a large nutrient pool in the plant (Felker and Bunch, 2009). Nitrate can be stored in the cladode (photosynthetic organ that constitutes the aboveground portion of the cactus pear plant) during periods when excess nitrate is available or during periods of slow growth, to be used later (Nerd and Nobel, 1995). Our results obtained from different experiments in this study strongly indicate the capability of mature leaves to store N for later use in *Phalaenopsis*, which is also a CAM plant (Hung, 2012).

PHALAEOPSIS ROOTS LIKELY HAVE THE FUNCTION OF N STORAGE. In Expt. 1, the ^{15}N concentration in roots receiving ^{15}N treatment decreased significantly ($P \leq 0.01$ by paired t test) between Week 4 and Week 8 after treatment (Table 1), showing that translocation of ^{15}N out of the roots took place several weeks after a single application of labeled fertilizer. In Expt. 4, out of the whole-plant ^{15}N content excess at 1, 2, 4, 8, 16, and 38 weeks after the application of ^{15}N -labeled fertilizer, the root ^{15}N content excess constituted 69%, 63%, 59%, 51%, 45%, and 35%, respectively (calculated from Fig. 4E); in other words, the proportion of ^{15}N in the roots out of the total ^{15}N absorbed was continually decreasing, demonstrating the likelihood of translocation of previously stored ^{15}N from the roots.

Between Week 8 and Week 16 in Expt. 4, the dry weights of roots, mature leaves, and newly grown leaves changed little, but significant amounts of N were accumulated in these organs, and the rates of accumulation were similar (Fig. 4A–C). The bulk of the N accumulated in these organs (i.e., roots, mature leaves, and newly grown leaves) probably came from recently absorbed fertilizer N, because regular Johnson's solution was applied regularly throughout the experimental period. At the same time, our data suggest that ^{15}N from the labeled fertilizer was still present in the substrate and was still absorbed and accumulated, because whole-plant ^{15}N content excess was still increasing between Week 16 and Week 38 (Fig. 4E). Considering the roots in particular, we can infer that accumulation of newly absorbed ^{15}N in the root tissue still took place between Week 8 and Week 16; this is because the roots still accumulated fertilizer N as shown by the increase in N content despite a lack of change in dry weight (Figs. 4A and 4C), whereas part of this recently absorbed N should be in the form of ^{15}N .

Although the accumulation of newly absorbed ^{15}N in roots between Week 8 and Week 16 can be inferred from this, ^{15}N content excess in roots remained at the same level during this period, indicating that some ^{15}N previously stored in the root must have been translocated elsewhere. The decrease in ^{15}N concentration in roots between Weeks 8 and 16 while ^{15}N concentration increased in the other organs (Fig. 4D) is consistent with outward translocation of previously stored ^{15}N from the

roots. The aforementioned observations from Expts. 1 and 4 indicate that *Phalaenopsis* roots are likely to have the function of N storage.

YOUNG ACTIVELY GROWING TISSUES IN PHALAEOPSIS SHOW STRONG SINK ACTIVITY FOR N. In Expt. 1, we found that regardless of site of application of the labeled fertilizer, newly grown leaves, which were not application sites of fertilizer for all treatments, consistently received the highest allocation of the fertilizer translocated from the application site; between 40% and 46% of the translocated ^{15}N was allocated to the newly grown leaves $[(4.5/11.2) \times 100\%, (6.9/15.0) \times 100\%$; calculated from Table 2]. This result shows that among all organs during the vegetative growth stage, newly grown leaves had the highest sink strength for N. Furthermore, results from Expt. 3 show that sink strength of *Phalaenopsis* leaves decreases with increasing leaf age, which is in agreement with what has been stated above.

We also found that fertilizer N applied to leaves was still translocated to roots (Table 1). Hence, from Expt. 1 we can see that if we isolate the roots out of their role as a conduit of nutrients from substrate to shoot, the roots by themselves show sink activity for N; and just as is observed in leaves where the actively growing young leaves show higher sink activity than mature leaves (Tables 1 and 2; Fig. 3), actively growing young roots show higher sink activity than older roots (Tables 1 and 2). This is not surprising, because roots as living organs have basic metabolism, which requires N; this requirement is especially high in young roots, which have high metabolic activity.

Results of Expts. 1, 3, and 4 indicate that during the vegetative stage, newly grown roots and newly grown leaves are strong sink organs for N in *Phalaenopsis* (Table 2; Figs. 3 and 4), whereas stalks and flowers are strong sinks during the reproductive growth period (Fig. 4). Newly grown roots, newly grown leaves, and reproductive organs are all organs with high metabolic activity, whereas metabolism requires N. Therefore, actively growing tissues with high metabolic activities are to be expected to have high N requirements. Similar results were found in blueberry, where the current season's stems and leaves receive the highest allocation of fertilizer N (Retamales and Hanson, 1989).

FERTILIZER APPLICATION THUS HAS A LONG-TERM EFFECT ON PHALAEOPSIS GROWTH. Plants received a single application of labeled fertilizer N in Expt. 4. Throughout the 38-week period, the plant still accumulated fertilizer N that had been applied at the beginning of the period (Fig. 4E). This result provides evidence of the high nutrient-holding capacity of the sphagnum moss substrate, which has also been demonstrated by the work of Yao et al. (2008).

Storage and partitioning of N have been shown with ^{15}N -labeling in other crops. In fruit trees, the woody trunk and roots are major storage organs for N. This storage function of woody tissues can perhaps be intuitively reasoned in the case of deciduous fruits, but it has been definitively shown with ^{15}N -labeling in peach trees, the old woody tissues of which provide the bulk of N during fruit set in spring, whereas before leaf fall they receive translocation of N from the foliage (Munoz et al., 1993). In greenhouse roses, a perennial crop with shorter cycles of growth and harvest, the N stored in old stems and foliage significantly contributes to the N requirement of new flowering shoots (Cabrera et al., 1995). In some orchids that have pseudobulbs, the pseudobulbs have been shown to serve the function as nutrient and water storage (Ng and Hew, 2000).

We have here shown with ^{15}N -labeling, the capability of the leaves of *Phalaenopsis* to store N. This ability may be coupled with ability to act as a reservoir of water as indicated by the water storage capacity of lower leaves of *Phalaenopsis* (Hung, 2012) and perhaps as a reservoir of other types of minerals and nutrients as well. Owing to the nutrient storage function in tissues and high nutrient-holding capacity of sphagnum moss substrate, fertilizer application thus has a long-term effect on *Phalaenopsis* growth.

In conclusion, although *Phalaenopsis* can absorb fertilizer N through both its roots and leaves, the roots have better absorption efficiency and translocate the absorbed N more readily compared with leaves. Nitrogen is a mobile nutrient. In many plants, N deficiency will result in transport of N from older tissues to young tissues. So the translocation of N is a common phenomenon. What is particular about *Phalaenopsis* is the fluidity with which N is translocated, even when N supply is not limiting. We have provided from these experiments numerical data indicating the presence of a significant N pool in *Phalaenopsis* tissues. A significant storage pool of various nutrients probably accounts for the great resilience of *Phalaenopsis* against nutrient deficiency stress in general.

Literature Cited

- Cabrera, R.I., R.Y. Evans, and J.L. Paul. 1995. Nitrogen partitioning in rose plants over a flowering cycle. *Sci. Hort.* 63:67–76.
- Chang, Y.C.A., W.L. Lin, J.Y. Hou, W.Y. Yen, and N. Lee. 2013. Concentration of 1-methylcyclopropene and the duration of its application affect anti-ethylene protection in *Phalaenopsis*. *Sci. Hort.* 153:117–123.
- Feigenbaum, S., H. Bielraj, Y. Erner, and S. Dasberg. 1987. The fate of ^{15}N labeled nitrogen applied to mature citrus trees. *Plant Soil* 97:179–187.
- Felker, P. and R.A. Bunch. 2009. Mineral nutrition of cactus for forage and fruits. *Acta Hort.* 811:389–394.
- Fernandez, V. and T. Eichert. 2009. Uptake of hydrophilic solutes through plant leaves: Current state of knowledge and perspectives of foliar fertilization. *Crit. Rev. Plant Sci.* 28:36–68.
- FloraHolland. 2013. Prijsinformatie FloraHolland week 52. 17 Jan. 2013. <http://www.floraholland.com/media/1075568/Ext_Week52_Prijsinformatie_Internet.PDF> [in Dutch].
- Fritz, A. 1978. Foliar fertilization—A technique for improved crop production. *Acta Hort.* 84:43–56.
- Haynes, R.J. and K.M. Goh. 1977. Review on physiological pathways of foliar absorption. *Sci. Hort.* 7:291–302.
- Hew, C.S. and J.W.H. Yong. 2004. The physiology of tropical orchids in relation to the industry. World Scientific Publ., Singapore.
- Hou, J.Y., T.L. Setter, and Y.C.A. Chang. 2010. Effects of simulated dark shipping on photosynthetic status and post-shipping performance in *Phalaenopsis* Sogo Yukidian 'V3'. *J. Amer. Soc. Hort. Sci.* 135:183–190.
- Hung, T.C. 2012. Meta analysis of photosynthetic pathway in *Phalaenopsis aphrodite* combining physiological approach and gene expression profiling studies. MS thesis, Natl. Taiwan Univ., Taipei, Taiwan [in Chinese with English abstract].
- Ichihashi, S., K. Miyata, K. Kato, T. Miwa, Y. Nakazawa, A. Suzuki, R. Kashima, and J. Kato. 2010. The effects of $\text{NH}_4\text{-N}$ and plant growth regulators on *Phalaenopsis* spiking and flowering. *Acta Hort.* 878: 335–346.
- Johnson, C.M., P.R. Stout, T.C. Broyers, and A.B. Carlton. 1957. Comparative chlorine requirements of different plant species. *Plant Soil* 8:337–353.
- Kannan, S. 1986. Physiology of foliar uptake of inorganic nutrients. *Proc. Indian Acad. Sci.* 96:457–470.
- Lajtha, K. and R.H. Michener. 1994. Stable isotopes in ecology and environmental science. Blackwell Scientific Publications, London, UK.
- Lee, C.H. and N. Lee. 1991. Characteristics of morphology and anatomy in root and leaf of *Phalaenopsis amabilis*. *J. Chinese Soc. Hort. Sci.* 37:237–248 [in Chinese with English abstract].
- Lei, H.Y. 2007. Changes of mineral composition and fertilizer requirement of *Phalaenopsis* during reproductive stages. MS thesis, Natl. Taiwan Univ., Taipei, Taiwan [in Chinese with English abstract].
- Munoz, N., J. Guerri, F. Legaz, and E. Primo-Millo. 1993. Seasonal uptake of ^{15}N -nitrate and distribution of absorbed nitrogen in peach trees. *Plant Soil* 150:263–269.
- Nerd, A. and P.S. Nobel. 1995. Accumulation, assimilation, and partitioning of nitrate in *Opuntia ficus-indica*. *J. Plant Nutr.* 18:2533–2549.
- Ng, C.K.Y. and C.S. Hew. 2000. Orchid pseudobulbs - 'False' bulbs with a genuine importance in orchid growth and survival! *Sci. Hort.* 83:165–172.
- Retamales, J.B. and E.J. Hanson. 1989. Fate of ^{15}N -labeled urea applied to mature highbush blueberries. *J. Amer. Soc. Hort. Sci.* 114: 920–923.
- Sandrock, D.R., T.L. Righetti, and A.N. Azarenko. 2005. Isotopic and nonisotopic estimation of nitrogen uptake efficiency in container-grown woody ornamentals. *HortScience* 40:665–669.
- Trepanier, M., M.P. Lamy, and B. Dansereau. 2009. *Phalaenopsis* can absorb urea directly through their roots. *Plant Soil* 319:95–100.
- U.S. Department of Agriculture. 2012. National agricultural statistics service: Floriculture crops 2011 summary. 17 Jan. 2013. <<http://usda01.library.cornell.edu/usda/current/FlorCrop/FlorCrop-05-31-2012.pdf>>.
- Wang, Y.T. 2000. Impact of a high phosphorus fertilizer and timing of terminating fertilization on flowering of a hybrid moth orchid. *HortScience* 35:60–62.
- Wang, Y.T. 2007. Potassium concentration affects growth and flowering of *Phalaenopsis* in a bark mix or sphagnum moss substrate. *HortScience* 42:1563–1567.
- Wang, Y.T. 2010. *Phalaenopsis* mineral nutrition. *Acta Hort.* 878: 321–333.
- Wang, Y.T. and E.A. Konow. 2002. Fertilizer source and medium composition affect vegetative growth and mineral nutrition of a hybrid moth orchid. *J. Amer. Soc. Hort. Sci.* 127:442–447.
- Westerman, R.L. and L.T. Kurtz. 1974. Isotopic and nonisotopic estimations of fertilizer nitrogen uptake by sudangrass in field experiments. *Soil Sci. Soc. Amer. Proc.* 38:107–109.
- Yao, H.Y., R.S. Chung, S.B. Ho, and Y.C.A. Chang. 2008. Adapting the pour-through medium extraction method to *Phalaenopsis* grown in sphagnum moss. *HortScience* 43:2167–2170.
- Yoneda, K., M. Usui, and S. Kubota. 1997. Effect of nutrition deficiency on growth and flowering of *Phalaenopsis*. *J. Jpn. Soc. Hort. Sci.* 66: 141–147 [in Japanese with English abstract].
- Yu, Y.C. 2012. Growth response and gene expression profiling in *Phalaenopsis* under nitrogen, phosphorus, and potassium deficiency. MS thesis, Natl. Taiwan Univ., Taipei, Taiwan.