Nitrogen Source for Inflorescence Development in *Phalaenopsis*: II. Effect of Reduced Fertilizer Level on Stored Nitrogen Use

Hadi Susilo and Yao-Chien Alex Chang
Department of Horticulture and Landscape Architecture, National Taiwan University, 1 Roosevelt Road Sec. 4, Taipei 10617, Taiwan

Additional Index Words. limited fertility, mineral mobilization, moth orchid, nitrogen-15, nitrogen storage, reproductive stage

Abstract. Plants of *Phalaenopsis* orchid are known for their great resilience and ability to flower under less than ideal conditions, including long periods without fertilization. Significant nutrient storage is thought to account for this characteristic; however, the use of stored nutrients in *Phalaenopsis* has not been fully studied. We used $^{15}$N-labeled Johnson’s solution to trace the use of stored nitrogen (N) and recently absorbed fertilizer N in *Phalaenopsis* given various fertilizer levels during forcing. By separately labeling fertilizer N applied to *Phalaenopsis* Sogo Yukidian ‘V3’ plants 6 weeks before and 6 weeks into forcing, we found in the inflorescence that the ratio of N derived from fertilizer applied 6 weeks before forcing to the N derived from fertilizer applied 6 weeks into forcing was 43% to 57%. With 90% reduction in fertilizer concentration during the reproductive stage, the ratio increased to 89% to 11%, indicating that stored N becomes a significant N source for inflorescence development when fertility becomes limited. Reducing fertilizer level during the reproductive stage from full-strength Johnson’s solution down to zero decreased the dry weight of newly grown leaves, reduced the number of flowers from 10.8 to 8.9, and slightly increased the time required between initiation of forcing and anthesis. However, the overall effect of reduced fertilization on the growth and flowering of *Phalaenopsis* Sogo Yukidian ‘V3’ plants in this study was slight, because under little or no fertilization, more stored N was mobilized and this was sufficient to meet most of the N demand for inflorescence development.

*Phalaenopsis* is among the world’s most important horticulture crops grown as flowering potted plants and is also grown for cut flower production. In recent years, *Phalaenopsis* is the highest grossing potted flowering plant in the world’s major floriculture markets (FloraHolland, 2013; U.S. Department of Agriculture, 2013). In addition to a long blooming period (Chang et al., 2013), *Phalaenopsis* is also noted for resilience against stresses (Hou et al., 2010; Hung, 2012; Lei, 2007), which makes it very versatile and adaptable, both during production and post-harvest.

Nitrogen is an important macronutrient in plants. The growth and flowering of *Phalaenopsis* are significantly affected by the N status in the plants (Lei, 2007; Yu, 2012). In *Phalaenopsis*, symptoms of N deficiency include reduced leaf number and leaf area, low chlorophyll content and reduced dry weight of leaves, and a greater incidence of leaf drop (Yoneda et al., 1997). N deficiency could also result in reduced flowering quality in *Phalaenopsis* (Peng, 2008; Wang, 2000; Wang and Gregg, 1994).

Although the effects of N deficiency in *Phalaenopsis* are known and have been reported, *Phalaenopsis* plants do show great resilience to N deficiency (Lei, 2007; Yu, 2012). Healthy plants can continue to grow and flower normally for a long time even under suboptimal N supply (Lei, 2007). In our previous study, we showed that this resilience to N deficiency is attributable to significant amounts of N being stored in the plants (Susilo et al., 2013). In another study, we quantified the contribution of fertilizer N stored throughout the vegetative stage of *Phalaenopsis* to the developing inflorescence when fertilizer supply was ample during forcing (Susilo et al., 2014). We hypothesized that reducing fertilizer level during the forcing period would increase the contribution of previously stored N to inflorescence development. To our knowledge there is no conclusive evidence to support this hypothesis in the current literature.

High rates of N before harvest are known to reduce the quality of some flowering crops (e.g., ter Hell and Hendriks, 1995). Consequently, some *Phalaenopsis* growers reduce or withhold fertilization altogether during the finishing stage of *Phalaenopsis*. Moreover, post-sale plants are often subjected to periods of no fertilization. Although effects and symptoms of N deficiency in *Phalaenopsis* have been reported in the literature (Lei, 2007; Peng, 2008; Wang and Gregg, 1994; Yoneda et al., 1997; Yu, 2012), how the stored N and currently applied fertilizer N are used in *Phalaenopsis* grown with reduced fertilizer level is still largely unknown. In the present study, we used $^{15}$N labeling to trace the use of stored N and newly absorbed fertilizer N under various fertilizer levels during the reproductive stage of *Phalaenopsis*.

Materials and Methods

Experimental Procedure. The objective of this experiment was to investigate how different rates of fertilizer application...
during the reproductive stage of *Phalaenopsis* would affect the use of N accumulated during the vegetative growth stage. Mature vegetatively propagated *Phalaenopsis* Sogo Yukidian ‘V3’ plants grown in sphagnum moss in 10.5-cm pots were divided into one group of seven plants and five groups of 20 plants each. The first group of seven plants was labeled “before forcing,” whereas the remaining five groups were labeled V 0%, V 10%, V 100%, F 10%, and F 100% (Fig. 1), where the first letter refers to the stage of $^{15}$N labeling [i.e., during the vegetative (V) or forcing (F) period] and 0%, 10%, and 100% refer to fertilization during the reproductive stage with 0%-,-, and 100%-strength Johnson’s solution (Johnson et al., 1957), respectively. The timing of $^{15}$N label application was designed so that all treatments had the same $^{15}$N absorption duration of 6 weeks.

A 6-week period of vegetative growth in a phytotron at 30/25 °C day/night temperature with maximum light intensity of 450 μmol·m$^{-2}$·s$^{-1}$ constituted the first phase of the experiment (Fig. 1). All plants received the same fertilization, which was provided by subirrigating each pot in a saucer filled with 200 mL full-strength Johnson’s solution when the surface of the sphagnum moss appeared dry. All plants were fertigated at the beginning of the experiment. The V 0%, V 10%, V 100% groups and the “before forcing” group received Johnson’s solution labeled with 11.25 atom% $^{15}$N at this first fertigation (Fig. 1). After 6 weeks of vegetative growth, plants in the “before forcing” group were harvested, divided into newly grown leaves (leaves produced since the start of the experiment), mature leaves (all leaves other than newly grown leaves), stem, and roots, measured for dry weight, and ground to fine powder for analysis of total N and $^{15}$N concentrations. The roots of plants in the remaining five groups were cleaned of the old substrate, washed, and the plants were repotted into fresh sphagnum moss medium to end $^{15}$N-labeling. Plants were then subjected to the second phase of the experiment.

A 12-week period of cultivation in a phytotron at 25/20 °C day/night temperature with maximum light intensity of 450 μmol·m$^{-2}$·s$^{-1}$ constituted the second, forcing phase of the experiment (Fig. 1). During this period, plants in the V 100% and F 100% groups were fertigated with full-strength Johnson’s solution, whereas those in the V 10% and F 10% groups were fertigated with 10%-strength Johnson’s solution. Plants in the V 0% group were irrigated with distilled water. Fertigation was done when the surface of the sphagnum moss appeared dry by subirrigating each pot with 200 mL of water or fertilizer solution as indicated in the various treatments. Six weeks into the forcing period, at which point the plants had spikes averaging 13 cm long, all plants were fertigated and the fertilizer solutions for the F 10% and F 100% groups were labeled with 11.25 atom% $^{15}$N (Fig. 1).

Six weeks after $^{15}$N treatment during the forcing period, the inflorescences having reached the visible bud stage, the SPAD values of the second and seventh leaves from the apex were measured for all plants with a chlorophyll meter (SPAD502; Minolta, Tokyo, Japan). Seven plants from each treatment were harvested, divided into newly grown leaves, mature leaves, stem, roots, and inflorescence, measured for dry weight, and analyzed for total N and $^{15}$N concentrations. The remaining 13 plants in each treatment were transferred to a Venlo-type greenhouse with an average temperature of 19.3 °C and maximum light intensity of 400 μmol·m$^{-2}$·s$^{-1}$ for observation.

### Culture conditions

| Repotting | Forcing | Sample collection | Flowering |
|-----------|---------|-------------------|-----------|
| 30/25 °C  | 25/20 °C| Flowering observation in a Venlo-type greenhouse |
| 6 weeks   | 12 weeks|                   | 18 weeks  |

**Fertilized with 100%-strength Johnson’s solution (100% J)**

| Repotting | Forcing | Sample collection | Flowering |
|-----------|---------|-------------------|-----------|
|          |         |                   |           |

**Fertilized with 0%, 10%, or 100%-strength Johnson’s solution**

| Repotting | Forcing | Sample collection | Flowering |
|-----------|---------|-------------------|-----------|
|          |         |                   |           |

### Treatments

1. **Before forcing**
   - Vegetative, 100% J
   - $^{15}$N harvested

2. **V 0%**
   - Vegetative, 100% J
   - Forcing, 0% J
   - Flowering observation, 0% J
   - $^{15}$N

3. **V 10%**
   - Vegetative, 100% J
   - Forcing, 10% J
   - Flowering observation, 10% J
   - $^{15}$N

4. **V 100%**
   - Vegetative, 100% J
   - Forcing, 100% J
   - Flowering observation, 100% J
   - $^{15}$N

5. **F 10%**
   - Vegetative, 100% J
   - Forcing, 10% J
   - Flowering observation, 10% J
   - $^{15}$N

6. **F 100%**
   - Vegetative, 100% J
   - Forcing, 100% J
   - Flowering observation, 100% J
   - $^{15}$N

---

**Fig. 1.** Culture conditions of *Phalaenopsis* Sogo Yukidian ‘V3’ and treatments for investigating the utilization of stored N under reduced fertilization in the reproductive stage.
of flowering. The dates of spiking, first flower anthesis, and first flower senescence were recorded and used to calculate the time to spiking, time to anthesis, and longevity of first flower. First flower diameter and total number of flowers were also recorded.

Fertilization regimens during the flowering observation period were the same as those during the forcing period (Fig. 1).

**15N-LABELING OF FERTILIZER.** The fertilizer solution used in this study was modified Johnson’s solution containing the following elements: 16 mM N, 2 mM phosphorus, 6 mM potassium, 4 mM calcium, 1 mM sulfur, 1 mM magnesium, 50 mM chloride, 25 mM boron, 5 mM manganese, 4 mM iron, 2 mM zinc, 0.5 mM copper, 0.1 mM molybdenum, and 0.1 mM nickel. The ammonium-to-nitrate ratio in the solution was 1:7. 15N-labeling was done by substituting half of the potassium nitrate in the Johnson’s solution with 60 atom% 15N-labeled potassium nitrate (ISOTEC, Miamisburg, OH) to attain a 15N concentration of 11.25 atom%.

**ANALYSIS FOR TOTAL N AND 15N ISOTOPE AND DATA CALCULATION.** Between 3 and 4 mg of the dried, ground samples were packaged into 8 × 5-mm tin capsules (Elemental Microanalysis, Okehampton, U.K.). The packaged samples were sent to the Stable Isotope Facility at the University of California, Davis for total N and 15N analysis using the same procedure as that described in Susilo et al. (2014). The atom percent 15N excess (atom% excess) was obtained by subtracting natural abundance of 15N (0.366 atom%) from the results of the 15N analysis. The 15N content excess = N content × weight% of excess 15N out of total N.

**STATISTICAL ANALYSIS.** The experiment was carried out in a completely randomized design. Data were subjected to analysis of variance to analyze the effect of treatments, and mean separation between treatments was obtained with the least significant difference test at $P \leq 0.05$ using CoStat software (Version 6.101; CoHort Software, Monterey, CA). SigmaPlot software (Version 10.0; Systat Software, San Jose, CA) was used to plot graphs in Figure 2, and the linear regression analysis was conducted using SAS software (Version 9.3; SAS Institute, Cary, NC).

**Results and Discussion**

**EFFECT OF VARIOUS FERTILIZER LEVELS ON DRY WEIGHT, TOTAL N, AND 15N LEVELS.** Low fertility during the reproductive stage generally decreased the dry weight of newly grown leaves, but had little effect on the dry weights of mature leaves, stem, roots, inflorescence, and the entire plant at the visible bud stage (Table 1). Reduced fertilization generally decreased N concentrations of newly grown leaves, roots, and inflorescence as well as whole-plant N concentration (Table 2). Reduced fertilizer also decreased SPAD values in upper and lower leaves (Table 2). Nitrogen contents of newly grown leaves, mature leaves, roots, inflorescence, and the whole plant declined as fertilizer concentration decreased (data not shown).

15N-labeling of fertilizer N was conducted at two stages in this experiment. In the treatment groups V 0%, V 10%, and V 100%, 15N concentrations and contents of the harvested samples indicate the fate of the stored N accumulated during the vegetative period, whereas 15N concentrations and contents in various plant parts in the treatment groups F 10% and F 100% show the fate of fertilizer N absorbed during the forcing period.

Regression analysis of 15N concentration at the visible bud stage of plants subjected to V 0%, V 10%, and V 100% treatments was conducted to compare the effect of lower fertilizer levels during the reproductive stage on the use of N stored during the vegetative stage. As the concentration of the Johnson’s solution decreased from 100% to 0% (i.e., from 16 to 0 mM N) during the reproductive stage, 15N concentrations in the newly grown leaves, stem, roots, inflorescence, and the whole plant increased linearly [$R^2 = 0.67$ to 0.89 (Fig. 2)]. The 15N concentration in the mature leaves increased from 0.668 to 0.758 atom% excess as fertilizer N concentration decreased from 16 to 0 mM. However, little correlation was found between

[Graph showing linear regression analysis for various plant parts with data points and regression equations.]

Fig. 2. Effect of fertilization with various nitrogen (N) concentrations during the reproductive stage on 15N concentration in Phalaenopsis Sogo Yukidian ‘V3’ harvested at the visible bud stage. 15N was applied 6 weeks before low-temperature forcing and the 15N label represented fertilizer N stored during the vegetative stage. Bars indicate se of the means (n = 7).
N concentration in the fertilizer and $^{15}$N concentration (Fig. 2B). The whole-plant $^{15}$N concentration increased from 0.98 atom% excess to 1.21 atom% excess as the concentration of fertilizer was reduced from full-strength to 0%-strength Johnson’s solution (Fig. 2F).

The $^{15}$N concentrations in the newly grown leaves, mature leaves, stem, and roots were lower under higher fertilizer levels because as more N was supplied and accumulated in these organs, the ratio of $^{15}$N increased, whereas the ratio of $^{15}$N to the total N decreased. Of particular interest, the inflorescence developed during the reproductive stage with stored N and recently absorbed fertilizer N being the sources of N. The observed increase in $^{15}$N concentration in the inflorescence with reduced fertilizer levels was because as N supply from the fertilizer was reduced during the reproductive stage, the proportion of stored $^{15}$N label that ended up in the inflorescence increased.

Plants in the “before forcing,” V 0%, V 10%, and V 100% treatments had similar whole-plant $^{15}$N content excess, because plants in these treatments received the same amount of $^{15}$N label during the vegetative stage (Table 3). The $^{15}$N content excess of the inflorescence differed little among the V 0%, V 10%, and V 100% treatments, but lower $^{15}$N content excess was found in the newly grown leaves of plants receiving V 0% treatment (Table 3). This was because during the reproductive stage, the inflorescence is a stronger sink for N than leaves in Phalaenopsis (Susilo et al., 2014) and thus the allocation of stored N to the inflorescence in the present study was less affected by changes in the fertilizer level. In contrast, as a result of the decreasing sink strength of newly grown leaves during the reproductive stage, the dry weight of the inflorescence was unaffected by reduced fertilization during the reproductive stage, whereas the dry weight of newly grown leaves when they were given less fertilizer during the reproductive stage. By the same reasoning, the dry weight of the inflorescence was unaffected by reduced fertilization during the reproductive stage, whereas the dry weight of newly grown leaves was lower (Table 1).

In the F 10% and F 100% treatments, the stage of $^{15}$N-labeling was during the forcing period. Whole-plant $^{15}$N content excess in the F 10% treatment was approximately one-tenth that in the F 100% treatment (Table 3), because plants in the F 10% treatment received one-tenth the amount of $^{15}$N label as in the F 100% treatment. Similarly, inflorescence $^{15}$N content excess in the F 10% treatment was approximately one-tenth that in the F 100% treatment (Table 3).

The effects of reduced fertilizer levels during forcing and flowering are small when N storage in plants is sufficient. As inflorescence development progressed beyond the visible bud stage, reducing fertilizer level resulted in lower bud count and slightly delayed the anthesis of the first flower, but had little effect on the time of spiking and the longevity of the first flower (Table 4). Flower diameter was unaffected by reduced fertilizer level, which is in agreement with the results obtained by previous workers (Lei, 2007; Peng, 2008; Wang, 2000; Wang and Gregg, 1994; Yu, 2012).

Reduced fertilizer level during the reproductive stage of Phalaenopsis Sogo Yukidian ‘V3’ reduced

---

**Table 1.** Dry weight of Phalaenopsis Sogo Yukidian ‘V3’ before forcing and at the visible bud stage after forcing under various fertilizer levels.

| Treatment | Newly grown leaves | Mature leaves | Stem | Roots | Inflorescence | Total |
|-----------|--------------------|---------------|------|-------|---------------|-------|
| Before forcing | 1.75 | 7.43 | 1.44 | 8.06 | — | 18.69 |
| V 0% | 2.74 b | 7.04 a | 1.62 a | 9.07 a | 2.59 a | 23.06 a |
| V 10% | 2.85 ab | 7.15 a | 1.70 a | 9.22 a | 2.64 a | 23.55 a |
| F 10% | 2.83 ab | 7.02 a | 1.73 a | 9.10 a | 2.44 a | 23.12 a |
| V 100% | 3.06 a | 7.27 a | 1.68 a | 8.70 a | 2.45 a | 23.16 a |
| F 100% | 3.02 a | 7.30 a | 1.65 a | 8.79 a | 2.50 a | 23.25 a |

- Plants in all treatments were fertilized with 100%-strength Johnson’s solution during the vegetative stage, but during the reproductive stage, they were fertilized with 0%- or 10%- strength Johnson’s solution as indicated. Labeling with Johnson’s solution enriched with 11.25 atom% $^{15}$N was done in the vegetative (V) period 6 weeks before forcing in the “before forcing” group as well as the V 0%, V 10%, and V 100% treatments, whereas in the F 10% and F 100% treatments, the $^{15}$N-labeling was done during the forcing (F) period at 6 weeks into forcing.

**Table 2.** Nitrogen concentration and relative chlorophyll concentration in Phalaenopsis Sogo Yukidian ‘V3’ before forcing and at the visible bud stage after forcing under various fertilizer levels.

| Treatment | Newly grown leaves | Mature leaves | Stem | Roots | Inflorescence | Whole plant |
|-----------|--------------------|---------------|------|-------|---------------|-------------|
| Before forcing | 2.63 a | 1.47 c | 0.97 a | 1.91 ab | — | 1.73 b |
| V 0% | 1.95 d | 1.63 b | 0.86 b | 1.44 c | 1.04 c | 1.47 d |
| V 10% | 2.24 c | 1.74 b | 0.79 c | 1.56 c | 1.07 c | 1.59 ed |
| F 10% | 2.19 c | 1.65 b | 0.78 c | 1.50 c | 1.07 c | 1.53 d |
| V 100% | 2.42 b | 1.87 a | 0.87 b | 2.11 a | 1.30 a | 1.90 a |
| F 100% | 2.14 c | 1.65 b | 0.83 bc | 1.79 b | 1.19 b | 1.66 bc |

**N concn (% dry wt)**

**SPAD value**

| Second leaf from apex | Seventh leaf from apex |
|----------------------|-----------------------|
| 76.1 b | 51.8 b |
| 72.4 ab | 50.3 b |
| 72.1 b | 50.8 b |
| 73.6 a | 57.6 a |
| 73.6 a | 59.1 a |

- Plants in all treatments were fertilized with 100%-strength Johnson’s solution during the vegetative stage, but during the reproductive stage, they were fertilized with 0%- or 10%-strength Johnson’s solution as indicated. Labeling with Johnson’s solution enriched with 11.25 atom% $^{15}$N was done in the vegetative (V) period 6 weeks before forcing in the “before forcing” group as well as the V 0%, V 10%, and V 100% treatments, whereas in the F 10% and F 100% treatments, the $^{15}$N-labeling was done during the forcing (F) period at 6 weeks into forcing.

- Newly grown leaves were those that developed after the start of the experiment, whereas mature leaves were the rest of the foliage.

- Means in the same column followed by different letters are significantly different by the least significant difference (LSD) test at $P \leq 0.05$ (n = 7). Dry weights before forcing were excluded from the LSD test.

---

J. AMER. SOC. HORT. SCI. 139(1):76–82. 2014. 79
the dry weight of newly grown leaves, the N concentration and content in the various organs as well as the number of flowers [Tables 1, 2, and 4 (data for N content not shown)], but these reductions were slight because N stored during the vegetative stage met a portion of the total N requirement during the reproductive stage.

Nitrogen deficiency in *Phalaenopsis* has been shown to produce significant effects such as leaf chlorosis (Yu, 2012), increase in leaf drop (Yoneda et al., 1997), reduced leaf growth

Table 3. ¹⁵N content excess in *Phalaenopsis* Sogo Yukidian ‘V3’ before forcing and at the visible bud stage after forcing under various fertilizer levels.

| Treatment* | Newly grown leaves¹ | Mature leaves¹ | Stem | Roots | Inflorescence | Whole plant |
|------------|---------------------|----------------|------|-------|---------------|-------------|
| Before forcing | 990 b⁴ | 805 b | 189 a | 2444 a | — | 4429 a |
| V 0% | 1057 b | 928 a | 172 b | 1809 b | 402 b | 4367 a |
| V 10% | 1226 a | 1009 a | 158 bc | 1871 b | 405 b | 4669 a |
| F 10% | 44 d | 56 d | 13 e | 228 c | 49 c | 390 c |
| V 100% | 1267 a | 970 a | 145 c | 1874 b | 359 b | 4617 a |
| F 100% | 553 c | 616 c | 99 d | 1915 b | 468 a | 3652 b |

¹Plants in all treatments were fertilized with 100%-strength Johnson’s solution during the vegetative stage, but during the reproductive stage, they were fertilized with 0%- or 10%- or 100%-strength Johnson’s solution as indicated. Labeling with Johnson’s solution enriched with 11.25 atom% ¹⁵N was done in the vegetative (V) period 6 weeks before forcing in the “before forcing” group as well as the V 0%, V 10%, and V 100% treatments, whereas in the F 10% and F 100% treatments, the ¹⁵N-labeling was done during the forcing (F) period at 6 weeks into forcing.

²Newly grown leaves were those that developed after the start of the experiment, whereas mature leaves were the rest of the foliage.

³Means in the same column followed by different letters are significantly different by the least significance difference test at P ≤ 0.05 (n = 7).

Table 4. Effects of various fertilizer levels during the reproductive stage of *Phalaenopsis* Sogo Yukidian ‘V3’ on some parameters of flowering.

| Treatment* | Time to spiking (d) | Time to anthesis (d) | Longevity of first flower (d) | First flower diam (cm) | Flowers (no.) |
|------------|---------------------|---------------------|-----------------------------|-----------------------|--------------|
| V 0% | 36.0 a⁴ | 134.0 a | 75.1 a | 12.0 a | 8.9 c |
| V 10% | 35.8 a | 129.0 bc | 74.3 a | 12.0 a | 9.4 bc |
| F 10% | 34.7 a | 131.3 ab | 76.1 a | 12.0 a | 9.6 b |
| V 100% | 35.5 a | 130.2 bc | 73.5 a | 12.0 a | 10.8 a |
| F 100% | 35.8 a | 126.8 c | 75.6 a | 12.1 a | 10.8 a |

⁴Plants in all treatments were fertilized with 100%-strength Johnson’s solution during the vegetative stage, but during the reproductive stage, they were fertilized with 0%- or 10%- or 100%-strength Johnson’s solution as indicated. Labeling with Johnson’s solution enriched with 11.25 atom% ¹⁵N was done in the vegetative (V) period 6 weeks before forcing in the “before forcing” group as well as the V 0%, V 10%, and V 100% treatments, whereas in the F 10% and F 100% treatments, the ¹⁵N-labeling was done during the forcing (F) period at 6 weeks into forcing.

⁵Means in the same column followed by different letters are significantly different by the least significant difference test at P ≤ 0.05 (n = 13).

(Yoneda et al., 1997; Yu, 2012), and reduced inflorescence length and bloom count (Wang, 2000). By contrast, severe N deficiency symptoms were lacking despite fertilizer reductions in this experiment. The lack of symptoms, on top of cultivar differences, was likely attributed to critical N concentration not being reached to cause significant effects and symptoms in plant tissues. Critically low potassium (K) concentrations are necessary for symptom development in *Phalaenopsis*. The concentration of K in plant tissues is typically way above concentrations at which deficiency symptoms begin to occur (Leigh and Wyn Jones, 1984). In *Phalaenopsis*, plants under K deficiency maintained normal-looking leaves, but it was only during flowering that severe symptoms such as leaf yellowing and necrosis as well as leaf abscission were observed (Wang, 2007; Yu, 2012). Very low K concentration (0.1% dry weight) was found in the symptomatic leaves, whereas in normal-looking leaves on the same plants, the concentration of K was much higher at 0.7% (Yu, 2012). These observations suggest the presence of a threshold K concentration in *Phalaenopsis* above which symptoms are lacking even when K storage is continually being depleted.

In the present study, N concentrations in newly grown leaves, roots, and the inflorescence decreased slightly as fertilizer concentrations became lower (Table 2). However, the range of values indicates that N concentrations in leaves were not low enough to produce drastic effects. Nitrogen concentrations as low as 0.6% to 0.9% were found in the leaves and roots of medium-sized *Phalaenopsis* Sogo Yukidian ‘V3’ plants after 32 weeks of fertilization regimen with zero N accompanied by chlorosis of leaves and stunted shoot growth (Yu, 2012). Low N concentration in a similar range was found in the work of Yoneda et al. (1997), in which excluding N from the fertilizer solution for 120 d produced deficiency symptoms in *Phalaenopsis*, with 0.6% and 0.8% N being found in the roots and leaves, respectively. By contrast, the lowest N concentration in roots or leaves was 1.44% in this study, which was found in the roots of plants given V 0% treatment (Table 2). On the same note, Lei (2007) found that excluding N from fertilizer solutions through the first flowering cycle had little effect on flowering, with N concentrations well over 1% being found in the shoot and roots, which were comparable to the N concentration range found in this study (Table 2). Thus, comparisons with the results obtained by others indicate that the critical N concentration for deficiency symptom development had not been reached in the present study.

Nitrogen level is tightly related to photosynthesis in many crops, because N availability affects photosynthetic N, which is contained in molecular components directly related to photosynthesis (Le Bot et al., 1998). Based on our observations in this study, if sufficient N is stored during the vegetative stage of *Phalaenopsis*, short-term lack of N probably would not affect physiological activity of the plants. In a previous study, when no N was supplied to 3-year-old seed-grown *Phalaenopsis* Musashino ‘90’ × Hakalau Queen ‘America’ plants, lower photosynthetic rates were found 120 d later, and the plants had lower dry weight compared with fully fertilized control plants (Yoneda et al., 1997). In the present study, reducing fertilizer N concentration from 16 to 0 ms for 84 d had little effect on dry weight of *Phalaenopsis* Sogo Yukidian ‘V3’ (Table 1), because stored N was still sufficient to meet the requirement of the plants and the critical tissue concentration for N deficiency had not been reached. Hence, photosynthesis was likely unaffected
and therefore the observed lack of change in dry weight in our experiment.

**Reduced fertilizer levels increased the use of stored N for inflorescence development.** In this study, information about the relative contribution under different fertilizer levels of stored N and recently absorbed N to the N accumulated in the inflorescence can be obtained by comparing inflorescence $^{15}$N content excess between the V 10% and F 100% treatments, as well as between the V 100% and F 100% treatments. In plants receiving full-strength Johnson’s solution, the ratio of N accumulated in the inflorescence at the visible bud stage, the ratio of N derived from fertilizer applied 6 weeks before forcing to the N derived from fertilizer applied 6 weeks into forcing was 43% to 57%, respectively [i.e., 359:468 μg (calculated from Table 3)]]. This is quite similar to the ratio that we found in another study (i.e., 31% to 69%), which was the corresponding ratio obtained from plants that were given ample fertilization (Susilo et al., 2014). When fertilizer level was reduced to 10%-strength Johnson’s solution, the ratio of N derived from fertilizer applied 6 weeks before forcing to the N derived from fertilizer applied 6 weeks into forcing became 89% to 11% [i.e., 405:49 μg (calculated from Table 3)]. By inference of the results of V 0% treatment, stored N constituted 100% of the N source for developing inflorescence when fertilizer was completely lacking during the reproductive stage. This indicates that as fertilizer level was reduced, more stored N, which was previously absorbed before spiking, was used for inflorescence development. The $^{15}$N concentration data are in good agreement with this observation, whereby decreasing fertilizer N concentration during the reproductive stage resulted in increasing proportion of stored N in the inflorescence (Fig. 2E).

The capacity for significant N remobilization under low availability of fertilizer explains the common observation that healthy *Phalaenopsis* plants can flower even without fertilization during forcing and flowering. We also observed this phenomenon in this study, in which plants in the V 0% treatment still yielded acceptable flowering even when fertilizer had been withheld for 30 weeks (Table 4). A similar case of fertilizer level affecting the extent of N remobilization from endogenous storage has been reported for the forage grass, *Lolium perenne*, in which decreasing N application concentration from 5.0 to 0.5 mm N resulted in greater translocation of $^{15}$N-labeled stored N from the roots and pseudostems to the leaves, as shown by a 7-fold increase of $^{15}$N concentration in leaves (Millard et al., 1990).

Storage of N enables uncoupling of growth and N uptake in deciduous trees, an important feature in the seasonal nature of their growth. The stored N pool meets the N requirement during the initial growth flush before N uptake occurs at the start of the growing season (Millard and Grelet, 2010). In the present study, we demonstrated a similar ability of *Phalaenopsis* plants to uncouple growth and N uptake when fertilization was limited or completely absent. However, stored N use is a significant feature in *Phalaenopsis* even when there is ample fertilizer supply. In our previous study, we found that even when significant absorption of labeled fertilizer N through the roots was evident during an 8-d period, the absorbed N only accounted for 12% of the N accumulated in the actively growing top leaf, whereas 88% came from remobilized stored N (Susilo et al., 2013). Even when fertilizer supply was ample, stored N was a significant N source for inflorescence development and constituted 43% of the total N accumulated in the inflorescence at the visible bud stage (Susilo et al., 2014). In this study, lack of fertilizer application to robust plants during forcing had little deleterious effect on inflorescence development, and we have demonstrated that stored N becomes an even more important source of N when fertility is limited.

In summary, the results of this study have shown that the great tolerance of *Phalaenopsis* to N deficiency stress is the result of the significant presence of a stored N pool, from which N is mobilized to a greater extent as fertility becomes limited. As long as N concentration does not go below a critical level, plants are affected only to a limited degree when fertilization is lacking with only slight decreases in leaf growth and bud count, whereas other quality parameters of growth and flowering were unaffected as demonstrated in the present study. Although acceptable flowering quality can be obtained without fertilizer application during the reproductive stage of *Phalaenopsis*, it is still important to provide sufficient N as well as other mineral elements during this stage to ensure optimal flowering quality and also to support subsequent growth when plants are cultivated over several cycles of flowering for cut flower production.

**Literature Cited**

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*. J. Amer. Soc. Hort. Sci. 138:117–123.

FloraHolland. 2013. Prijsinformatie FloraHolland week 30. 17 Sept. 2013. <http://www.floraholland.com/media/1773468/Prijzinformatieweek30.PDF> [in Dutch].

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].

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.

Le Bot, J., S. Adamowicz, and P. Robin. 1998. Modeling plant nutrition of horticultural crops: A review. Sci. Hort. 74:47–82.

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].

Leigh, R.A. and R.G. Wyn Jones. 1984. A hypothesis relating critical potassium concentrations for growth to the distribution and functions of this ion in the plant cell. New Phytol. 97:1–13.

Millard, P. and G. Grelet. 2010. Nitrogen storage and remobilization by trees: Ecophysiological relevance in a changing world. Tree Physiol. 30:1083–1095.

Millard, P., R.J. Thomas, and S.T. Buckland. 1990. Nitrogen supply affects the remobilization of nitrogen for the regrowth of defoliated *Lolium perenne* L. J. Expt. Bot. 41:941–947.

Peng, Y.C. 2008. The uptake, partitioning, and uses of nitrogen in *Phalaenopsis* Sogo Yukidian ‘V3’. MS thesis, Natl. Taiwan Univ., Taipei, Taiwan [in Chinese with English abstract].

Susilo, H., Y.C. Peng, and Y.C.A. Chang. 2014. Nitrogen source for inflorescence development in *Phalaenopsis*: I. Relative significance of stored and newly absorbed nitrogen. J. Amer. Soc. Hort. Sci. 139:69–75.

J. Amer. Soc. Hort. Sci. 139(1):76–82. 2014.
Susilo, H., Y.C. Peng, S.C. Lee, Y.C. Chen, and Y.C.A. Chang. 2013. The uptake and partitioning of nitrogen in *Phalaenopsis* Sogo Yukidian ‘V3’ as shown by $^{15}$N as a tracer. J. Amer. Soc. Hort. Sci. 138:229–237.

ter Hell, B. and L. Hendriks. 1995. The influence of nitrogen on keeping quality of pot plants. Acta Hort. 405:138–147.

U.S. Department of Agriculture. 2013. Floriculture crops 2012 summary. 17 Sept. 2013. <http://usda01.library.cornell.edu/usda/current/FlorCrop/FlorCrop-04-25-2013.pdf>.

Wang, Y.T. 2000. Impact of a high phosphorus fertilizer and timing of termination of fertilization on flowering of a hybrid moth orchid. HortScience 35:60–62.

Wang, Y.T. 2007. Potassium nutrition affects *Phalaenopsis* growth and flowering. HortScience 42:1563–1567.

Wang, Y.T. and L.L. Gregg. 1994. Medium and fertilizer affect the performance of *Phalaenopsis* orchids during two flowering cycles. HortScience 29:269–271.

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.