

# The Acidification of Sphagnum Moss Substrate during *Phalaenopsis* Cultivation

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**Abstract.** Sphagnum moss has been used as the major substrate for cultivating *Phalaenopsis* spp. in China, Japan, and Taiwan. With a lengthened duration of cultivation, the pH of the moss gradually declines. It is not understood what causes this decline in substrate pH. Using the vegetatively propagated *Phal.* Sogo Yukidian ‘V3’, this study investigated if substrate, fertilization, light, and plant roots could be the cause of pH decline in the substrate. The results showed that, although increasing fertilizer concentration resulted in a low initial pH (pH measured by the pour-through technique at first fertilization), fertilization itself was not the primary cause of the long-term pH decline. Regardless of whether the sphagnum moss was fertilized, the pH of the substrate without plants increased as time progressed, whereas the pH of the substrate in which living *Phalaenopsis* plants were growing declined with time. Although the magnitude and course of pH decline were different in various substrates, the pH of sphagnum moss, artificial textile fiber, and pine bark substrates in which living plants were growing declined with time. Whether the substrate was exposed to light (clear pots) or not (opaque pots) had no effect on substrate pH, indicating that algae were not a factor in pH decline. Therefore, the roots of *Phalaenopsis* may be the major contributor to substrate pH decline during production.

*Phalaenopsis* has become one of the most important floral crops in the world. It is the highest valued potted plant sold at auctions in The Netherlands and the most valued potted flowering plant in the United States (United States Department of Agriculture, 2010). In Taiwan, the export value of *Phalaenopsis* increased from \$64.0 million in 2009 to \$82.6 million in 2010 (Bureau of Foreign Trade,

2011), a 29% annual growth, with increasing quantities being exported.

Wang and Konow (2002) showed the influence of root substrate and fertilizer on substrate pH. The addition of 20% sphagnum peat to fir bark resulted in a lower initial pH and more severe decline in substrate pH than fir bark alone (Wang, 1998; Wang and Konow, 2002). Since the late 1980s, sphagnum moss has been used as the sole substrate for producing *Phalaenopsis* in Taiwan. Previous research showed that the pH of the moss in *Phalaenopsis* containers declines over time (Chen, 2006; Lei, 2007; Peng, 2008; Yao, 2007; Yu, 2004). Some orchid growers consider that this drop in pH may be caused by the decomposition of the moss. However, the decline in substrate pH during production may be the result of the characteristics of the moss, fertilizer applications, and the presence of roots.

The nature of the root substrate affects initial pH value and determines the degree of acidification during production. Tsai (2000) grew *Oncidium* Gower Ramsey in coconut husks, tree fern roots, sphagnum moss, or a mixture of these materials and found acidification (pH decline) in all of these substrates. Coconut husks and tree fern roots had higher initial pH and the decline in pH was less than the other two. Sphagnum moss had the lowest initial pH of 4.2 and the mixture of the three materials had the most acidification with pH dropping from 5.9 to 3.1. Previous research showed that, as fertilizer concentration in-

creased, pH of the root substrate declined (Kowalczyk et al., 2008; Wang, 1996, 2010). In general, repeated applications of a fertilizer having nitrate as its main nitrogen (N) source causes root substrate pH to rise, whereas one having its N mainly from ammonium or urea leads to lower pH (Argo and Biernbaum, 1997; Argo and Fisher, 2002; Peng, 2008; Pinton et al., 2007).

Other than anchoring the plant and absorbing water and nutrients, roots also release organic and inorganic substances to alter the rhizosphere. The tendency to secrete such substances and the type of substances released are affected by a number of factors such as plant species and nutrient concentration (Pinton et al., 2007). As the plant releases exudates to the rhizosphere through anion channels, the H<sup>+</sup>-ATPase on cell membrane discharges a proton to balance the electrical charge, causing the pH of the rhizosphere to decline (Kollmeier et al., 2001; Neumann et al., 1999; Sakaguchi et al., 1999).

The objective of this study was to determine the effects of fertilizer, characteristics of substrates, light exposure to substrate, and plant species on the change of substrate pH during the production of *Phalaenopsis*. An *oncidium* (*Oncidium* Sw.) hybrid was used in one experiment to determine if there was a difference among orchid species in causing the pH decline in the sphagnum moss substrate.

## Materials and Methods

**Plant materials.** Except for Expt. 5, all other experiments used *Phalaenopsis* Sogo Yukidian ‘V3’ plants that were 16 months out of the flask and planted in sphagnum moss in soft, clear plastic pots. These plants were removed from their original 10.5-cm pots, cleaned of the moss, and replanted in 10.5-cm pots (825 mL in vol.) that were filled with moist Chilean sphagnum moss (200 g fresh weight/pot; 25 g dry weight/pot). The moss was soaked overnight in tap water and the excess water was removed in a spin-drying machine at 1200 rpm for 1 min. Plants were placed and grown in a greenhouse with pad and fan cooling. The average day/night temperatures were 23.2/21.1 °C and the average photosynthetic photon flux was 165 μmol·m<sup>-2</sup>·s<sup>-1</sup> when measured between 1100 HR and 1300 HR.

A 15.0N–2.2P–12.5K water-soluble fertilizer (Peters Excel 15-5-15 Cal-Mag; The Scotts Co., Marysville, OH) was used at a rate of 0.67 g·L<sup>-1</sup> (100 mg·L<sup>-1</sup> N) in Expt. 3, whereas 1.33 g·L<sup>-1</sup> (200 mg·L<sup>-1</sup> N) was used in all other experiments. The pH value of the fertilizer solution was adjusted to 6.0 with NaOH before application, resulting in electrical conductivity (EC) values of 0.7 and 1.4 dS·m<sup>-1</sup> for 0.67 g·L<sup>-1</sup> and 1.33 g·L<sup>-1</sup> of the fertilizer, respectively. This fertilizer contained (by weight), among other nutrients, 1.20% ammonium, 11.75% nitrate, 2.05% urea, 5% soluble calcium, and 2% soluble magnesium. Plants were fertigated when the substrate became dry; no additional irrigation was given between fertigations.

The pour-through technique for the collection of leachate samples from the root substrate

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followed that of Yao et al. (2008). Sixty milliliters of distilled water was poured evenly over the surface of the moss in pots 30 min after fertigation. A pH meter (Microcomputer pH/mv/TEMP meter 6171; JENCO, Portland, OR) and a conductivity meter (IQ170; IQ Scientific Instruments, Carlsbad, CA) were used for measuring the pH and EC, respectively, of the collected leachate samples.

**Expt. 1: Effects of the presence of plants and fertilization on acidification of the sphagnum moss.** This experiment was a  $2 \times 2$  factorial arrangement having two fertilizer treatments (with or without fertilization) each with or without a plant in the pot. The four treatment combinations were 1) with a plant and fertilization (*Phal.+*/*Fer.+*); 2) no plant but with fertilization (*Phal.-*/*Fer.+*); 3) with a plant but no fertilization (*Phal.+*/*Fer.-*); and 4) no plant and no fertilization (*Phal.-*/*Fer.-*). Plants that were fertilized were given 200 mL of a solution containing  $1.33 \text{ g}\cdot\text{L}^{-1}$  of the 15.0N–2.2P–12.5K fertilizer every 3 weeks. Those that were not fertilized were given distilled water at the same amount and frequency. Leachate samples were collected by the pour-through technique 30 min after fertilization or watering. Each treatment had 12 single-pot replications. This experiment lasted for 30 weeks.

**Expt. 2: Effects of light exclusion, presence of plants, and fertilizer application on substrate acidification.** Sphagnum moss that received fertilizer in Expt. 1 (*Fer.+*) had more algae; therefore, light exclusion was imposed to determine if algae had any effect on pH decline in the moss substrate. This experiment was a  $2 \times 2 \times 2$  factorial combination of the presence or absence of light (light and darkness), with or without fertilization (*Fer.+* and *Fer.-*), and the presence or absence of a plant in pot (*Phal.+* and *Phal.-*) for a total of eight treatments. Clear, soft plastic pots 10.5 cm in diameter were used for the light treatment, whereas similar pots made of black plastic were used for the darkness treatment. A single pot represented an experimental unit that was replicated 12 times per treatment. The substrate surface was covered with aluminum foil with several small holes to prevent light from reaching the moss surface and yet allow for water evaporation. The cultural practices were similar to that of Expt. 1. Pots were fertilized or watered every 6 weeks as a result of the slow water loss. Leachate samples were collected 30 min after fertigation for pH and EC determinations. This experiment lasted for 24 weeks.

**Expt. 3: Effects of plant and nutrient solution on solution pH.** This experiment used short-term hydroponic culture as a tool to determine the effect of roots on pH. This would eliminate the possible effect of moss on substrate pH. An air-driven periodic immersion bioreactor (Development Center for Biotechnology, Taipei, Taiwan) was used as the hydroponic culture container. To support the plant and to avoid root rot resulting from long periods of soaking in water, 400 g of glass beads (Guo-Zhou Ltd. Co., Taipei, Taiwan) 6 to 9 mm in diameter were placed at the bottom of each container. The experimental design

was similar to that of Expt. 1, except there was no moss. There were four treatments in total (with or without a plant in the bioreactor and with or without fertilization) and each treatment consisted of a single bioreactor that was replicated six times.

Plants that received fertilization were given a nutrient solution that contained  $0.67 \text{ g}\cdot\text{L}^{-1}$  of a 15.0N–2.2P–12.5K fertilizer, whereas those receiving no fertilization received distilled water at each irrigation. Both the nutrient solution and distilled water were adjusted to pH 6 with NaOH. At the onset of this experiment, plastic bottles were filled with 500 mL of the nutrient solution or water, which were wrapped with aluminum foil to keep out light, for the entire experimental period. The original growing substrate was removed and deteriorated roots were trimmed off, and then the plants were washed under running tap water to completely remove the sphagnum moss. The roots were then immersed in distilled water for 30 min and allowed to air-dry overnight. The root system was immersed in the designated liquid for 30 min each and every day between 1500 HR and 1600 HR. Two hours after the drained and recovered liquid was collected, the pH and EC were determined. The treatments were continued for 23 d.

**Expt. 4: Acidification of various growing substrates.** Plants were cultured in sphagnum moss (SM), artificial textile fiber (TF; artificial moss), or processed bark of *Pinus radiata* (PB; Orchinata, No. 5; Pacific Wide Group, Christchurch, New Zealand) to investigate the effect of substrate on the acidification. Textile fiber is a waste product from the manufacture of textile using an equal volume mixture of polyamide (nylon 6) and polyethylene terephthalate and consists of loosely twisted threads (Chang et al., 2006). All materials were soaked in water overnight. Sphagnum moss and TF were dried by spinning for 1 min in a spin-drying machine at 1200 rpm. Excess water was drained off of the bark before planting. All plants were fertigated every 3 weeks. Pots that were filled with bark became dry after 5 to 6 d and were given distilled water but not so much as to cause leaching. This experiment lasted for 27 weeks.

**Expt. 5: Genus effect on substrate acidification.** This experiment was to elucidate if plant species contributes to substrate acidification. Vegetatively propagated plants of *Phalaenopsis* Sogo Yukidian 'V3' (average fresh weight 275 g) and *Oncidium* Gower Ramsey (five pseudobulbs and 322 g in average fresh weight) were planted in 10.5-cm soft, clear plastic pots that were filled with Chilean sphagnum moss. Pots that were filled with moss, but had no plants, were used as controls. Fertigation was applied every 3 weeks and this experiment lasted for 18 weeks. Each treatment had 12 single-pot replications.

**Experimental design and statistical analysis.** All experiments were conducted in a completely randomized design. Data were analyzed with Costat 6.303 (CoHort Software, Monterey, CA) using analysis of variance. Figures were made with SigmaPlot 10.0 (Systat Software Inc., Chicago, IL).

## Results

**Expt. 1: Effects of presence of plants and fertilization on acidification of the sphagnum moss.** The initial pH (pH measured by the pour-through technique at the first fertilization) for *Phal.-*/*Fer.-* was 4.8 and declined to 3.9 in Week 12 but fluctuated between pH 4.1 and 5.3 between Weeks 12 and 30 (Fig. 1A). *Phal.+*/*Fer.-* had a starting pH of 5.0 that declined to 3.2 in Week 12 and fluctuated between 3.4 and 4.0 thereafter with an end pH of 3.5 (Fig. 1A). *Phal.-*/*Fer.+* had a low initial pH of 3.7 and it fluctuated between 3.4 and 3.7 until the end of this experiment (Fig. 1A). *Phal.+*/*Fer.+* had a starting pH of 3.7 that dropped to 2.9 in Week 15 and remained at this level until Week 30 (Fig. 1A). These results showed that fertilization (*Fer.+*) resulted in lower initial pH of the moss substrate and the presence of plants (*Phal.+*) caused a faster and more severe acidification than moss without a plant (*Phal.-*).

Fertilization (*Fer.+*) resulted in high starting EC of  $1.33 \text{ dS}\cdot\text{m}^{-1}$  in the moss (Fig. 1B). Beginning in Week 6, the EC of *Phal.-*/*Fer.+* exceeded that of *Phal.+*/*Fer.+*, reaching  $1.87 \text{ dS}\cdot\text{m}^{-1}$  by Week 30 (Fig. 1B). The EC of *Phal.-*/*Fer.+* increased with time, indicating the accumulation of salts after each fertigation. The EC of *Phal.+*/*Fer.+* went up to  $1.60 \text{ dS}\cdot\text{m}^{-1}$  in Week 6 but declined in Week 9 and reached  $1.27 \text{ dS}\cdot\text{m}^{-1}$  in Week 30, lower than that of *Phal.-*/*Fer.+* (Fig. 1B). The decrease in EC over time in fertilized pots reflects the likely uptake of nutrient ions by the plant

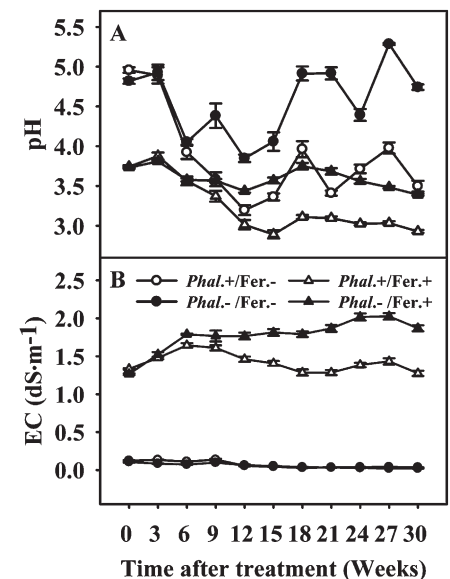


Fig. 1. Changes in pH (A) and electrical conductivity (EC) (B) values in sphagnum moss as affected by planting *Phalaenopsis* Sogo Yukidian 'V3' and fertilizer application ( $1.33 \text{ g}\cdot\text{L}^{-1}$  Peters Excel 15.0N–2.2P–12.5K Cal-Mag). "*Phal.+*/*Fer.-*" means with *Phalaenopsis* planted and without fertilization. "*Phal.-*/*Fer.-*" means without *Phalaenopsis* planted and without fertilization. "*Phal.+*/*Fer.+*" means with *Phalaenopsis* planted and with fertilization. "*Phal.-*/*Fer.+*" means without *Phalaenopsis* planted and with fertilization. Bars indicate SE of the means;  $n = 12$ .

rather than adsorption by the substrate or losses through leaching, especially when compared with pots in which there were no plants. Electrical conductivities for the two Fer- treatments were 0.12 dS·m<sup>-1</sup> initially and ended at 0.04 dS·m<sup>-1</sup> (Fig. 1B).

**Expt. 2: Effects of light exclusion, presence of plants, and fertilizer application on substrate acidification.** The results showed that the initial pH of the substrate receiving fertilization (3.4) was lower than that of the substrate given only water (4.1). Regardless of fertilization or exposure to light, pots having a plant all had lower pH than those without. Substrate acidification was not observed in pots without a plant (data not shown). Plants in clear pots that were exposed to light had greater root number and root dry weight of 6.3 g, whereas those in black pots that were covered with foil had fewer roots with a dry weight of 4.0 g (data not shown), suggesting that light possibly promoted root growth. However, light and the presence of algae had no effect on substrate pH at Week 24 (Table 1). The presence of plants or fertilization caused the pH of the moss substrate to decline.

**Expt. 3: Effects of plant and nutrient solution on solution pH.** The initial pH of the *Phal.-/Fer.-* solution was 7.1, which gradually increased to 7.6 at the end of this experiment (Fig. 2A). The *Phal.+/Fer.-* treatment had a starting pH of 6.6 and the pH dropped to 6.4 by Day 12 (Fig. 2A). The pH for *Phal.-/Fer.+* started at 6.6 and increased with time to a final pH of 7.0. The pH in bioreactors that had a plant and were fertilized began at 6.5 that declined to 6.1 after 18 d (Fig. 2A). These results strongly suggest that the presence of roots resulted in the pH decline in the bioreactor nutrient solution.

The EC of the nutrient solution was 0.73 dS·m<sup>-1</sup> for all fertilization treatments at the onset of this experiment. By the time the experiment was ended, solution EC increased to 0.96 dS·m<sup>-1</sup> regardless of whether there was a plant in the bioreactor (Fig. 2B). The increase of EC is probably caused by salt residue left from solution evaporation. The initial solution EC for the two treatments that did not receive fertilizer was 0.02 dS·m<sup>-1</sup> that increased to 0.16 dS·m<sup>-1</sup> in bioreactors with a plant and to 0.10 dS·m<sup>-1</sup> in bioreactors without a plant.

Table 1. The analysis of variance for effects of light, *Phalaenopsis* planting, and fertilization on substrate acidification.

Source	df	Type III SS	MS	F	P	
<b>Main effects</b>						
Light	1	0.094940	0.094940	1.1773	0.281	NS
<i>Phal.</i>	1	18.134939	18.134939	224.8898	<0.001	***
Fer.	1	6.135195	6.135194	76.0820	<0.001	***
<b>Interaction</b>						
Light* <i>Phal.</i>	1	1.224310	1.224310	15.1866	<0.001	***
Light*Fer.	1	0.908242	0.908242	11.2630	0.001	**
<i>Phal.</i> *Fer.	1	4.806800	4.806800	59.6087	<0.001	***
Light* <i>Phal.</i> *Fer.	1	0.452311	0.452311	5.6091	0.020	*
Error	85	6.854334	0.080639			
Total	92	38.979406				

NS, \*, \*\*, \*\*\*Non-significant and significant at  $P \leq 0.05, 0.01, \text{ and } 0.001$ , respectively.

**Expt. 4: Acidification of various growing substrates.** The initial pH values of SM, TF, and PB were 3.9, 6.4, and 4.8, respectively (Fig. 3A). By Week 27, the pH of SM dropped to 2.9, a decline of one pH unit and the lowest pH among the three substrates. The pH in TF declined quickly by more than two pH units to 3.9 by Week 27. The magnitude of acidification in PB (from 4.8 to 4.2) was less than in the other two substrates (Fig. 3A).

The substrate EC was lowest in PB and changed little during the experiment (Fig. 3B). EC in SM and TF gradually increased during the first 6 weeks and that became higher in SM after Week 12. At the end of this experiment, SM had an average EC of 1.8 dS·m<sup>-1</sup>, whereas TF had an EC of 1.55 dS·m<sup>-1</sup>.

**Expt. 5: Genus effect on substrate acidification.** No acidification was observed in the SM without a plant, in which the pH increased slightly from 3.7 at the beginning to 4.0 after 18 weeks (Fig. 4A). The pH of SM with a *Phalaenopsis* planted declined from 3.6 at the onset of this experiment to 3.3 at the end. The pH of the SM with *Oncidium* increased slightly from 3.6 to 3.9 by Week 9 but then declined to 3.3 by the end of this experiment (Fig. 4A).

As time progressed, substrate solution EC of all three groups gradually increased (Fig. 4B). This is particularly true for the substrate without a plant, increasing from 1.09 initially to 1.93 dS·m<sup>-1</sup> at the end. The EC of the SM with *Phalaenopsis* reached 1.44 dS·m<sup>-1</sup> and that with *Oncidium* increased to 1.67 dS·m<sup>-1</sup> at the end (Fig. 4B).

## Discussion

Drop of pH of SM substrate over time is commonly observed in the cultivation of *Phalaenopsis*. Factors that may cause the acidification of the moss include fertilizer application, characteristics of the substrate, and the physiological nature of the plant itself. The results from these five experiments are used to discuss the major cause of substrate acidification and the other factors involved.

Whether there was a *Phalaenopsis* plant in the pot or not, the initial pH of the moss was always lower with fertilization than without (Fig. 1A). Yao (2007) also showed that as fertilizer concentration increased, pH of the moss after the initial fertilization declined

more. This may be the result of the ion exchange between the substrate and the nutrient solution. Sphagnum moss is a natural material, which has numerous negatively charged sites that attract H<sup>+</sup>. On fertilizing, the cations

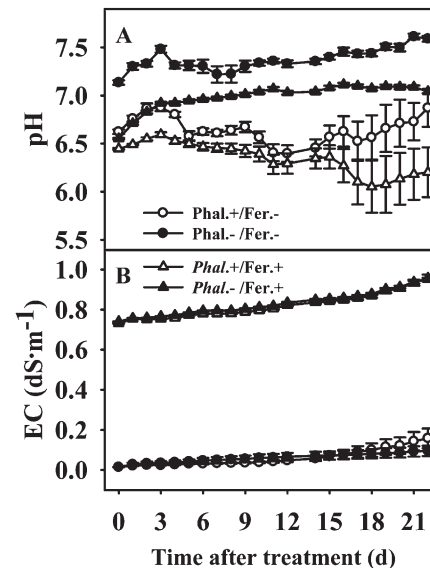


Fig. 2. Changes in pH (A) and electrical conductivity (EC) (B) values in hydroponic solution as affected by planting *Phalaenopsis* Sogo Yukidian 'V3' and fertilizer application (0.67 g·L<sup>-1</sup> Peters Excel 15-5-15 Cal-Mag). "*Phal.+/Fer.-*" means with *Phalaenopsis* planted and without fertilization. "*Phal.-/Fer.-*" means without *Phalaenopsis* planted and without fertilization. "*Phal.+/Fer.+*" means with *Phalaenopsis* planted and with fertilization. "*Phal.-/Fer.+*" means without *Phalaenopsis* planted and with fertilization. Bars indicate SE of the means; n = 6.

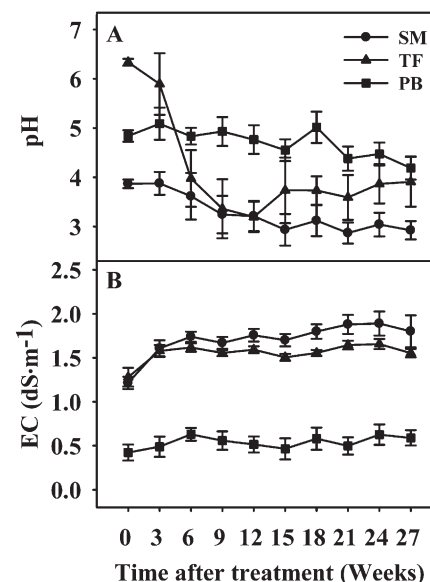


Fig. 3. Changes in substrate pH (A) and electrical conductivity (EC) (B) as affected by planting *Phalaenopsis* Sogo Yukidian 'V3' with three substrates. SM = sphagnum moss; TF = artificial textile fiber; PB = pine bark. Bars indicate SE of the means; n = 20.



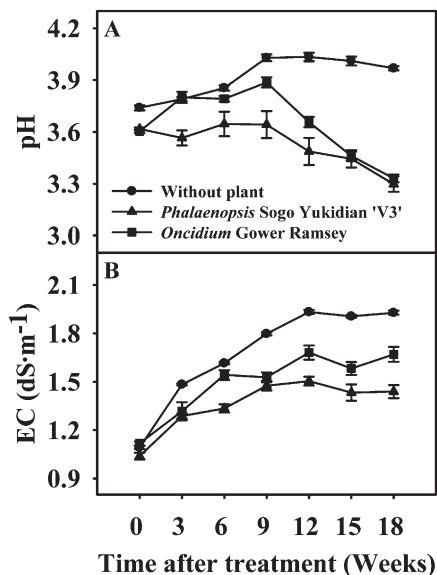


Fig. 4. Changes in substrate pH (A) and electrical conductivity (EC) (B) in sphagnum moss as affected by orchid species. Bars indicate SE of the means; n = 12.

in the fertilizer replace the  $H^+$  on these sites. Therefore, as fertilizer concentration increases, more  $H^+$  is released by the moss, causing the pH of the moss substrate to decline more.

Peters Excel 15.0N–2.2P–12.5K water-soluble fertilizer was used in this study. Seventy-eight percent of the N in this fertilizer is in the nitrate form; therefore, it is a basic fertilizer. Prolonged use of a basic fertilizer high in nitrate caused the substrate pH to increase (Argo and Fisher, 2002). This is the result of cotransportation of  $H^+$  by roots as nitrate ions are absorbed to elevate substrate pH. On the contrary, when roots absorb ammonium ions, protons are released, causing a decline in substrate pH (Argo and Biernbaum, 1997; Argo and Fisher, 2002; Pinton et al., 2007). In the current study, instead of increasing, the pH of the moss substrate in pots with a plant being fertilized with the high nitrate fertilizer (*Phal.+/Fer.+*) declined (Fig. 1A). Peng (2008) applied fertilizer solutions with various  $NO_3^-:NH_4^+$  ratios (0:100, 25:75, 50:50, 75:25, or 100:0) to *Phalaenopsis* plants potted in SM and found a lowering of pH in every treatment. Therefore, the type of fertilizer and the nitrate to ammonium ratio are not the main cause of substrate pH decline during *Phalaenopsis* cultivation.

Among the three materials used, the artificial moss had the highest initial pH, which was followed by the bark mix and then the SM (Fig. 3A). The difference in pH may have to do with the cation exchange capacity (CEC) and base saturation of these materials. At a pH range of 3 to 4, the CEC of SM is  $26 \text{ meq}\cdot\text{g}^{-1}$  (Kubota et al., 1993), whereas bark has a higher CEC of  $100 \text{ meq}\cdot\text{g}^{-1}$  (Pacific Wide Group, 2009). The higher CEC of bark means that it has more cation exchange sites. However, the bulk of the cation exchange sites in bark have been occupied by  $Ca^{2+}$  and  $Mg^{2+}$  ions added in the liming process by the manufacturer

(Pacific Wide Group, 2009), and thus fewer  $H^+$  ions are bound at these sites. Live SM has the ability to produce unesterified polyuronic acid (Clymo, 1964), and it grows in very acidic habitats; therefore, it is not surprising that the dried moss contains a large amount of exchangeable  $H^+$ . The artificial moss is an inert material with limited CEC (Chang et al., 2006) that partly accounts for the highest pH observed among the three materials (Fig. 3A).

The bark used in this study was produced by the Pacific Wide Group. Its technical descriptions indicate a low pH of 3.5 in the raw bark. After aging treatments, its pH increased to 5.5 to 6.5 and is supposed to stay in that range for 9 months (Pacific Wide Group, 2009). This was not observed in this study and the pH of the bark also declined with time (Fig. 3A). Sphagnum moss is an organic material; therefore, pH change in SM substrate may be contributed by decomposition process. On the contrary, the artificial moss is a stable material with a near neutral pH that degrades very slowly. However, both pH of SM and the artificial moss started to decline significantly at the beginning of Week 3 (Fig. 3A), at the time new roots were observed to start growing. This indicates that the onset of substrate acidification may be closely associated with root growth.

Although fertilizer application and the nature of the substrate both contribute to substrate pH decline, they are not the major causes. Because pH declined in all pots with a plant in them regardless of which substrate was used and the decline in pH was slight in pots without a *Phalaenopsis* plant, it is clear that roots are the main contributor of substrate pH decline (Figs. 1A, 2A, and 3A).

Although the rate of acidification was different, the pH of the SM substrate dropped whether planted with a *Phalaenopsis* or an *Oncidium* (Fig. 4A). During the tissue culture of *Dendrobium candidum*, medium acidification started during the first 2 weeks of culture. In a study by Wang and Ha (2007), as time progressed, the pH of the agar medium dropped from an initial pH of 5.6 to a final pH of 4.2. Therefore, it is apparent that other epiphytic Orchidaceae species also possess the ability to acidify the rhizosphere.

Previous work showed that the decline in substrate pH is related to the absorption of minerals. Pinton et al. (2007) pointed out that under phosphorus (P)-deficient conditions, the roots of most dicots secrete carboxylates in exchange for P-complex to increase P absorption. In balancing the electrical charge inside and outside of the roots,  $H^+$  is released to the substrate, resulting in acidification. When *Zea mays* is deficient of potassium, the root releases more sugars, carboxylates, and amino acids; for every carboxylate that the root releases, it also secretes a  $H^+$ , resulting in pH decline in the rhizosphere (Krafczyk et al., 1984). Some dicots, under low iron (Fe) conditions, release  $H^+$  to the rhizosphere to activate the iron reductase on the cell membrane to increase the reduction of  $Fe^{3+}$  to  $Fe^{2+}$  for absorption (Strategy I); grasses release phytosiderophores (PS) through the ion channel simultaneously with  $H^+$ , then take

up metal–PS complex by specific transporters in the plasma membrane (Strategy II) (Pinton et al., 2007). Regardless of whether plants use Strategy I or Strategy II for increasing ion absorption,  $H^+$  is released to acidify the growing substrate.

Although substrate acidification may be caused by root response to the lack of certain minerals in the rhizosphere, Lei (2007) reported that the SM substrate became acidified in two flowering cycles during which plants were given various ratios of N, P, and K. However, when N, P, and K were not applied, the pH of the moss hardly changed and plants did not show symptoms of being deficient of minor mineral nutrients. Therefore, the acidification of the substrate, in which *Phalaenopsis* is planted, may not be caused by lack of mineral nutrients. The results of this study show that the roots of *Phalaenopsis* may be the main cause of substrate acidification, which may have something to do with how originally the epiphytic roots absorb mineral nutrients in their natural habitat. Growth of *Phalaenopsis* is normal in a substrate with low pH; therefore, raising substrate pH may not be needed in the production of *Phalaenopsis*.

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