Timing is Crucial for Plug Seedling Substrate Testing

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SUMMARY. Many plug seedling growers complain about the inadequacy of substrate testing as a measure of nutritional status because results are too variable. We conducted two experiments to test a model system of sampling substrate at a set time after fertilization. *Petunias* (*Petunia ×hybrida* Hort. ex Vilm. var. *multiflora* 'Primetime White') were grown in 288-cell plug trays. Six fertilizer regimes were used consisting of a factorial arrangement of three fertilizer cycles (at each, every other, and every third irrigation) and two leaching fractions (0% and 20%). Fertilizer or water was applied at 0900 HR daily, and then 24 hours later in Expt. 1, and 1 hour later in Expt. 2, substrate solutions were sampled and analyzed. Samples taken after waterings were used to assess the dilution and leaching effects of water on substrate nutrient concentrations. In Expt. 2, additional substrate samples were taken at various hours after fertilizing to test the effect of plant depletion of the substrate. Substrate nutrient concentration curves constructed from data drawn at a fixed time after fertilizations, but not after waterings, were logical and

could be interpreted. When data from a fixed time after fertilizations and waterings were plotted together, the curves could not be interpreted. Data from samples taken at various hours after fertilization in Expt. 2 revealed large reductions in concentrations, often after only 4 hours. Overall, leaching and dilution effects from watering in combination with the increased time span from fertilizing to sampling resulted in nutrient concentrations that could not be interpreted. Substrate testing can be effective for plug seedling production, but samples need to be taken 1 to 2 hours after fertilizations.

The plug industry has grown rapidly from its beginning 22 years ago (Firth, 1994) to >7 billion plugs produced annually in North America in 1993 (Knox, 1993). However, substrate testing and foliar analysis systems have not been developed for monitoring the nutrient status of these crops. Plug production presents growers with unique cultural problems attributable to small container sizes, such as lack of stability in substrate moisture content, pH changes, poor aeration, varying nutrient level, and increased total salt levels (Koranski, 1985). Because seedlings are highly sensitive in the plug production stage and because plug substrate volumes are so small and unbuffered, even a small miscalculation in fertilizer application can jeopardize an entire crop.

Therefore, it is imperative that plug seedling growers be able to monitor nutrient status. Three systems exist: visual diagnosis, substrate testing, and foliar analysis. Visual diagnosis is not desirable as a first line of monitoring because plant deficiency and toxicity symptoms or injuries must be present to use the system. Foliar analysis procedures and standards have not been developed for plug seedlings. Tentative substrate standards, not based on research, have been proposed for plug seedlings (Warncke and Krauskopf, 1983). These standards have prompted many plug growers to try substrate testing, only to abandon it later due to erratic results not subject to interpretation. Of the three monitoring systems, substrate testing offers the best immediate potential because indications of nutritional problems can occur in the substrate before they occur in tissue nutrient levels or in plant

symptoms (Ross, 1991). The problem of erratic substrate test results experienced by plug growers is likely due to radical shifts in nutrient concentration brought on by rapid removal of nutrients by growing seedlings and to dilution and leaching effects of water application. This is a greater problem with plug crops than other crops because of the abnormally large plant biomass : substrate volume ratio in plug seedling trays. In this study, a model system for timing of substrate sampling a short time after fertilizer application was tested to determine the feasibility of obtaining substrate nutrient concentrations that can be interpreted.

Materials and methods

Two experiments were conducted on *Petunia ×hybrida* Hort. ex Vilm. var. *multiflora* 'Primetime White' (Goldsmith Seeds, lot 137A-32) grown in 288-cell plug trays. Substrate consisted of 3 sphagnum moss (≤6 mm) : 1 perlite (≤3 mm) (v/v) amended with dolomitic limestone at 6 g·L⁻¹. Experiment 1 was sown on 19 Oct. 1993 and Expt. 2 on 17 Apr. 1994. After being sown, trays were watered and placed in a dark germination chamber at 24 °C. On day 4, they were placed in a randomized complete-block design in a glass greenhouse at 35° N latitude in Raleigh, N.C. The day and night temperatures were set at 22 °C. Fertilizer treatments commenced 14 and 5 d after sowing in Expts. 1 and 2, respectively. Six fertilizer treatments were used, consisting of a factorial arrangement of three fertilizer cycles (each, every other, and every third irrigation) and two leaching fractions (0% and 20%). There were three replications in the first experiment and four in the second. An experimental unit consisted of one plug tray. A commercial water-soluble fertilizer (20N-4.4P-16.6K) was applied to all treatments in both experiments (Table 1). Higher concentrations were applied in the treatments that had less frequent fertilizer applications.

Plants were fertilized or watered daily at 0900 HR. To achieve the desired leaching percentages, one extra tray was included in each plot of the experiment. Just before each fertilizer or water application, these extra trays were watered to the point of leachate appearance, and the volume of water required to reach this point was re-

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corded. Either this volume or this volume + 20% was applied to the corresponding treatment trays. All water and fertilizer was applied through a TeeJet nozzle (model TP8004LP; Spraying Systems Co., Wheaton, Ill.) that provided a flat spray pattern. The nozzle was held sufficiently high above the plug tray to achieve a spray width equal to the width of the tray. Water was supplied to the nozzle at 70 kPa from a calibrated, inverted, three-L plastic soda bottle that was pressurized with CO₂.

Various fertilizer cycles throughout the crop production period were selected in each treatment for substrate sampling. A fertilizer cycle was defined as the period from one fertilizer application to the point just before

the subsequent fertilizer application, including the water applications within this period. Substrates were sampled at a fixed time after the fertilizer application and after any water applications that might have occurred within the cycle to determine the effects of water on nutrient dilution and leaching. In the first experiment, substrates were always sampled 24 h after applying fertilizer or water. Shoots of 12 plants were cut off, and the associated substrates from these 12 plugs were removed and dried in a 70 °C oven for 24 h. Soil solutions were extracted by the saturated media extract method (Warncke, 1986). In Expt. 2, shoots of 12 seedlings were cut off immediately after fertilization or irrigation. One hour later, substrate was removed from

these plugs. Soil solutions were squeezed out by placing the substrate in a single layer of cheesecloth and twisting the cloth to apply pressure. The two soil sampling procedures, saturated media extract and squeeze, were used in these experiments to evaluate the universality of

a timed sampling procedure regardless of sampling technique.

In addition to periodic substrate sampling 1 h after fertilizer or water application in the Expt. 2, two fertilizer cycles were selected for additional samples. One cycle was at mid-crop and the other near the end of the crop. This was done to measure the time course of nutrient removal from substrate by seedlings. When fertilizer was applied at every irrigation (treatments 1 and 4), substrates were sampled within these two cycles at 1, 4, 8, and 24 h. When fertilizer was applied every second irrigation (treatments 2 and 5), substrates were sampled at 1, 4, 8, 24 (just before watering), 25 (1 h after watering), and 48 h after fertilization. When fertilizer was applied every third irrigation (treatments 3 and 6) substrates were sampled at 1, 4, 8, 24, 25, 48, 49, and 72 h. To ensure adequate substrate solution volume, substrate that was sampled ≥8 h after the 0900 HR fertilization or irrigation was brought to saturation with water, without leaching, 1 h before sampling. After saturating the substrate, shoots were cut off, and 1 h later, the substrate solutions were squeezed out.

To assess the efficacy of restricting substrate sampling to a fixed time after fertilization, the following procedure of graphic presentation was used. Data points for substrate nutrient con-

Table 1. Fertilization treatments applied in the two experiments.

Treatment	Leaching (%)	Fertilizer frequency (d) ^z	N (mg·L ⁻¹)	
			Expt. 1	Expt. 2
1	0	1	75	100
2	0	2	100	115
3	0	3	125	125
4	20	1	100	50
5	20	2	125	100
6	20	3	150	115

^z1 = fertilized at every irrigation, 2 = at every second irrigation, and 3 = at every third irrigation.

Fig. 1. Experiment 1 substrate solution concentrations of NO₃-N 24 h after applying fertilizer (solid points) or water (open points). Fertilizer was applied at (A) each irrigation, (B) at every second irrigation, and (C) at every third irrigation. The leaching percentage was 20% in A–C. The solid curves connect only the points 24 h after fertilizations. The dash curves connect all points taken 24 h after fertilizer and water applications. The solid horizontal line indicates the lower end, and the dashed horizontal line the upper end of the tentative adequate range of NO₃-N concentrations as set forth by Warncke and Krauskopf (1983). Vertical bars represent ±SE within dates and treatments (n = 3).

Fig. 2. Experiment 1 substrate solution levels of electrical conductivity (EC) and specific nutrients from treatment 5 in which fertilizer was applied at every second irrigation and fertilizer and water were applied with a 20% leaching percentage. Solid points denote data collected 24 h after a fertilization, and open points indicate data collected 24 h after a watering. The solid curves connect only the points 24 h after fertilizations. The dash curves connect all points taken 24 h after fertilizer and water applications. The solid horizontal line indicates the lower end, and the dashed horizontal line indicates the upper end of the adequate range of nutrient concentrations as set forth by Warncke and Krauskopf (1983). Vertical bars represent $\pm$ SE within dates and treatments (n = 3).

centrations after fertilizations and waterings were plotted on the same graphs and then were connected two ways. First, all points were connected to show the erratic pattern of nutrient concentrations over time, and then, only the points after fertilizations were connected to show smooth concentration curves that can be interpreted.

Substrate solutions were analyzed for pH, electrical conductivity (EC), $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, Ca, Mg, and K. EC and pH were measured using a pH/conductivity/TDS/temperature meter (model 695; Extech Instruments, Waltham, Mass). Colorimetric analyses were performed for $\text{NO}_3\text{-N}$ (Caltaldo et al., 1975), $\text{NH}_4\text{-N}$ (Chaney and Marbach, 1962), and $\text{PO}_4\text{-P}$ (for concentrations $<10\text{ }\mu\text{g}\cdot\text{mL}^{-1}$; Murphy and Riley, 1962; for concentrations $>10\text{ }\mu\text{g}\cdot\text{mL}^{-1}$; Chapman and Pratt, 1961) on a UV/VIS spectrophotometer (model lambda 3; Perkin-Elmer, Norwalk, Conn.). Analyses of Ca, Mg, and K were run on an atomic absorption spectrophotometer (model 373; Perkin-Elmer, Norwalk, Conn.).

On the last substrate sampling date, the shoots of 40 plants from each of the experimental trays were harvested, rinsed under tap water, then soaked in 0.2 N HCl for 1 min, and finally rinsed in deionized water. The

samples were dried at 70 °C and weighed. The tissues were analyzed for Ca and Mg content using atomic absorption spectroscopy.

Treatments were arranged in a randomized complete-block design with one plug tray per experimental unit. Data were subjected to an analysis of variance (SAS Inst., Cary, N.C.).

Results and discussion

Graphs were drawn for the substrate solution concentrations of each nutrient variable within each treatment. Only representative curves are presented due to the large number of curves and because the curves for each macronutrient and EC were similar.

Substrate solution $\text{NO}_3\text{-N}$ concentrations within select treatments in Expt. 1 are presented in Fig. 1. When fertilizer was applied at each irrigation (Fig. 1A), logical curves consistent with the theoretical growth of a seedling were obtained. Initially, $\text{NO}_3\text{-N}$ concentrations in the substrate increased because uptake by the plant was low compared to fertilizer delivery rate. As plant uptake of $\text{NO}_3\text{-N}$ increased, the curves leveled off. Subsequently, substrate concentrations decreased when plants were sufficiently large to remove $\text{NO}_3\text{-N}$ faster than it was delivered.

Substrate concentrations of $\text{NO}_3\text{-N}$

in treatment 5, fertilized every second irrigation (Fig. 1B), were variable because substrate concentrations occurring 24 h after fertilizations and waterings were plotted in this curve. The combination of dilution from watering and the increased time span from fertilization to sampling after watering lead to declines in $\text{NO}_3\text{-N}$ concentrations. In the day-14–15 fertilizer cycle, the $\text{NO}_3\text{-N}$ concentration was adequate [based on the tentative adequate levels set forth by Warncke and Krauskopf (1983)] after the fertilizer application but was low 24 h after a water application. In the day-22–23 cycle, the $\text{NO}_3\text{-N}$ concentration was excessive after fertilization but adequate 24 h after a water application.

When two waterings occurred between fertilizer applications, greater decreases occurred in substrate $\text{NO}_3\text{-N}$ concentration within fertilizer cycles (Fig. 1C). Substrate $\text{NO}_3\text{-N}$ concentrations decreased from adequate to deficient in the day-14–16 cycle and in the day-26–28 cycle.

Similar deviations in the substrate were obtained with all other nutrients and EC (Fig. 2). Salt concentrations deviated more as a consequence of watering as the crop matured (Fig. 2A). Ammonium declined from excessive to acceptable (Fig. 2B), K from adequate to deficient (Fig. 2D), and

Fig. 3. Experiment 2 substrate solution concentrations of $\text{NO}_3\text{-N}$ 1 h after applying fertilizer (solid points) or water (open points). The fertilizer application frequency and leaching percentage were (A) every irrigation at 0% (B) every second irrigation at 20%, and (C) every third irrigation and 0%. Solid curves connect only the points 1 h after fertilizations. Dash curves connect all points taken 1 h after fertilizer and water applications. The solid horizontal line indicates the lower end and the dashed horizontal line indicates the upper end of the adequate range of $\text{NO}_3\text{-N}$ concentrations as set forth by Warncke and Krauskopf (1983). Vertical bars represent $\pm\text{SE}$ within dates and treatments ($n = 4$).

Fig. 4. Experiment 2 substrate solution levels of electrical conductivity (EC) and specific nutrients at various times within individual fertilizer cycles at mid-crop (13 d after sowing) or late crop (34 d after sowing). The solid horizontal line indicates the lower end, and the dashed horizontal line indicates the upper end of the adequate range of nutrient concentrations for seedlings as set forth by Warncke and Krauskopf (1983). Vertical bars represent $\pm$ SE within dates and treatments ($n = 4$).

Mg from adequate to deficient (Fig. 2F) from 24 h after a fertilization to 24 h after the following water application. Phosphate concentrations (Fig. 2C) were always above and Ca (Fig. 2E) always were below the tentative adequate ranges. Nutrient decreases were more pronounced as the crop matured.

Deviations in substrate concentrations of each nutrient in samples taken after a watering lead to conflicting assessments regarding the concentrations of fertilizer applied to the crop. Within one fertilizer cycle, the recom-

mendation could be to lower, sustain, or increase fertilizer concentrations depending on whether the sample was taken after a fertilizer or a water application. However, when the substrate concentrations that occurred after fertilizations only were plotted, smoother curves resulted that could be interpreted. The problem, therefore, becomes a question of timing: should samples be taken after fertilization or after watering? The number of waterings between fertilizations in commercial production systems varies with age of crop and weather condi-

tions. Thus, sampling after fertilization appears to offer more consistent nutrient concentration data.

Data from Expt. 2 were consistent with Expt. 1, even though substrates were extracted by the saturated media extract method 24 h after fertilization and watering in Expt. 1 and by the squeeze method 1 h after fertilization and watering in Expt. 2. Curves for substrate concentrations of NO₃-N 1 h after fertilizations from select treatments (Fig. 3A) could be interpreted easily. When one watering (Fig. 3B) and two waterings (Fig. 3C) were

interspersed between fertilizer applications, the curves were erratic and subject to multiple, opposing interpretations. However, when only the fertilizer points were connected, smoother curves resulted. The same erratic substrate nutrient concentration curves occurred for the other nutrients analyzed in all treatments (curves not shown because they had similar shapes to those in Expt. 1). Within a fertilization cycle, the substrate concentration could be excessive or adequate or, in other cycles, adequate or deficient depending on whether the substrate was measured after a fertilization or a watering. These results reinforce the decision to collect samples only after a fertilizer application, but the question still remains as to whether the samples should be taken at a set time interval after a fertilizer application.

To answer this question, substrate nutrient concentrations occurring during the mid- and late crop fertilizer cycles in which extra samples were drawn were plotted. Some representative curves are presented in Fig. 4. Substrate salt levels decreased with time and as a consequence of watering (Fig. 4A). Nitrate concentration shifted from adequate to deficient during the first 4 h after fertilization in treatment 2 (Fig. 4B). In treatment 3, $\text{NO}_3\text{-N}$ shifted from excessive to adequate during the first 4 h and then to deficient by the end of the 3-d fertilization cycle (Fig. 4C). Deviations also occurred in $\text{NH}_4\text{-N}$, which shifted from excessive to safe (Fig. 4D); in K, which shifted from adequate to low (Fig. 4E); and in Ca, which ranged from deficient to extremely deficient (Fig. 4F).

Substrate solution Ca levels were always below the suggested range of 80 to 199 $\text{mg}\cdot\text{L}^{-1}$ (Figs. 2E and 4F), and the Mg levels were often <30 to 69 $\text{mg}\cdot\text{L}^{-1}$ (Fig. 2F). However, quality plug seedlings were produced. Calcium whole-shoot concentrations ranged from 0.68% to 1.37%, and Mg concentrations ranged from 0.66% to 0.89% (data not shown), while normal ranges are thought to be 0.6% to 4.8% for Ca and 0.3% to 1.4% for Mg (Dole and Wilkins, 1988). The paradox of apparently adequate Ca and Mg plant accumulation in substrate deemed to be deficient in these nutrients suggests that tentative Ca and Mg substrate standards are incorrect and need to be adjusted downward.

Standards need to be established for all essential mineral nutrients for plug seedling production. At least two sets of standards might be needed due to the different fertilizer regimes used by growers: one set of standards for crops fertilized with dilute solutions at every irrigation and at least one additional set of standards for crops where waterings are interspersed between applications of more concentrated fertilizer.

Substrate testing can be a viable tool for assessing nutritional status of plug seedling crops when sampling (using either the saturated paste or squeeze method) is conducted at precise times. Based on this study, a desirable sampling time would be 1 to 2 h after fertilization. A shorter period than 1 h between fertilizing and sampling might be inadequate for nutrient equilibration in the substrate solution, but periods >2 h would reflect measurable levels of plant nutrient uptake.

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Effect of Bottom Heat, IBA Dipping Duration, and Wounding on Rooting of Stem Cuttings of *Milicia excelsa*

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SUMMARY. The effect of bottom heat, wounding, and duration of stem basal IBA dip on macropropagation of *Milicia excelsa* was investigated. Bottom heat enhanced root dry mass and accelerated root initiation. Percentage rooting and root dry mass were not affected by wounding and duration of stem basal treatment. However, wounding interacted with bottom heat to affect dry mass ($P < 0.05$). Root biomass was 60% higher from wounded cuttings than from nonwounded cuttings under the nonheated condition. Chemical names used: 1H-indole-3-butyric acid (IBA).

Milicia excelsa Welw. (Iroko) is a major tropical forest species and a commercially important timber species in Ghana (Wagner and Cobbinah, 1993). Until recently, *M. excelsa* was only propagated sexually, but research is underway to develop procedures for vegetative propagation.

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