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Article

Optimizing manganese and iron delivery for contrasting cultivars of subirrigated greenhouse-grown pot chrysanthemums

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Abstract

Excessive fertilizer use in greenhouse floricultural operations results in low-nutrient use efficiency by plants and poses environmental risk. Here, we optimized the usage of fertilizer manganese (Mn) and iron (Fe) by modern cultivars of subirrigated pot chrysanthemum. Mn and Fe (approximately 100% to 6% of industry standards) were provided in an otherwise balanced nutrient solution during vegetative growth, and all nutrients were removed during reproductive growth. Two experiments were conducted for each nutrient in a naturally lit research greenhouse using a split-plot design with four blocks arranged randomly. Mn (5.00–0.3125 μmol L^{-1}) or Fe (10.56–0.66 μmol L^{-1}) was the main plot and cultivar ("Milton Dark Pink", "Williamsburg Purple", and "Olympia White") was the subplot. The cultivars exhibited contrasting phenotypes. However, any treatment effects on plant yield and inflorescence development and quality were minor, so that Mn or Fe use efficiency increased approximately 16-fold with decreasing supply. Even though leaf Mn, zinc, and calcium levels were occasionally correlated inversely with decreasing Fe delivery, the leaf Mn (44.8–121.8 mg kg^{-1}) and Fe (68.5–121.8 mg kg^{-1}) levels were always considered acceptable. These findings contribute to the development of a low-input practice that would improve the sustainability of floricultural crop production.

Key words: chrysanthemum, greenhouse floriculture, nutrient delivery, nutrient use efficiency, subirrigation

Résumé

L'application d'une quantité excessive d'engrais pour la production de fleurs en serre réduit l'efficacité avec laquelle la plante assimile les oligoéléments tout en engendrant un risque pour l'environnement. Les auteurs ont optimisé l'assimilation du manganèse (Mn) et du fer (Fe) des engrais par les cultivars modernes de chrysanthème, cultivés en pot avec irrigation souterraine. Pour cela, ils ont fourni du Mn et du Fe (de cent à six pour cent de la norme recommandée par l'industrie, environ) aux plantes en plus d'une solution équilibrée en nutriments durant la période végétative, puis ont supprimé l'additif quand est survenue la période de la reproduction. Pour chaque oligoélément, les auteurs ont procédé à deux expériences en tiroir à quatre blocs aléatoires dans une serre de recherche à éclairage naturel. L'administration de Mn (5,00 à 0,3125 μmol par litre) ou de Fe (10,56 à 0,66 μmol par litre) correspondait au traitement principal, le traitement secondaire étant le type de cultivar (Milton Dark Pink, Williamsburg Purple ou Olympia White). Les variétés utilisées se caractérisaient par leurs phénotypes contrastants. Le traitement n'a eu que des effets mineurs sur le rendement et le développement des inflorescences de même que la qualité des fleurs, si bien que l'assimilation du Mn ou du Fe ne s'est améliorée que d'environ 16 fois, avec la diminution de leur concentration. Bien que la teneur des feuilles en Mn, en zinc et en calcium soit parfois inversement corrélée à la diminution de la quantité de Fe administrée, la concentration de Mn (de 44,8 à 121,8 mg par kg) et de Fe (de 68,5 à 121,8 mg par kg) dans les feuilles a toujours été jugée acceptable. Ces résultats concourront à l'élaboration d'une méthode qui réduira l'apport des oligoéléments en vue d'une meilleure pérennité de la floriculture. [Traduit par la Rédaction]

Mots-clés : chrysanthème, floriculture en serre, apport de nutriments, assimilation des oligoéléments, irrigation souterraine

Introduction

Closed subirrigation systems are popular in indoor floricultural production because they eliminate the generation of leachate, allow the recycling of nutrient-rich feedwater, and minimize the risks of contaminating local water resources with nitrogen (N), phosphorus (P), boron, and molybdenum (Ontario Ministry of the Environment 2012; MacDonald et al. 2013; Ferrarezi et al. 2015; Maguire et al. 2018). Opportunity

exists for optimizing nutrient use efficiency (NUE) by popular ornamental species grown with closed subirrigation. NUE comprises two components: the efficiency of nutrient acquisition (ENA, also known as nutrient capture or nutrient uptake) and the efficiency of nutrient utilization (ENU, also associated with nutrient remobilization) (López-Arredondo et al. 2017). ENA is primarily determined by root architecture and production of membrane transporters; the nutrient content of the plant or shoot is expressed as a function of the supply in the nutrient solution or the amount of nutrient(s) added directly to the root medium. ENU expresses biomass production in the plant or shoot as a function of the nutrient content within the plant or shoot. Providing that a reduction in nutrient supply is not excessive, the ENA generally increases during vegetative growth with decreasing supply, whereas the ENU increases during reproductive growth (White 2012; MacDonald et al. 2014; López-Arredondo et al. 2017; Shelp et al. 2020).

Fertilizer recommendations generally focus on the delivery of macronutrients, especially N, P, and potassium (K). Current commercial grower recommendations recommend that pot chrysanthemums in a soilless media are fertilized at 21.4–28.6 mmol L^{-1} N, with the possibility of reducing the rate by 25%–50% or eliminating it during the final 2–3 wk of the crop cycle in subirrigation systems (Green Leaf Plants 2015). Many commercial and research fertilizer formulations (e.g., Peter's Professional Peat-Lite Neutral Cal-Mag 17-3-17, ICL Fertilizers, Dublin, OH, USA; Fusion Plant-Prod 17-5-17, Master Plant-Prod, Brampton, ON, Canada; the Hoagland solution (Hoagland and Arnon 1938); and the Sonneveld solution (Sonneveld and Kreij 1987)), often with similar macronutrient composition (in mmol L^{-1}: 10.5–21.4 N, 1.7–2.7 P, and 6.0–7.7 K) are available for chrysanthemum production. Our previous research on the nutrition of pot chrysanthemums grown in the greenhouse under low- and high-ambient light conditions as would typically be seen in a southwestern Ontario winter and summer, respectively, demonstrated that the production of market-quality plants and flowers is not negatively affected by removing the entire nutrient supply at bud break, and by dramatically reducing macronutrient supplies during vegetative growth (i.e., N, P, K, sulphur (S), calcium (Ca), and magnesium (Mg) levels as low as 4.625, 0.65, 1.9, 0.56, 1.69, and 0.38 mmol L^{-1}, respectively, without adjusting the micronutrient levels) (MacDonald et al. 2014; Shelp et al. 2017, 2020, 2021*a*, 2021*b*; Sutton et al. 2019; Duncan Stephens et al. 2021).

The four fertilizers mentioned above contain a relatively broad concentration range of the micronutrient metals zinc (Zn; 0.76–13 μmol L^{-1}), copper (Cu; 0.31–12.7 μmol L^{-1}), manganese (Mn; 5–16 μmol L^{-1}), and iron (Fe; 15–32 μmol L^{-1}) at 10.5–21.4 mmol L^{-1} N. Chelating agents such as ethylenediaminetetraacetic acid are used to maintain the solubility of these micronutrient metals (Albano and Merhaut 2012). However, when the chelates are released to the environment, they persist and maintain the capacity to extract and solubilize heavy metals from sediments. Thus, we aim to reduce the micronutrient levels in common premixed fertilizers to minimize environmental impacts and maximize cost savings.

Previously, Zn and Cu at 0.875 and 0.19 μmol L^{-1}, respectively, were supplied in combination with optimized macronutrient levels, during vegetative growth only, without adverse effects on chrysanthemum plant and flower quality (Shelp et al. 2021*a*). In the present paper, our modified delivery strategy was used to study, under two indoor growing seasons, the response of three modern pot chrysanthemum cultivars to lower Mn (from 5.0 to 0.3125 μmol L^{-1}) or Fe (from 10.56 to 0.66 μmol L^{-1}) levels in combination with an optimized macronutrient regimen.

Mn performs specific cellular functions in endoplasmic reticulum, Golgi apparatus, mitochondria, plastids, and peroxisomes, whereas the vacuoles serve as a reservoir to regulate cellular Mn homeostasis (Das et al. 2019; Alejandro et al. 2020). There are only a small number of Mn-containing enzymes (e.g., the Mn_4Ca cluster in the O_2-evolving/water-splitting complex of photosystem II, Mn-superoxide dismutase, oxalate oxidase, purple acid phosphatase). However, Mn activates more than 30 enzymes involved in a variety of pathways, including the biosynthesis of chlorophyll, amino acids, carotenoids and gibberellins, and the remobilization of nitrogen. Fe functions mainly in the chloroplasts and mitochondria, and is largely stored as ferritins in nonphotosynthetic plastids of leaves or sequestered into globoid structures within protein storage vacuoles (Regvar et al. 2011; Jeong et al. 2017). It is involved in many biological processes including photosystems I and II, and many other components of photosynthesis, respiration and the biosynthesis of chlorophyll and ethylene.

Chrysanthemum growth is little affected by mild Mn deficiency, but the whole plant may turn uniformly pale green (Lunt et al. 1964). With more severe deficiency, interveinal chlorosis of the younger leaves is distinct, and flower bud development is delayed and flower size smaller. Growth may be slightly stunted under Fe deficiency, with interveinal chlorosis starting at the plant top and proceeding to the base (Roorda Van Esinga and Smilde 1980). Small necrotic spots may appear on older leaves, coalescing into large areas covering the blade. Flower bud formation is delayed and flowers remain small with a pale colour.

Many transporters associated with the acquisition, translocation, storage, and remobilization of Mn and Fe have broad specificity for several essential divalent cations (Jeong et al. 2017; Alejandro et al. 2020; He et al. 2021). Examples include: the utilization of Fe, Mn, and Zn by the plasma membrane-localized Iron-Regulated Transporter in *Arabidopsis thaliana* (L.) Heynh. (*At*IRT1) (Korshunova et al. 1999); the utilization of Mn, Fe, Zn, and Cu by the plasma membrane-localized Yellow Stripe-Like (*At*YSL1) transporter in *Arabidopsis* (Waters et al. 2006); the utilization of Mn, Fe, and Zn by the tonoplast-localized Natural Resistance-Associated Macrophage Protein in *Noccaea caerulescens* (J. & C. Presl) F.K. Mey. (*Tc*NRAMP4) (Oomen et al. 2009); and the utilization of Mn, Zn, and calcium (Ca) by the *Arabidopsis* Golgi-localized P_2 A-type ATPase or ER-type Calcium ATPase (*At*ECA3) (Mills et al. 2008). Such processes might account for improvements in Mn and Fe use efficiency in intact chrysanthemums grown in a low-input production system. Therefore, the tissue levels of Zn, Cu and Ca, as well as Mn and Fe were monitored in our investigations.

Materials and methods

Plant growth conditions

Cultivation and growth conditions for *Chrysanthemum morifolium* Ramat. ("Milton Dark Pink", "Williamsburg Purple", and "Olympia White") have been extensively described elsewhere (Shelp et al. 2017, 2020; Sutton et al. 2019). Briefly, the unrooted cuttings were dipped in a mixture of Stim-Root liquid (0.1% indole butyric acid) and B9 (0.25% daminozide) (Plant Products, Brampton, ON, Canada), and stored overnight in a 4 °C cooler. Each cutting was then inserted into a peat Jiffy Plug amended with 30% minerals (Model CF Hort. Plug 343040-26, Jiffy Products (N.B.) Ltd., Shippagan, NB, Canada), and maintained in the vegetative state on a misting bench in a naturally lit greenhouse. From 12 to 21 days, the misting tent was removed and the cuttings were supplied every 2 days with a fertilizer solution containing 17.8 mmol L^{-1} N.

Rooted cuttings were dipped in a 1% solution of Safer's Insecticidal Soap (Woodstream Canada Corporation, Brampton, ON, Canada) and individually potted in 10 cm diameter pots. The commercial soil medium was a mixture of peat moss and perlite (50:50 by volume) without a preplant fertilizer charge; the initial pH was adjusted to 5.70–6.15 with ground limestone (BM 6, Berger, Boisbriand, QC, Canada). Saturated medium extracts contained 2.9–3.4 μmol L^{-1} Fe and <0.55 μmol L^{-1} Mn. The potted soil was saturated with deionized water, then the pots were placed into 16 troughs in a computer-controlled ebb-and-flow subirrigation system housed in a naturally lit greenhouse with a set day/night temperature of 25 °C and humidity of 50%. Two experiments were conducted for both Mn and Fe (Mn Expt. 1: July–October 2020; Mn Expt. 2: January–March 2021; Fe Expt. 1: October–December 2020; Fe Expt. 2: February–April 2021). Greenhouse equipment failure forced the Fe Expt. 1 to be restarted when only one cultivar of chrysanthemum was available.

Plants were exposed to 1 wk of long days (12 h light:12 h dark cycle) and vegetative growth was maintained by implementing a night break with supplemental lighting (Solar Max Spectrum LED, BML Horticulture, Austin, TX; 20–50 μmol m^{-2} s^{-1} photosynthetic photon flux density at pot level) from 0030 to 0230 h. Then, the plants were pinched to equal heights to promote branching and switched to short days (10 h light:14 h dark) to induce flowering. Blackout curtains were used throughout the experiment to maintain the light:dark cycle; to compensate for any shading from the blackout curtain infrastructure, the supplemental lighting was also used under the low ambient light conditions present in the winter months. Plants were fertigated as needed every 1–3 days with the nutrient solutions described below until bud emergence, when the cultivars had a fully formed set of buds (5–6 wk). Subirrigation pumps were activated at 1100 h for 5 min to create a 2–3 cm deep irrigation head, which was allowed to drain back into the adjacent 40 L tanks for recycling. Nutrient solution was replenished as needed, every 1–2 wk. Post bud emergence, all plants were supplied with deionized water only in the same manner. Greenhouse pests were managed using an integrated pest management system (Swirskii-System, Biobest Group, Westerlo, Belgium), in combination with a Beleaf 50SG (ISK Biosciences Corporation, Concord, OH, USA) spray, once per crop cycle.

Experimental design

Each experiment was conducted on side-by-side benches with plants arranged in a split-plot randomized complete block design with 4 blocks and 10 plants per treatment. Each treatment appeared once per block. The outermost plants on each bench served as border plants and were not included in the analysis. They received a modified Sonneveld solution (7.1 mmol L^{-1} N, 1.0 mmol L^{-1} P, 3.5 mmol L^{-1} K, 0.58 mmol L^{-1} Mg, 2.6 mmol L^{-1} Ca, 0.87 mmol L^{-1} S, 3.5 μmol L^{-1} Zn, 10.56 μmol L^{-1} Fe, 5.0 μmol L^{-1} Mn, 0.75 μmol L^{-1} Cu, 5 μmol L^{-1} B, and 0.5 μmol L^{-1} Mo) (Sonneveld and Kreij 1987), prior to flower bud break (stage 2 of bloom development; Duncan Stephens et al. 2021), and then deionized water from bud break to harvest. The nutrient regimen was identical in the treatment rows, with the exceptions that Mn was replaced by 5, 2.5, and 1.25 (Mn Expt. 1) or 1.25, 0.625, and 0.3125 μmol L^{-1} Mn (Mn Expt.2), and Fe was replaced by 10.56, 5.28, and 2.64 (Fe Expt. 1) or 2.64, 1.32, and 0.66 μmol L^{-1} Fe (Fe Expt. 2). The concentration of Mn or Fe supplied during vegetative growth was considered as the main plot, while cultivar ("Milton Dark Pink" or "Williamsburg Purple") was the subplot (with the exception of Fe Expt. 1, which used only one cultivar, "Olympia White").

Beginning at bud emergence, bloom development was assessed weekly by counting the buds and rating their maturity on a scale from 1 to 6 (Duncan Stephens et al. 2021; Fig. S1). Plants were harvested at the onset of flower senescence, and shoot height, shoot fresh mass (FM), and flower FM were determined, along with a final assessment of bloom development (i.e., bud/infloresence count and stage, bloom diameter). Shoots and inflorescences were dried in a 95 °C oven for at least 3 days and dry mass (DM) of each was determined. The plants were examined for visual symptoms of nutrient deficiency throughout the experiment. In each experiment (with the exception of Mn Expt. 1), plant greenness was assessed as an estimate of chlorophyll content (Radhamani et al. 2016) in three young leaves of each plant (every other plant in Fe Expt. 1) using a SPAD 502DL Plus Chlorophyll Meter (Spectrum Technologies, Inc., Aurora, IL, USA) at bud emergence and immediately prior to harvest.

Leaf nutrient analysis

On the day the nutrient supply was removed, three recently matured, fully expanded leaves were collected from each plant and pooled according to treatment replicate (Hill Laboratories 2019). After drying in a 95 °C oven for at least 3 days, samples were individually ground into a fine powder using a Waring 7011 G blender (Waring Commercial, Torrington, CO, USA). Samples were then sent to the Agriculture and Food Laboratory at the University of Guelph for nutrient analysis. The samples were microwave acid digested, and diluted to an appropriate volume with nanopure water before measurement using an inductively coupled plasma–optical

emission spectrometry method developed and validated in-house (based on USEPA Method 200.7 revision 4.4; United States Environmental Protection Agency).

Statistical analysis

All statistical analyses were conducted using SAS version 9.4 (SAS Institute Inc., NC, USA) with the PROC GLIMMIX method ($\alpha = 0.05$). Normality and homogeneity of variance were confirmed before further statistical analyses were performed. Initially, cultivars were analysed together to compare responses to the main effect, and then individually for comparison of the main effect. Repeated measures analysis was used to compare data across time (i.e., inflorescence development), using a compound symmetry covariance structure. Variance was separated into fixed effects (Mn or Fe treatment and cultivar), random effects (block), and all relevant interactions within and between the fixed and random effects. Analyses of variance (ANOVAs) were performed and when effects were significant ($P \leq 0.05$), the means were compared to each other using Tukey's honest significant difference test using the slice function.

Results

Growth of two chrysanthemum cultivars supplied with moderate levels of Mn

Summary of significant effects

The Mn supply was reduced by 94% across the two experiments. There were no visual symptoms of Mn deficiency (i.e., interveinal chlorosis on young leaves, tan sunken spots in the chlorotic areas, reduced and retarded growth, delayed flowering, smaller flowers) in either experiment regardless of the Mn treatment (Fig. S2A–S2H). Greenness of young leaves at bud emergence (Table S1) and final harvest (data not shown) was not affected by treatment. The only treatment effect on the morphological characteristics at final harvest was with the stage of inflorescence development in Expt. 2 (Table S1). A cultivar effect was evident for most of the morphological characteristics measured; the only exceptions were the number of buds/inflorescences and the shoot DM in Expt. 2. There were treatment (Expt. 2 only), time and cultivar effects, as well as time–cultivar interactions, on inflorescence development (Table S2), and cultivar effects on the leaf levels of most nutrients in both the experiments (Table S3). Because of the presence of several treatment–cultivar interactions on the morphological characteristics, the cultivars were individually analysed below.

Morphological characteristics

In general, the shoot height, shoot DM, inflorescence DM, bloom diameter, and inflorescence/bud number at final harvest, and greenness of young leaves at bud emergence (Table 1) and final harvest (Table S4) were unaffected by the Mn treatment, regardless of cultivar. However, some differences occurred (Table 1). In Expt. 2, bloom diameter and inflorescence development were slightly affected in "Milton Dark Pink" and "Williamsburg Purple", respectively, though the response was not linearly correlated with the Mn supply.

Bud and inflorescence development

In both experiments, the bud/inflorescence number in each cultivar was similar across the treatments from bud emergence until final harvest, though occasionally some values were different (Table S5). The stage of bud/inflorescence development was determined weekly from bud emergence (stage 1) to harvest. Bud/inflorescence development was generally uniform across all treatments, regardless of cultivar (Fig. 1, Table S6); indeed, there were no differences among the treatments at final harvest (Table 1).

Leaf nutrient composition at bud emergence

Regardless of the experiment and cultivar, leaf tissue levels of the macronutrients, including Ca, and the micronutrients, including Fe, Zn, and Cu, were generally unaffected by the 16-fold range in Mn supply (Table 2, Tables S7 and S8). The only macronutrient exception was the slightly elevated S level in "Milton Dark Pink" with the lowest Mn supply in Expt. 2. The only micronutrient exception was the 30% decrease in Mn in "Williamsburg Purple" with the reduction in Mn supply from 5.00 to 2.5 μmol L^{-1} in Expt. 1. Overall, the leaf Mn levels ranged from 51 to 116 mg kg^{-1} DM across all cultivars and experiments.

Growth of two chrysanthemum cultivars supplied with moderate levels of Fe

Summary of significant effects

The Fe supply was reduced by 94% across the two experiments. There were no visual symptoms of Fe deficiency (i.e., interveinal chlorosis on young leaves, stunted growth, delayed flowering) in either experiment regardless of Fe treatment (Fig. S2I–S2P). Treatment did not affect greenness at bud emergence regardless of cultivar (Table S9). There was no treatment effect on the morphological characteristics (Table S9), bud/inflorescence development (Table S10), or macronutrient composition (Table S11). There were several treatment effects on micronutrient composition (Table S11). Cultivar effects were only possible in Expt. 2 because Expt. 1 used one cultivar only. In Expt. 2, cultivar had an effect on all morphological characteristics at final harvest (Table S9), as well as bud/inflorescence development (Table S7). Cultivar also affected most nutrient concentrations (Table S11). Because treatment–cultivar interactions were observed for several of the characteristics monitored, the cultivars were analysed individually below.

Morphological characteristics at final harvest

In both experiments, the treatment did not affect the morphological characteristics (shoot height, shoot DM, inflorescence DM, bloom diameter, inflorescence development

Table 1. Morphological characteristics of two chrysanthemum cultivars supplied with varying levels of Mn up to bud emergence in Summer/Fall 2020 (Expt. 1) and Winter/Spring 2021 (Expt. 2).

Cultivar	Mn supply (μmol L^{-1})	Shoot height (cm plant^{-1})	Shoot DM (g plant^{-1})	Inflor. DM (g plant^{-1})	Bloom dia. (cm plant^{-1})	Inflor. dev. (stage plant^{-1})	Bud/inflor. (No. plant^{-1})	Greenness (SPAD value)
				Expt. 1				
"Milton Dark Pink"	5.00	27.39	4.93	2.05	6.21	3.6	36.1	–
	2.50	27.28	4.78	1.99	6.19	3.5	34.5	–
	1.25	28.66	5.20	2.16	6.35	3.5	37.0	–
"Williamsburg Purple"	5.00	28.30	5.59	2.34	5.00	3.3	32.2	–
	2.50	28.73	5.84	2.49	5.07	3.2	33.3	–
	1.25	28.33	5.51	2.38	5.10	3.2	32.1	–
				Expt. 2				
"Milton Dark Pink"	1.25	24.08	3.87	1.66	7.06	4.1*b*	20.7	45.12
	0.625	25.23	4.15	1.78	7.10	4.1*ab*	22.2	45.16
	0.3125	24.74	4.01	1.78	7.12	4.2*a*	20.5	45.12
"Williamsburg Purple"	1.25	22.85	4.67	2.33	5.60*ab*	4.0	21.5	51.03
	0.625	22.04	4.46	2.24	5.58*b*	4.1	20.7	52.09
	0.3125	22.80	4.50	2.22	5.68*a*	4.1	20.1	51.98

Note: Data represent the mean of four treatment replicates; each replicate consists of 10 individual plants. In Expt. 2 greenness was based on 10 individual plants per treatment; the SPAD value for a plant is the average of measurements from three young leaves at flower bud emergence. Means that are significantly different ($P \leq 0.05$) within columns and cultivars according to the Tukey's honest significant difference test are designated by different letters. Abbreviations: dev., development; dia., diameter; DM, dry mass; inflor., inflorescence; shoot DM, stems, petioles, leaves, and inflorescences.

and number of inflorescences/buds at final harvest, and leaf greenness at bud emergence and final harvest), regardless of cultivar (Table 3, Table S4).

Bud and inflorescence development

In Expt. 1 the bud/inflorescence number of "Olympia White" remained constant from bud emergence to harvest across all treatments. In contrast, in Expt. 2 the number increased in both "Milton Dark Pink" and "Williamsburg Purple" from emergence to harvest, though there was no treatment effect (Table S12). Nevertheless, the stage of inflorescence development increased with time in the three cultivars across both experiments, though there were no treatment effects (Fig. 2, Fig. S13).

Leaf nutrient composition at bud emergence

Regardless of the experiment and cultivar, the leaf macronutrient levels at bud emergence were generally unaffected by the 16-fold range in Fe supply (Table 4, Table S14). The only effects of treatment were on Mg and Ca levels in "Milton Dark Pink" in Expt. 2, which increased slightly with the reduction in Fe supply from 2.64 to 0.66 μmol L^{-1}. Leaf Fe was not different among the treatments in either experiment (Table 4, Table S15). Overall, the Fe concentrations ranged from 68.5 to 103.0 mg kg^{-1} DM across all cultivars and treatments. Differences were evident in the concentration of some of the other micronutrients. The leaf Mn level was negatively correlated with the Fe supply in both experiments, regardless of cultivar, increasing approximately 16%–31% with the reduction in Fe. In contrast, the leaf Cu level was correlated with the Fe supply in both "Olympia White" and "Milton Dark Pink", respectively, in experiments 1 and 2, decreasing by approximately 11%–16% with the reduction in Fe. The leaf Mo level was also affected by the Fe supply in Expt. 2; however, the response was different between the two cultivars. In "Milton Dark Pink," the lowest Mo level was found with 0.66 μmol L^{-1} Fe supply, whereas in "Williamsburg Purple", the highest level was found with 0.66 μmol L^{-1} Fe supply.

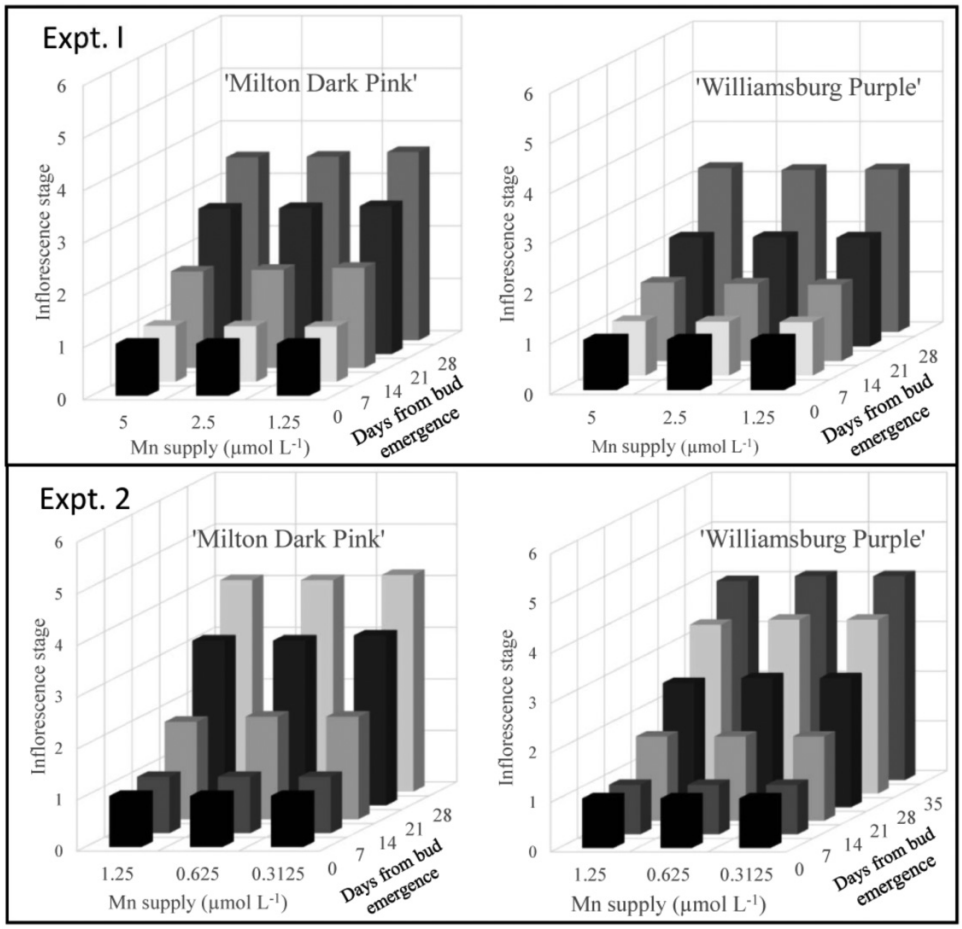
Discussion

Strategic timing and rates of Mn and Fe fertilization improve use efficiency in subirrigated, greenhouse-grown pot chrysanthemums

The common practice in commercial greenhouses is to supply nutrients in excess to avoid deficiency symptoms which, if present, would render a plant unsaleable. These levels are higher than necessary for maximal growth, but not high enough to be toxic. Fertilizer recommendations are often based on outdated cultivars and irrigation techniques and focus on macronutrients, especially N, P, and K. For example, Green Leaf Plants (2015) recommends a complete fertilizer at 21.4–28.6 mmol L^{-1} N with added micronutrients for greenhouse pot chrysanthemums but suggests that the rate can be reduced by 25%–50% or eliminated during the final 2–3 weeks of the crop cycle with subirrigation systems. The Sonneveld and Hoagland solutions, commonly used when growing plants in soilless media, contain 5 μmol L^{-1} Mn and 15–25 μmol L^{-1} Fe at 10.5–18.5 mmol L^{-1} N (Hoagland and Arnon 1938; Sonneveld and Kreij 1987).

MacDonald et al. (2014) demonstrated that eliminating nutrient delivery altogether during the reproductive growth of

Fig. 1. Inflorescence development of two chrysanthemum cultivars supplied with varying levels of Mn up to bud emergence. The stage of inflorescence development was determined weekly from bud emergence (stage 1) to harvest. Statistical treatment of the data are shown in Table S6.



a subirrigated pot chrysanthemum ("Yellow Favor") does not affect the quality of the plants and flowers. Subsequent studies with many chrysanthemum cultivars ("Olympia White", "Covington Yellow", "Milton Dark Pink", "Williamsburg Purple", "Kingsville Yellow", "Milton Orange", and "Newport Bronze"), typically replicated over two growing seasons, further optimized the delivery of N, P, K, Ca, Mg, S, Zn, and (or) Cu by combining nutrient removal during reproductive growth with reduced nutrient delivery during vegetative growth, resulting in overall decreases of 75%–87.5% in nutrient delivery, without adversely affecting flower quality (Shelp et al. 2017, 2020, 2021*a*, 2021*b*; Sutton et al. 2019; Duncan Stephens et al. 2021). While the cultivars being compared often exhibited contrasting phenotypes (e.g., biomass accumulation, bloom diameter, tissue nutrient levels), the NUE always increased with decreasing nutrient delivery, and any treatment effects on morphological characteristics were minor, likely to be unnoticed by consumers.

In the present study, three cultivars of chrysanthemum were supplied with an optimized macronutrient regimen, and control supplies of Zn, Cu, B, and Mo during vegetative growth. While there were cultivar differences in morphology and tissue nutrient levels, the levels of N (4.5%–6.53% DM), P (0.67%–1.02% DM), K (4.77%–6.60% DM), Ca (1.14%–1.76% DM), Mg (0.39%–0.69% DM), Zn (26.3–60.8 mg kg^{-1} DM), Cu (2.7–9.1 mg kg^{-1} DM), B (36.5–74.8 mg kg^{-1} DM), and Mo (1.2–4.0 mg kg^{-1} DM) across all treatments are in general agreement with sufficiency guidelines for chrysanthemums in the extension literature (4.0%–6.5% DM N, 0.2%–1.2% DM P, 1.0%–10.0% DM K, 0.5%–4.6% DM Ca, 0.1%–1.5% DM Mg, 5–250 mg kg^{-1} DM Zn, 5–50 mg kg^{-1} DM Cu, and 20–200 mg kg^{-1} DM B) (Ontario Ministry of Agriculture, Food and Rural Affairs 2014; Plank et al. 2018; Hill Laboratories 2019). They are also in agreement with estimates of the critical deficiency concentrations in leaves of dicotyledonous plants (i.e., 1–5 mg kg^{-1} DM Cu and 0.1–1.0 mg kg^{-1} DM Mo, depending on the species; see Broadley et al. 2012). According to these guidelines, Cu is the only nutrient that may be low. However, common symptoms of Cu deficiency in chrysanthemum are leaf margin desiccation and suppression of flowering (Roorda van Ensinga and Smilde 1980), neither of which was seen. Furthermore, previous research from our laboratory demonstrated that leaf Cu levels as low as 3.7 mg Cu kg^{-1} DM are not associated with visual signs of deficiency (Shelp et al. 2021*a*).

Our previous research generally involved the replication of experiments over two growing seasons, and the treatment

Table 2. Composition of select nutrients from diagnostic leaves of two chrysanthemum cultivars supplied with varying levels of Mn until flower bud emergence in Summer/Fall 2020 (Expt. 1) and Winter/Spring 2021 (Expt. 2).

Cultivar	Mn supply (μmol L^{-1})	Leaf concentration Mn	Fe	Zn	Cu	Ca
		(mg kg^{-1} DM)				(% DM)
		Expt. 1				
"Milton Dark Pink"	5.00	65.5	86.5	32.5	5.6	1.57
	2.50	61.5	76.5	36.0	5.9	1.63
	1.25	51.0	94.3	38.3	5.7	1.66
"Williamsburg Purple"	5.00	115.5*a*	86.5	29.8	5.9	1.32
	2.50	77.3*b*	82.5	27.8	5.4	1.33
	1.25	78.5*b*	86.0	34.0	6.1	1.34
		Expt. 2				
"Milton Dark Pink"	1.25	60.0	97.3	46.8	8.0	1.68
	0.625	58.5	121.8	49.5	8.1	1.68
	0.3125	56.5	94.8	52.8	9.1	1.76
"Williamsburg Purple"	1.25	68.3	98.8	32.5	6.4	1.34
	0.625	62.0	94.0	32.0	6.4	1.30
	0.3125	68.8	92.3	33.5	6.9	1.29

Note: Data represent the mean of four treatment replicates; each replicate is based on a single analytical determination of a subsample taken from the pooled tissues of 10 individual plants. Means that are significantly different ($P \leq 0.05$) within columns and cultivars according to the Tukey's honest significant difference test are designated by different letters.

Table 3. Morphological characteristics of three chrysanthemum cultivars supplied with varying levels of Fe up to bud emergence in Summer/Fall 2020 (Expt. 1) and Winter/Spring 2021 (Expt. 2).

Cultivar	Fe supply (μmol L^{-1})	Shoot height (cm plant^{-1})	Shoot DM (g plant^{-1})	Inflor. DM (g plant^{-1})	Bloom dia. (cm plant^{-1})	Inflor. dev. (stage plant^{-1})	Bud/inflor (No. plant^{-1})	Greenness (SPAD value)
				Expt. 1				
"Olympia White"	10.56	29.98	5.71	2.55	5.97	3.7	27.7	46.99
	5.28	29.70	5.73	2.55	6.03	3.7	29.4	46.26
	2.64	30.05	6.11	2.72	6.03	3.7	31.2	47.02
				Expt. 2				
"Milton Dark Pink"	2.64	31.63	6.42	2.18	7.68	3.1	31.1	50.37
	1.32	31.39	6.57	2.26	7.62	3.0	32.6	49.09
	0.66	31.38	6.60	2.31	7.64	3.1	31.7	50.40
"Williamsburg Purple"	2.64	27.18	7.49	2.52	5.78	2.5	36.4	55.64
	1.32	27.15	8.21	2.85	5.83	2.5	37.8	55.81
	0.66	27.90	7.66	5.55	5.83	2.5	36.1	55.56

Note: Data represent the mean of four treatment replicates; each replicate has 20 (Expt. 1) or 10 (Expt. 2) individual plants. Greenness was based on 10 individual plants per treatment in both experiments. SPAD value for each plant is the average of measurements from three young leaves at flower bud emergence. None of the means within columns and cultivars are significantly different ($P \leq 0.05$) according to the Tukey's honest significant difference test. Abbreviations: dev., development; dia., diameter; DM, dry mass; inflor., inflorescence; shoot DM, stems, petioles, leaves, and inflorescences.

responses were similar across the seasons, as well as the two culivars under consideration. Therefore, when symptoms of Mn or Fe deficiency were absent from the first experiment, it was decided to further reduce their levels in the second experiment. Overall, the Mn and Fe supplies were reduced by approximately 94% during vegetative growth, compared to the industry standards, and approximately 97% over the crop cycle. There were no signs of uniform yellowing, interveinal chlorosis, delayed flower bud development, smaller flower size with pale colour, or small necrotic spots that coalesce into large desiccating areas (Lunt et al. 1964; Roorda van Eysinga and Smilde 1980). Across all experiments, the Mn levels ranged from 44.8 to 121.8 mg kg^{-1} and the Fe levels ranged from 68.5 to 121.8 mg kg^{-1}, which are within the ranges considered acceptable for normal plant growth (25–375 mg kg^{-1} Mn and 20–750 mg kg^{-1} Fe; Ontario Ministry of Agriculture, Food and Rural Affairs 2014; Planck et al. 2018; Hill Laboratories 2019). Comparison of these

Fig. 2. Inflorescence development of three chrysanthemum cultivars supplied with varying levels of Fe up to bud emergence. The stage of inflorescence development was determined weekly from bud break (stage 1) to harvest. Statistical treatment of the data are shown in Table S13.

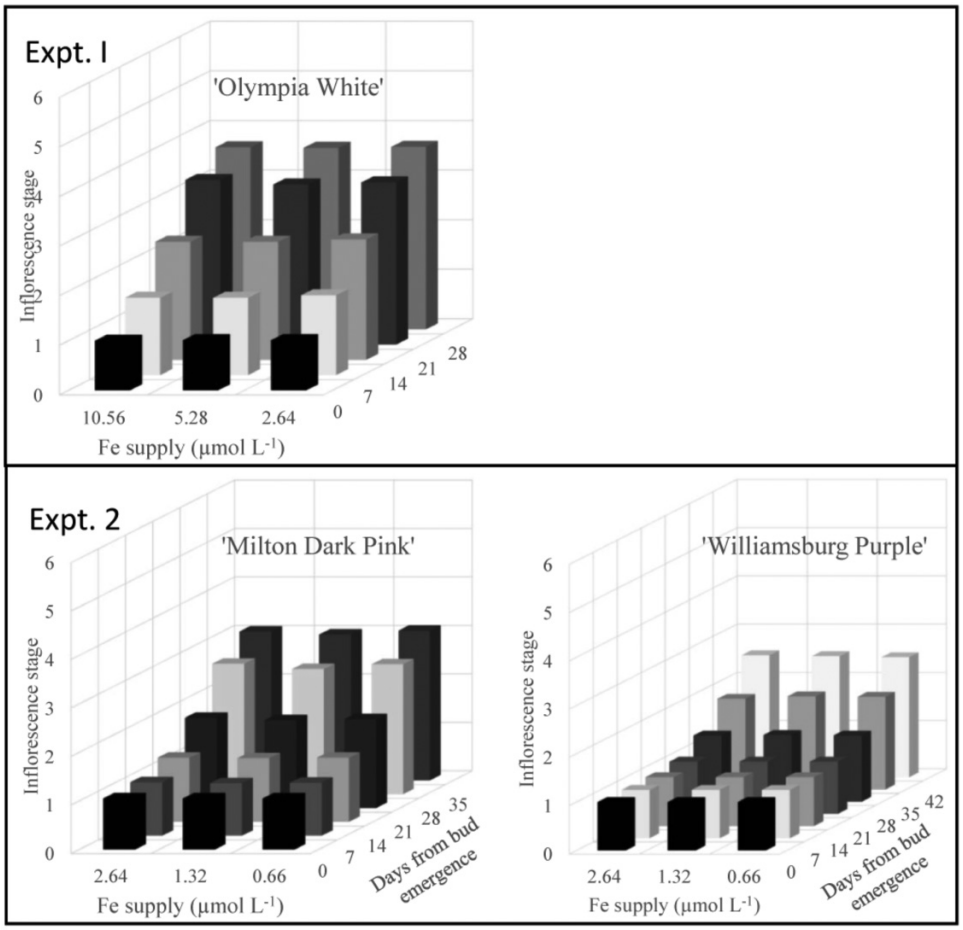


Table 4. Composition of select nutrients from diagnostic leaves of two chrysanthemum cultivars supplied with varying levels of Fe until flower bud emergence in Summer/Fall 2020 (Expt. 1) and Winter/Spring 2021 (Expt. 2).

		Leaf concentration				
Cultivar	Fe supply (μmol L^{-1})	Fe	Mn	Zn	Cu	Ca
			(mg kg^{-1} DM)			(% DM)
		Expt. 1				
"Olympia White"	10.56	103.0	44.8c	26.3	4.3a	1.19
	5.28	96.0	52.8b	29.8	4.1a	1.20
	2.64	90.8	58.8a	29.0	3.6b	1.21
		Expt. 2				
"Milton Dark Pink"	2.64	81.3	70.8b	43.0b	3.8a	1.44a
	1.32	74.8	80.0a	49.0a	3.4b	1.47ab
	0.66	74.5	82.8a	50.8a	3.5ab	1.55b
"Williamsburg Purple"	2.64	74.8	63.5b	35.0	2.9	1.14
	1.32	71.0	66.8ab	41.5	2.7	1.15
	0.66	68.5	73.8a	46.0	2.7	1.17

Note: Data represent the mean of four treatment replicates; each replicate is based on a single analytical determination of a subsample taken from the pooled tissues of 10 individual plants. Means that are significantly different ($P \leq 0.05$) within columns and cultivars according to the Tukey's honest significant difference test are designated by different letters.

findings with the relatively stable morphological results leads to the conclusion that the Mn and Fe use efficiencies were improved approximately 16-fold with decreasing supply, and 32-fold over the crop cycle, in comparison to industry standards, without adversely affecting the plant and flower quality of either cultivar. This was done by removing the entire nutrient suite during the reproductive growth stage and lowering the Mn and Fe supplies during the vegetative growth stage.

Mechanisms for improved Mn and Fe use efficiencies

Increasing NUE as a function of decreasing nutrient supply can result from improvements in ENA and (or) ENU. Nutrient budgets have been used to demonstrate that increased ENA is the primary mechanism to obtain sufficient N, P, and S for the growth of chrysanthemums on decreasing supplies of the respective macronutrient (MacDonald et al. 2014; Shelp et al. 2017, 2020; Sutton et al. 2019). The NRAMP and IRT protein families in dicotyledonous plants have members that are involved in the transport of Mn and Fe from the rhizosphere into root cells. For example, *At*NRAMP1 is a plasma membrane-localized, metal/H^+ symporter that mediates Mn uptake by epidermal and cortical cells in roots (Cailliatte et al. 2010; Castaings et al. 2016). Under Mn deficiency, *AtNRAMP1* expression is moderately upregulated, and the *atnramp1* mutant grows poorly and accumulates less Mn than the WT, suggesting that this protein is a high-affinity Mn uptake transporter (Cailliatte et al. 2010). Active Mn uptake may also be mediated by the high-affinity Fe transporter *At*IRT1 (Korshunova et al. 1999; Castaings et al. 2016). In response to Fe deficiency, the nuclear *AtFIT* gene is activated, inducing the upregulation of *AtFRO2* and *AtIRT1* (Connolly et al. 2002; Varotto et al. 2002). Better knowledge of the affinities and kinetics of these Mn and Fe transporters would be useful in assessing their importance with decreasing nutrient delivery, especially when the reduction is insufficient to elicit visible signs of deficiency (Griffiths and York 2020).

The possibility of enhanced ENU with decreasing Mn or Fe supply cannot be excluded. Temporary stores of Mn and (or) Fe in the roots and source leaves could be remobilized by the upregulation of tonoplastic and Golgi network efflux proteins *At*ZIP1 and *At*NRAMP2,3,4 (Thomine et al. 2000; Lanquar et al. 2005, 2010; Milner et al. 2013; Bastow et al. 2018; Gao et al. 2018). Remobilization could also be increased by upregulating the plasma membrane-localized proteins *At*YSL1 and *At*YSL3, which transport Mn and Fe between translocation streams (Waters et al. 2006). Notably, remobilization would be especially important during the reproductive growth of chrysanthemums as Mn and Fe can be removed at flower bud emergence with no adverse effects on plant and flower quality (this study; Shelp et al. 2017, 2020, 2021*a*, 2021*b*; Sutton et al. 2019; Duncan Stephens et al. 2021).

The methods employed here did not allow for a nutrient balance to estimate the relative contribution of the nutrient solution vs. cuttings, pot mix and Jiffy plug; or, to assess the relative importance of ENA and ENU in sustaining the growth of flowering chrysanthemums when the micronutrient supply (Mn, Fe, Zn, Cu) was reduced during vegetative growth and then the entire nutrient supply was removed during reproductive growth (c.f. Shelp et al. 2020 vs. Shelp et al. 2021*a*). Furthermore, a nutrient balance would be useful for studying remobilization only if the contents of various plant parts change in response to the treatments. To overcome this limitation, isotopes of Mn and Fe could be supplied to the root systems or a leaf (via the leaf flap feeding method) at various times throughout the vegetative and reproductive growth stages to monitor the simultaneous movement of labelled and unlabelled Mn or Fe (Biddulph et al. 1959; Tsukamoto et al. 2006; Valentinuzzi et al. 2020). This would allow the uptake, storage, and translocation of Mn and Fe to be tracked as a function of plant development. Gene expression analysis via quantitative polymerase chain reaction of potential Mn and Fe transporters in both roots and leaves would give greater insight into mechanisms responsible for Mn and Fe uptake and remobilization when the supplies are lowered, albeit not excessively lowered so that visible and morphological symptoms of deficiency become apparent. Other factors such as root architecture and release of root storage pools, could have also contributed to improved NUE, but these possibilities are beyond the scope of this study.

Potential interactions among Mn, Fe, Zn, Cu, and Ca

Transporters such as NRAMP1, IRT1, YSL1, and ECA3 have broad specificity for essential divalent cations (Jeong et al. 2017; Alejandro et al. 2020; He et al. 2021; Rai et al. 2021). This non-specificity and the related antagonism could explain the inverse correlation, observed in some cases here, of the leaf Mn, Zn, and Ca levels with the decreasing Fe supply (Rai et al. 2021). They could also support the existence of crosstalk between interconnected pathways for the regulation of metal homeostasis (Rai et al. 2021). Nonetheless, these findings indicate that the balance of divalent cations in the nutrient solution might be an important consideration when nutrient delivery during the vegetative growth stage approaches the low sufficiency range.

The dilution of commercial fertilizers to achieve optimal macronutrient levels can result in micronutrient levels that are inadequate to sustain plant growth and quality, or exacerbate the adverse effects of an unsuitable micronutrient balance. Interestingly, there is quite a range in the approximate ratios of Zn:Cu (from 2:1 to 10:1), Fe: Mn (from 1:1 to 5:1), and B: Mo (15:1 to 40:1) among common commercial (Fusion Plant Products 15-5-17; Peters Professional 17-3-7 Peat-Lite Neutral Cal-Mag, ICL Fertilizers, Dublin, OH, USA)) and the more historical Sonneveld and Hoagland solutions. To date, the levels of Zn, Cu, Mn, and Fe have all been successfully reduced in a research setting to below the levels obtained in the commercial setting with the dilution of Fusion Plant Products 15-5-7 (this study; Shelp et al. 2021*a*, 2021*b*). Thus, it may be possible to further reduce in a research setting the levels of Zn, Cu, Mn, and Fe, and to proceed with the

optimization of the two remaining essential micronutrients, B and Mo, which have been shown to accumulate in greenhouse-influenced waterways in southwestern Ontario (Ministry of Environment 2012; Maguire et al. 2018). It may however, become necessary to fine-tune the micronutrient balance. For example, Fe-deficient plants accumulate additional Cu in leaf tissues (Waters et al. 2012), and higher Cu levels in the nutrient solution, relative to Zn, reduce the availability of Zn to plants, and vice versa (Imtiaz et al. 2003). Once completed, the delivery strategy can be validated in a commercial setting using modern cultivars. Together, this research should indicate whether the current commercial fertilizers need to be reformulated to meet the needs of the modified subirrigation strategy for chrysanthemums.

Prospects for the floricultural industry

Subirrigation systems are popular environmentally friendly means of floricultural greenhouse production (MacDonald et al. 2013; Ferrarezi et al. 2015; Semananda et al. 2018). Because fertilizer recommendations are generally based on overhead irrigation systems and outdated cultivars, an opportunity exists to optimize nutrient delivery and potentially lower nutrient inputs. To date, our modified delivery strategy for modern cultivars of chrysanthemum has been optimized with macronutrients in a research setting using a closed subirrigation system (Shelp et al. 2017, 2020; Sutton et al. 2019), and then validated in a commercial setting using an open subirrigation system where the spent nutrient solution is released directly into the municipal sewage system in compliance with the Nutrient Management Act (Shelp et al. 2021*b*). In both cases, macronutrient delivery to modern chrysanthemum cultivars can be reduced in the subirrigation solution by approximately 75% during vegetative growth, compared to common fertilizer formulations, and then removed entirely during reproductive growth. Therefore, reducing the fertilizer rate results in proportional cost savings (Shelp et al. 2021*b*), and decreases the risk of environmental contamination.

It would also be of interest to study the timing and optimization of nutrient delivery using drip irrigation, another common environmentally friendly method for irrigating indoor floricultural crops (Lévesque et al. 2009). Unlike subirrigation, drip irrigation is scalable to any size, and reducing nutrient delivery would lower the need for overirrigation. This research would validate the new fertilization rates and the modified delivery strategy with a different irrigation system, enabling our research to reach more growers.

In Canada, there were almost five million indoor potted chrysanthemums produced in 2020 (Statistics Canada 2021). Our chrysanthemum research could encourage growers to adjust the timing and application of fertilizers, leading to input and cost reductions without sacrificing plant quality. Ultimately, our innovative strategy could be adopted for all potted ornamental crops grown indoors across Canada, which totalled in excess of 80 million plants in 2020, thereby improving the overall sustainability of the floricultural industry.

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Supplementary material

Supplementary data are available with the article at https://doi.org/10.1139/CJPS-2021-0286.

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