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Validating integrative nutrient diagnostic norms for greenhouse cut-roses

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ABSTRACT

Efforts to optimize fertilizer use efficiency in intensively managed greenhouse rose crops led to studies to establish and validate norms for their integrated nutrient diagnosis. The present study experimentally validates the practical usefulness of previously established DRIS (Diagnosis and Recommendation Integrated System) and CND (Compositional Nutrient Diagnosis) norms for soil-grown roses in the Bogota Plateau of Colombia. Corrective fertilization treatments, based on a control fertigation solution, were designed based on preliminary diagnosis by DRIS methodology, and applied over two growth and flowering flushes in an experimental plot within a commercial rose crop. These integrative nutrient diagnoses methods detected microelement imbalances in rose leaf tissues, including excesses of iron (Fe) and deficiencies of zinc (Zn), copper (Cu) and manganese (Mn). Conventional soil and foliar analyses had some contrasting interpretation diagnoses on these nutrients. Implementation of corrective changes, based on DRIS results, to the supply of these micronutrients in fertilization treatments improved the elemental (individual) and mean nutrient balance indices in rose leaf tissues, and led to gradual increases in average stem length and the fraction of harvested flowers with longer stems (> 70 cm) over the course of two flowering cycles. These DRIS and CND methods highlighted a significant Fe:Mn interaction in rose crops, likely affected by the supply and ratio of micronutrients in the fertigation solutions, inherently low Mn levels in the soils of the region, and a major role of the dominant rootstock in use. The use of integrative nutrient diagnosis methods, based on relationships between all nutrient elements and flower yield as the primary response criterion, offer an enhanced capacity to identify those elements with the highest probability of generating a positive flower yield response when correcting their supply in rose fertilization programs.

1. Introduction

Integrated nutritional diagnostic systems, like the Diagnosis and Recommendation Integrated System (DRIS) and Compositional Nutrient Diagnosis (CND), are useful in predicting nutritional imbalances that may affect the productivity and quality of crops ([Agbangba et al., 2011](#page-8-0); [Anjaneyulu and Raghupathi, 2010;](#page-8-1) Dias et al., 2010; [Llanderal and Contreras, 2018;](#page-8-2) [Lucena, 1997](#page-8-3); [Parent, 2011](#page-8-4); [Sánchez et al., 2018;](#page-9-0) [Serra et al., 2010](#page-9-1)). The DRIS and CND nutrient diagnostic standards, or norms, calculated for a crop must be validated, theoretically first, and eventually undergo an experimental or practical validation to certify their usefulness. In general, the fastest way to experimentally validate new integrated nutrient diagnostic standards is to apply them to previous or ongoing fertilization studies and determine subsequent crop responses in terms of balance indices (Coleman et al., 2003). This experimental validation should be performed after diagnosing the limiting elements, applying subsequent corrected fertilization tests and then evaluating posterior crop responses [\(Dos Anjos Reis](#page-8-5) [and Monnerat, 2003](#page-8-5)).

Among ornamental horticulture crops, greenhouse-grown cut roses (Rosa \times spp. L) are an intensively managed cropping system, characterized by receiving very large inputs of water, fertilizers and agrochemicals [\(Cabrera and Solis-Perez, 2017](#page-8-6); [Cabrera et al., 2009](#page-8-7)). With the objective of contributing to the optimization of fertilizer use in this crop, while sustaining productivity and minimizing costs and environmental impacts, we are conducting studies to establish and validate norms for integrated nutrient diagnosis techniques through DRIS and CND techniques. We have generated and theoretically validated these

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Table 1

Fertilization treatments designed after iterative assessments of leaf tissue DRIS analyses in an experimental crop of 'Freedom' roses (on R. × 'Natal Briar'). Preliminary leaf analyses (prior to start for study) were employed to determine fertigation treatments for the first flower production cycle. Treatments for the second production cycle were generated from DRIS analyses applied at the end of the first flower production cycle. The fertigation treatments were applied over an 11-week experimental period in each flower production cycle.

 x A fertigation solution used by commercial growers was used as control solution in these studies. All concentrations in mg¹⁻¹.

^y Weekly supplemental foliar application of $30 \text{ mg } L^{-1}$ copper (as Cu-EDTA).

^z Weekly supplemental foliar application of 30 mg L⁻¹ copper (as Cu-EDTA) and 15 mg L⁻¹ zinc as (as Zn-EDTA).

norms for cut roses growing on soil beds within greenhouses in the Bogota Plateau of Colombia [\(Franco-Hermida et al., 2017](#page-8-8), [2016](#page-8-9), [2013](#page-8-10)). These norms were generated from a large database of flower productivity, nutrient analyses from plant tissues and soil samples from rose cultivars grafted on $Rosa \times 'Natal Briar'.$

The present study was conducted to experimentally validate the practical utility of these previously generated DRIS and CND norms for rose leaf tissues. For this purpose, controlled greenhouse fertilization experiments were carried out to test the corrective recommendations provided by the DRIS system, which included evaluation of the impact of fertigation program corrections on rose flower productivity and quality. The ultimate goal of these studies is to generate practical and useful integrated nutrient diagnostic norms that allow for relatively quick adjustments and optimization of fertilization programs in commercial rose crops.

2. Materials and methods

2.1. Experimental plot and crop management practices

The validation study was carried out in a plot within a commercial greenhouse rose crop located in Chía (Cundinamarca), Colombia (4.8970 °N, 74.0601 °W). This commercial crop was considered adequately nourished and showed no apparent symptoms of nutrient disorders. The experimental plot area consisted of 15 soil beds, each 1 m wide by 30 m long, occupying a total greenhouse area of 925 m^2 . The soil, a loamy-clayey texture with good drainage, was classified as Typic Haplanthrepts [\(IGAC, 2000](#page-8-11)). The soil beds were heavily amended with organic matter (7.1 %), and chemical analyses revealed the following average results: pH 6.3; electric conductivity (EC) 2.1 dS $\mathrm{m}^{-1};$ cation exchange capacity (CEC) 25.9 $\mathrm{cmol_c\,kg^{-1}};$ and nutrient contents (in mg·kg−¹) of: 35 ammonium-nitrogen (NH4-N), 177 nitrate-nitrogen (NO3-N), 192 phosphorous (P), 987 potassium (K), 3910 calcium (Ca), 868 magnesium (Mg), 176 sulfur (S), 125 iron (Fe), 10.4 manganese (Mn), 9.4 zin. (Zn), 10.7 copper (Cu) and 3.5 boron (B).

Two-year-old rose plants of the cultivar 'Freedom' grafted on R . \times 'Natal Briar' were growing in the 30 m^2 soil beds, each with 206 rose plants, representing a planting density of 6.9 plants/m², and fertigated through drip irrigation. The nutrient solution used to irrigate this commercial crop had the following composition (in mg L^{-1}): 140–180 N, 25–40 P, 100–200 K, 100–150 Ca, 40–70 Mg, 0.5–1.0 Mn, 0.5–4.0 Fe, 0.1–1.0 Cu, 0.2–1.5 Zn, and 0.5–1.0 B. The average maximum and minimum daily temperature within the plastic-covered greenhouse was 14 and 29 °C. The crop was managed through pruning practices that produced cyclical flushes of growth and flowering ([Cabrera, 2002](#page-8-12); [Cabrera et al., 2009](#page-8-7); Calatayud et al., 2008).

2.2. Foliar and soil analysis

All preliminary and follow-up leaf tissue and soil sampling and analyses conducted throughout the study were performed as follows. Composite leaf samples were collected from randomly chosen plants throughout the entire population prior to the start of the experimental runs, and thereafter from each experimental unit/bed (three composite replicates per treatment). These foliage samples were from the fifth compound leaves from flower stems at the phenological stage corresponding to the opening/reflexing of sepals and visible exposure of petal color (about 8 weeks from harvest/pruning of previous flowering flush). Leaf samples were dried and ground, and analyzed in the Soil and Plant Laboratory of GR Chía S.A (Chía, Colombia). The following methodologies were used: dry digestion and atomic absorption spectroscopy for Ca, Mg, Na, K, Fe, Cu, Mn and Zn; colorimetry for P; wet digestion and micro-Kjeldahl analyses for N and turbidimetry for S ([Silva Mojica et al., 1990\)](#page-9-2).

Composite soil samples were collected from soil cores (2.5 cm diameter x15 cm deep) taken in a zigzag pattern from the entire plot prior to the start of the experimental runs, and thereafter from each experimental bed (three composite soil samples per treatment, with each composite sample made up of 16 soil cores). The soil samples were collected on the same dates when, and beds where, the leaf samples were collected. Following drying and grinding of soil samples, complete analysis of extractable nutrients were carried out according to the methodologies of the Instituto Geográfico Agustín Codazzi ([IGAC,](#page-8-13) [2007\)](#page-8-13).

2.3. Assessment of crop nutrient status to establish fertigation treatments

The previously generated, and theoretically validated, DRIS and CND norms ([Franco-Hermida et al., 2013](#page-8-10)) were experimentally validated in this study over the course of two growth and flowering cycles of 11-weeks each. Several fertigation treatments were used in each of two flowering cycles [\(Table 1\)](#page-1-0), and these were based on preliminary/ initial DRIS diagnoses. The preliminary nutrient concentration of soil and leaf samples collected from the experimental rose plot were assessed and categorized (very high, high, normal, low or very low) by conventional soil nutrient range (SNR) and foliar nutrient range (FNR) criteria [\(Ortega, 1997\)](#page-8-14). These values and their interpretation diagnosis (id) are found in the rows labeled "Before T" in [Table](#page-2-0) 2.

The DRIS index for each element (e.g. I_N , I_P , I_K , etc.) was also calculated from these preliminary foliar tissue analyses, shown in the first row labeled "Before T" in [Table 3.](#page-3-0) The absolute value of the DRIS index for each element was contrasted against the mean nutrient balance index (NBIm), calculated by the potential fertilization response

Table 2

Concentration of essential nutrient elements in soil and leaf tissues samples, and their interpretation diagnosis (id) by soil nutrient range (SNR) and foliar nutrient range (FNR) criteria in fertilization treatments used to validate integrative DRIS and CND nutrient diagnostic norms for greenhouse rose crops.

z The interpretation diagnosis (id) categories are: VH= very high; H= high; N= normal; L= low; VL= very low (according to [Ortega, 1997\)](#page-8-14).

^y Units: Soil analysis in mg kg⁻¹; Foliar analysis: N, P, K, Ca, Mg, S in %; Fe, Mn, Zn, Cu and B in mg kg⁻¹. Average nutrient values followed by different letter indicates significant differences according to the Tukey test ($\alpha \le 0.05$).

methodology proposed by [Wadt \(1996\)](#page-9-3) and [Wadt and De Novais](#page-9-4) [\(1999\).](#page-9-4) The magnitude of the absolute difference, and the sign, identify the elements deviating from the NBIm in an excessive or deficient manner. According to this DRIS methodology, the elements that were outside the limits imposed by NBIm were Fe, Cu and Zn (first row in [Table 3\)](#page-3-0), and their adjustment, aiming at zeroing the individual and mean nutrient balance indexes offers the greatest probability of yield responses. Therefore, the highest probabilities for significant rose yield responses were initially (before the start of treatments) identified as the restricting of Fe and increasing of Cu and Zn supplies in the crop's fertilization program. This led to the fertilization treatments employed in the first experimental flowering cycle.

2.3.1. Fertilization treatments for first flowering cycle

Employing a commercial fertigation solution as a control, modifications were made to its formulation to generate four differential fertigation treatments, aimed to "correct" during a first experimental flowering cycle the anomalous DRIS nutrient indexes of Fe, Cu and Zn. Two of these fertigation treatments were supplemented once a week with foliar applications of chelated Cu and Zn. The treatments are described below, and the applied nutrient concentrations shown in [Table 1](#page-1-0).

Control = Fertigation solution used by commercial growers.

 $T1 =$ Base solution with a 60 % reduction in Fe concentrations $(<$ Fe $)$

T2= Base solution with a 60 % reduction in Fe, and 15 % and 30 % increases in Zn and Cu concentrations, respectively (\leq Fe, $>$ Zn, $>$ Cu).

T3= Base solution with a 60 % reduction in Fe, and 15 % and 30 % increases in Zn and Cu concentrations, respectively (\leq Fe, $>$ Zn, $>$ Cu), plus weekly supplemental foliar Cu foliar applications (30 mg L⁻¹ Cu as Cu-EDTA).

T4 = Base solution with a 60 % reduction in Fe, and 15 % and 30 % increases in Zn and Cu concentrations, respectively (\leq Fe, $>$ Zn, $>$ Cu), plus weekly foliar Cu and Zn applications (30 mg L−¹ Cu as Cu-EDTA and 15 mg L^{-1} Zn as Zn-EDTA).

For this first flowering cycle the rose plants were fertilized with

these treatments over an 11-week experimental period, culminating with the harvest of all the flowers. About 8 weeks after the start of this first flowering flush (cycle), the developing flower shoots reached the phenological stage corresponding to the start of opening/reflexing of sepals and having visible exposure of petal color. Composite leaf tissue and soil samples (three replicates per treatment) were collected at this stage, dried, ground and subjected to nutrient analyses, followed by their interpretation with the previously cited conventional (SNR, FNR) and integrative (DRIS, CND) nutrient diagnostic procedures.

2.3.2. Fertilization treatments for second flowering cycle

The DRIS diagnosis at the end of the first flowering flush were used to design a new, reduced, set of fertigation treatments to be used during a second, subsequent 11-week flowering cycle. In addition to the original control fertigation solution, only two fertigation treatments were employed in this second cycle [\(Table 1](#page-1-0)), and their justification, which included changes in Fe, Mn, Zn and Cu concentrations and elimination of foliar Zn and Cu applications, is presented in the Results and Discussion (specifically section [3.2.4\)](#page-5-0).

Nine weeks from the onset of this second flowering cycle, the developing flower shoots reached the developmental stage corresponding to the start of reflexing of sepals and visible exposure of petal color. Composite leaf tissue and soil samples were again collected at this stage (three replicates per treatment), dried, ground and subjected to nutrient analyses, followed by subsequent interpretation by conventional (SNR, FNR) and integrative (DRIS, CND) diagnostic methods.

2.4. Measurements of flower yield and quality response variables

Crop yield responses to the fertilization treatments were assessed by tracking changes in total flower productivity in each bed, and expressed in harvested stems per unit area (m^2) . The flower yields were extrapolated to annual productivity, the variable originally used to generate the DRIS and CND norms ([Franco-Hermida et al., 2013](#page-8-10)). This was done by transforming the results of each flowering cycle to harvested flowers/m² year¹. In addition, the length of the harvested flower shoots

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 $(\alpha \le 0.05)$.

was graded according to commercial export standards, quantifying the stems found in three classes: 70 s (70−79 cm), 40 s (40−49 cm) and non-exportable (NE; < 40 cm). During the second production cycle the diameter of the harvested flower stems was measured just above the cutting point.

2.5. Experimental design and statistical analyses

The experimental design was completely randomized, with tree replications per treatment. Each replication consisted of one 30 m^2 (1 m x 30 m) soil bed having 206 rose plants. The statistical analyses were performed using SAS V8 ® software employing ANOVA procedures with Tukey mean comparison tests, and correlations. The DRIS and CND standards were considered experimentally validated if the fertilization treatments achieved significant increases ($\alpha \le 0.05$) in the measured response variables compared with the control treatment.

3. Results and discussion

3.1. Preliminary soil and crop nutrient diagnosis

Results of both preliminary and subsequent experimental soil and foliar analyses, and their interpretation diagnosis (id) by conventional soil nutrient range (SNR) and foliar nutrient range (FNR) criteria are presented in [Table 2](#page-2-0). The preliminary data, before the start of the study, are shown in the rows labeled "Before T". According to these SNR and FNR methods, they considered as normal the concentrations of N, Ca, Mg and B, both in soil and in leaf tissue samples, whereas Mn was diagnosed as low. Conversely, there were discrepancies in the SNR and FNR diagnosis for P, K and Zn, categorizing their concentrations as low in soils, but normal in leaf tissues [\(Table 2](#page-2-0)). Iron and Cu in soil samples were diagnosed by SNR as low and high, respectively, but reversed to high and low, respectively, in leaf tissues according to FNR. These observations highlight the challenge to a horticulture diagnostician or crop consultant to carefully consider the methodologies used to collect soil and leaf tissue samples, their processing, the laboratory methods used to extract and quantify each element and/or its recorded analyte (e.g. total concentration versus biologically active), along with the interpretation standards or guides that are employed [\(Lucena, 1997](#page-8-3); [Mills](#page-8-15) [and Jones, 1996](#page-8-15)). Nevertheless, the use of both conventional soil and plant tissue analyses improves on the ability of a diagnostician to determine the nutrient status of a crop, and provide advise on the treatments to correct incipient nutrient disorders [\(Mills and Jones, 1996](#page-8-15)).

Elemental DRIS and CND indices $(I_j, \text{ with } j = \text{specific nutrient})$ for each nutrient and the mean balance index (NBIm and CNDm r^2 , respectively), calculated (according to [Wadt and De Novais, 1999](#page-9-4)) for preliminary and experimental leaf nutrient data are shown in [Table 3](#page-3-0), along with their respective interpretation diagnosis. The elemental and NBIm DRIS indices data from the preliminary leaf samples (first row in [Table 3\)](#page-3-0) are also graphically presented in [Fig. 1,](#page-4-0) which quickly highlights which nutrients are diagnosed as normal, in excess or in deficit; e.g. within, above or below the NBIm boundaries, respectively.

According to DRIS methodology, the N, P, K, Ca, Mg, S, Mn, and B concentrations in the preliminary leaf samples were deemed normal (within the mean balance index, NBIm, calculated from all nutrients), thereby suggesting the status of these elements would not be significantly affecting rose flower production at that time (before start of experiments). Conversely, the elements Fe, Cu and Zn exceeded (positively or negatively) the NBIm limits ([Fig. 1;](#page-4-0) first row in [Table 3\)](#page-3-0), and their adjustment, aiming at zeroing the individual and mean nutrient balance indexes, theoretically offers the greatest probability of significant yield responses [\(Wadt, 1996;](#page-9-3) [Wadt and De Novais, 1999](#page-9-4)). Therefore, we hypothesized that restricting Fe and increasing Cu and Zn supplies in the rose crop's fertilization program would lead to a balanced crop nutrient status and improvements in flower productivity, guiding to the fertilization treatments employed in the first

Fig. 1. DRIS indices for individual nutrients (bars), and mean nutrient balance index (NBIm, dashed lines) for leaf tissues of rose plants ('Freedom' grafted on $R. \times$ 'Natal Briar') prior to the start of experimental treatments to validate integrative DRIS and CND norms for greenhouse rose crops.

experimental flowering cycle [\(Table 1](#page-1-0)).

There were some discrepancies between the different nutrient diagnostic methods applied to the crop prior to the start of the experiment ("Before T" rows in [Tables 2 and 3\)](#page-2-0). The contents of Fe were low in soil according to SNR, but the three foliar diagnosis methods (FNR, DRIS, CND) qualify it as high to excessive. Conversely, Cu and Zn contents were considered high according to the soil analysis, but the foliar diagnoses methods presented Cu content as low or very low, and normal or low for Zn content. Conceivably the antagonisms existing among these soil cationic microelements, and their effect on plant absorption, might explain this situation ([Barber, 1995;](#page-8-16) [Fageria, 2001](#page-8-17); [Marschner,](#page-8-18) [1995\)](#page-8-18). Higher levels of Fe and P on leaf tissues can be interacting with Cu and Zn, and determining the negative (low to very low) diagnosis of the latter [\(Fageria, 2001](#page-8-17); Grattan [and Grieve, 1998](#page-8-19)). This interactive effect is only evident on the DRIS and CND methods, but was not detected by the conventional FNR method.

The conventional foliar and soil critical levels proposed by [Ortega](#page-8-14) [\(1997\),](#page-8-14) used for the diagnosis of data in [Table 2](#page-2-0), do not consider crop productivity and were obtained by calculating only average and standard deviation of each element from a large database of pre-existing analyses for rose crops. However, their interpretation about the crop nutrient status ([Table 2](#page-2-0)) coincided in 8 of the 11 determined elements with the results generated by the integrative DRIS and CND methods ([Table 3\)](#page-3-0). This observation still confers to the conventional FNR methods an adequate utility for a relatively quick and uncomplicated interpretation of tissue analyses.

3.2. Responses and diagnosis of the first flower production cycle

3.2.1. Soil analysis

The soils in the floriculture region in the Bogota Plateau are commonly reported to have low levels of Mn [\(IGAC, 2000](#page-8-11)), which effectively was the diagnosis for the soil samples collected prior to the start of this study (first row in [Table 2](#page-2-0)). The Fe soil concentrations were similarly interpreted as low by SNR criteria, despite continuous applications of macro- and micronutrients to these soils through intensive fertigation practices. Furthermore, modifications in the concentrations of Fe, Zn and Cu in the applied fertigation solutions used during this study [\(Table 1\)](#page-1-0) were not sufficient as to significantly alter their soil concentrations over the entire experimental period [\(Table 2\)](#page-2-0). This apparent lack of response in soil micronutrient concentrations with changes in the applied fertigation treatments suggests a significant nutrient buffering capacity in this soil ([Sillanpää, 1982\)](#page-9-5), likely enhanced by its high organic matter content, product of the continuous amendments applied to soil-grown flower crops ([Cabrera and Solis-](#page-8-6)[Perez, 2017](#page-8-6)). Moreover, these observations support the contention that conventional dry soil analyses are not sensitive enough to predict responses or changes in crop nutrient status and productivities in intensively managed crops. It has been proposed that intensively fertigated greenhouse crops should be studied and evaluated through the use of extracted aqueous soil solutions instead of dry soil samples ([Cadahía, 2005](#page-8-20); [Franco-Hermida et al., 2017](#page-8-8); [Incrocci et al., 2017](#page-8-21); [Ramos-Miras et al., 2011](#page-8-22)), but their use is not widespread due to logistical and labor difficulties for their extraction, and lack of appropriate reference levels.

3.2.2. Foliar analysis

Reductions on the Fe supplies in fertigation solutions led to decreases in leaf Fe concentrations on all treatments with respect to the control solution ([Table 2\)](#page-2-0), and interestingly also corresponded with significant and concomitant reductions on the foliar concentration of Mn. Interactions and imbalances of micronutrients are reportedly some of the most important factors affecting yields of crops ([Rietra et al.,](#page-8-23) [2017\)](#page-8-23). Thus, changes in the supply of individual metallic microelements often aggravates their cation-cation competition and interactions in soil solution, differentially affecting their uptake and accumulation in plant tissues ([Franco-Hermida et al., 2017](#page-8-8), [2013](#page-8-10)). Despite the strongly antagonistic interaction reported for Fe and Mn in many crops ([Fageria, 2001](#page-8-17); [Marschner, 1995](#page-8-18); [Rietra et al., 2017\)](#page-8-23), reducing Fe supply in the fertigation solutions (T1 to T4) in the first rose production cycle did not produce increases in leaf Mn concentrations, but quite the contrary, these were reduced with respect to the control nutrient solution [\(Table 1\)](#page-1-0). Conversely, increasing the supply of Cu and Zn, in fertigation solutions and with foliar supplemental applications, in treatments T2, T3 and T4, significantly increased their foliar content with respect to the control nutrient solution and the first treatment (T1) where only Fe supply was reduced. These observations point out the often complex nature of the interactions between nutrients in the soil (Barber, 2005; [Fageria, 2001;](#page-8-17) [Marschner, 1995\)](#page-8-18), and more so with microelements, whose fertilization (application) rates are very high compared to their seasonal or annual nutrient removal [\(Rietra et al.,](#page-8-23) [2017\)](#page-8-23), particularly in intensively managed flower crops like roses ([Cabrera and Solis-Perez, 2017\)](#page-8-6).

The combined reduction in Fe supply with increases on Cu and Zn supplies through fertigation and foliar supplements (T4) also resulted in a significant decrease in P foliar concentration ([Table 2](#page-2-0)). Significant interactions of P with Fe and Zn, and to a lesser degree with Cu, have been reported for several crops at both the soil (solution) level and at the plant metabolic level [\(Fageria, 2001](#page-8-17); [Grattan and Grieve, 1998](#page-8-19); [Rietra et al., 2017](#page-8-23)).

3.2.3. DRIS and CND indices

The rose plants from the control nutrient solution maintained the initial foliar nutrient indices observed for each element $(I_N, I_P, I_K,$ etc.; [Fig. 1\)](#page-4-0) through the first flowering cycle, highlighting the significant deviations of Fe, Zn and Cu outside of the boundaries of the calculated mean nutrient balance index values in both DRIS (NBIm) and CND $(CNDm r²)$ ([Table 3](#page-3-0)).

Compared to the control nutrient solution, the imposition of the four experimental fertilization treatments over the first flowering cycle significantly reduced the nutrient indices of Fe (I_{Fe}) in both DRIS and CND diagnostic methods, yet these I_{Fe} still exceeded the mean nutrient balance indices [\(Table 3](#page-3-0)). While no statistical differences were found among the experimental treatments, each gradual modification to the nutrient solution or foliar supplementation – from T1 to T4 – showed a decreasing trend in I_{Fe} . The introduction of supplementary Zn supplies through both fertigation and foliar applications (treatments T2, T3 and T4) improved the Zn status of the leaf tissues, expressed in I_{Zn} that were positioned within the limits of both NBIm and CNDm r² [\(Table 3](#page-3-0)). Similar to Zn, increasing the supplies of Cu through fertigation (treatment T2) brought desirable increases in the foliar Cu index, I_{Cu} ([Table 3\)](#page-3-0), but its values exceeded the upper boundaries of both NBIm and CNDm r^2 with supplemental foliar applications (T3 and T4). A major observation made across all four experimental treatments was the significant reduction in leaf Mn index, I_{Mn} . We contend that this Mn response is largely and closely tied to an imbalance with Fe, despite not following the classical antagonistic interaction reported for many crops (Barber, 2005; [Fageria, 2001](#page-8-17); [Marschner, 1995](#page-8-18); [Rietra et al., 2017\)](#page-8-23).

According to the tenets of DRIS and CND, a balanced crop nutrient status, technically found in a high-yielding population, occurs when the elemental index values for all the nutrients are contained within the normality or boundary limits set by NBIm and CNDm r^2 , and these in turn approach or reach a balance of zero ([Franco-Hermida et al., 2017,](#page-8-8) [2013;](#page-8-8) [Parent, 2011](#page-8-4); [Wadt and De Novais, 1999\)](#page-9-4). On this basis, fertigation treatment T2 [\(Table 1\)](#page-1-0), where the concentrations of Fe were reduced 60 % (from 4 to 1.6 mg L^{-1}), and Zn and Cu were increased 15 % and 30 % (from 1 to 1.15 and 1 to 1.3 mg L^{-1} , respectively), provided the nutrient concentrations and ratios in the rootzone that, under the conditions of this first flower production cycle, led to a foliar nutrient balance approaching that found in a high-yielding population.

3.2.4. Flower yield and quality

The applied fertilization treatments did not produce significant flower yield responses with respect to the control, nor in the distribution of stem length grades [\(Table 4\)](#page-5-1). It should be noted, however, the numerically larger flower yields and long stem grades observed in T2 and T4 compared to the control fertigation solution. It has been reported that flower and biomass yields in rose crops often take considerable time to respond to fertilization treatments, particularly for macronutrients and salinizing ions (Na, Cl), whose concentrations are often buffered by stored reserves that can dynamically be remobilized or translocated from mature to new tissues ([Cabrera and Solis-Perez,](#page-8-6) [2017;](#page-8-6) [Cabrera et al., 2009](#page-8-7)). However, the expectation for micronutrients, with significant limitations in translocation and phloem mobility [\(Marschner, 1995;](#page-8-18) [Mills and Jones, 1996\)](#page-8-15), is that changes and/or limitations in their supply should more readily produce differential responses in their accumulation and balance in developing shoots (i.e. rose flower shoots), and expressed more quickly in flower/biomass yields, nutrient status and quality attributes.

A combined assessment of the nutrient status responses provided by the DRIS and CND methods ([Table 3\)](#page-3-0), and the trends in flower

Table 4

Flower yields and harvested stem measurements from fertilization treatments used to validate DRIS and CND nutrient diagnostic norms for greenhouse rose crops.

Treatment	Yield (flowers) m^{-2} year ⁻¹)	Stem Grade ^x			Stem Length	Stem Diameter ^y
		70 s (%)	40 s $(\%)$	NE (%)	(cm)	(mm)
First Production cycle ²						
Control	83.9	36.2	10.3	10.6		
T1	86.4	31.3	10.6	10.1		
T ₂	91.2	36.3	9.7	11.4		
T ₃	83.8	32.7	10.8	10.8		
T ₄	88.2	36.5	10.4	10.7		
Second production cycle						
Control	84.8	23.1 _b	12.8a	12.6	89.5 _b	7.5
T1'	87.8	32.4a	8.4 b	9.5	99.1a	7.4
T2'	94.0	32.3a	8.6 b	9.0	98.5 a	7.5

 x Fraction (%) of harvested flower with stems lengths 70 s ($>$ 70 cm), 40 s (40−49 cm) and non-exportable (NE) discards (< 40 cm). The remaining percentage corresponds to intermediate quality grades.

^y Length measured from the distal stem cut to the base of the flower peduncle. Diameter measured just above the distal stem cut. Length and stem measurements of floral carried out 8 weeks after pruning.

 z Absence of letters accompanying the average values indicates that there are no significant differences according to the Tukey test ($\alpha \leq 0.05$).

productivity ([Table 4](#page-5-1)), indicate that reductions in Fe, and increases in Zn and Cu supplies in treatment T2 were the closest to approach the nutrient balance that could significantly increase flower yields. This led to the decision to carry over this treatment, now labeled T1', along with the control fertigation solution, into a second flower production cycle ([Table 1\)](#page-1-0). As Mn was the element that emerged as deficient in all treatments (T1 to T4) in the first flower cycle, increasing its supply by 20 % in the carryover T1′ fertigation treatment, was added as an additional treatment, labeled as T2′. The original fertigation treatment that only restricted Fe supplies in the fertigation solution (T1), and the two treatments receiving foliar applications of Cu and Zn (T3, T4) were discarded, as they did not improve (i.e. reduced) the nutrient balance indices NBI m and CND m r^2 , and actually lead to exceedingly high I $_{\rm Cu}$.

3.3. Responses and diagnosis of the second production cycle

3.3.1. Soil analysis

Similar to the first flowering cycle, soil nutrient concentrations were not significantly affected by changes in the supply and balance of Fe, Mn, Cu and Zn provided by the experimental treatments (T1′, T2′; [Table 1](#page-1-0)) with respect to the control fertigation solution ([Table 2](#page-2-0)). This supports the previous assertion that diagnostics systems based on dry soil analyses are not good predictors of potential responses or changes in crop nutrient status and productivity in intensively managed flower crops (Franco-Hermida et al., 2017).

3.3.2. Foliar analysis

Leaf concentrations of P were significantly lower, while Zn and Cu concentrations were higher in treatments T1′ and T2′ with respect to the unmodified control nutrient solution ([Table 2](#page-2-0)). These responses for P, Zn and Cu observed in T1′ were expected to emulate those observed in the same treatment (T2) during the first flowering cycle, and actually maintained leaf Fe concentrations stable, still diagnosed as normal by conventional FNR criteria [\(Ortega, 1997](#page-8-14)). Foliar Mn concentrations once again showed a decreasing trend when associated with reduced soil solution supplies of Fe and increased Zn and Cu supplies (T1′). However, a modest increase in the supply of Mn in the fertigation solution T2′ was sufficient to cause sufficient change in its soil solution ratio with (the antagonistic) Fe, as to effectively increase its concentration in leaf tissues [\(Table 2\)](#page-2-0), almost reaching the normal or sufficiency levels recommend for rose crops by FNR criteria ([Ortega,](#page-8-14) [1997\)](#page-8-14).

3.3.3. DRIS and CND indices

The fertigation treatments used in the second flowering cycle significantly reduced the elemental DRIS and CND indices for iron, I_{Fe} ([Table 3\)](#page-3-0). The elemental indices for P, Zn and Cu mirrored the behavior described by their foliar nutrient concentrations ([Table 2\)](#page-2-0), in conformity with the common antagonisms reported among these nutrients in other crops ([Fageria, 2001;](#page-8-17) [Rietra et al., 2017\)](#page-8-23).

The elemental Mn indices (I_{Mn}) observed in T1' in the second flowering cycle paralleled the behavior of the similar T2 in the first flowering cycle ([Table 3\)](#page-3-0), with significantly depressed, or deficient, values in the combined presence of reduced Fe and increased Cu and Zn supplies in the fertigation solution. Conversely, the increased Mn supply in fertigation solution $T2'$ corrected the I_{Mn} , bringing it closer to be within the limits of the mean nutrient balance index values in both DRIS and CND, and actually contributing to a significant reduction in the DRIS NBIm index [\(Table 3](#page-3-0)). In this sense, the diagnostic capacity of CND was not able to detect and adjust differences in the micronutrient balance between fertigation treatments T1′ and T2′, reflected in their having similar CNDm r² values.

3.3.4. Flower yield and quality

The total flower productivity recorded in fertigation treatments T1' and T2′ was not significantly different from the control nutrient

solution. However, changes in the foliar nutrient balance (i.e. changes in both elemental and mean nutrient balance indices) generated by these treatments significantly affected the distribution of flower stem grades during this second flowering cycle [\(Table 4](#page-5-1)). The proportions of harvested stems in the 70 s ($>$ 70 cm) and 40 s ($>$ 40 cm) length grades significantly increased and decreased, respectively, in both T1′ and T2′ treatments, ultimately reflected in significantly larger average stem lengths for this flowering cycle with respect to the control treatment.

The length of rose floral stems is determined mainly by two components, the cell number (differentiation) and cellular elongation (growth) in the shoot internodes produced within a flowering cycle ([Morisot et al., 1998](#page-8-24)). With a judicious pruning management (Calatayud, et al., 2008), and under the near optimum radiation and temperature conditions often found in commercial greenhouses in the Bogota Plateau, significant deviations in rose flower stem development would expectedly be more related to conditions caused by improper or stressful irrigation and fertilization practices. The DRIS procedures in this study certainly allude to a significant involvement of micronutrient imbalances in the observed rose stem length responses. Incipient to severe deficiencies of the micronutrients Zn and Mn in other Rosaceae woody crops like stone (Prunus spp.) fruit trees (peaches, plums, nectarines) are known to affect cellular elongation, leading to shortened internodes, reduced root elongation and small leaves [\(Johnson and](#page-8-25) [Uriu, 1989;](#page-8-25) [Marschner, 1995](#page-8-18); [Swietlik, 2002\)](#page-9-6). Correction of the Zn, Cu, and Mn imbalances detected by DRIS, by simply doing relatively small changes (increases) in their supply in the fertigation formulations were sufficient to significantly change the overall nutrient status of the rose plants and increase the elongation of the harvested flower shoots ([Table 4](#page-5-1)). These responses highlight the enhanced capacity of integrative nutrient diagnosis systems like DRIS to detect "hidden" or unsuspected nutrient disorders or imbalances effectively affecting crop productivity and/or quality [\(Barker and Pilbeam, 2007;](#page-8-26) [Mills and](#page-8-15) [Jones, 1996](#page-8-15)).

3.4. Overall assessment of nutrient diagnosis systems

As previously mentioned, some discrepancies were observed in the interpretation diagnosis of conventional SNR and FNR methods with respect to the adequacy or sufficiency of specific nutrients in soils and rose leaf tissues. Interestingly, despite their long and extensive utilization in horticultural crops, SNR and FNR interpretation standards have been generated with little to no consideration to yields or productivity, and rather have been more reliant on visual symptoms of nutrient disorders ([Lucena, 1997;](#page-8-3) [Mills and Jones, 1996](#page-8-15)). Nevertheless, and despite this significant oversight, the interpretation provided by the FNR method about the preliminary rose crop nutrient status ([Table 2\)](#page-2-0) coincided for most (8 of 11) of the evaluated nutrients with the diagnosis generated by DRIS and CND [\(Table 3](#page-3-0)).

The diagnostic norms used by DRIS and CND are based on actual yield or productivity parameters, whereby the nutrient status from a high-yielding population is used to evaluate the relationships between all elements, generating relative values and ratios that are ultimately expressed in individual (elemental) and integrative (global or mean) indices of balance associated with maximum yields [\(Franco-Hermida](#page-8-8) [et al., 2017, 2013;](#page-8-8) [Parent, 2011;](#page-8-4) [Wadt and De Novais, 1999\)](#page-9-4). Application of the theoretically generated DRIS and CND norms to a new sample (with results from a complete foliar nutrient analyses) will generate a diagnosis that highlights those elements whose correction or adjustment, through the implementation of changes in the fertilization program, aimed at zeroing the individual and mean nutrient balance indexes, offers the greatest probability of yield responses. DRIS methodology was used to assess the nutrient status of this experimental rose crop, and guide the formulation of the fertilization treatments employed in both flowering cycles [\(Table 1](#page-1-0)). While the individual treatments did not produce significant differences in flower yields with respect to the control fertigation solution [\(Table 4](#page-5-1)), a significant

Fig. 2. A. Correlation between cut flower productivity and global nutrient balance indices for DRIS (A) and CND (B) after two flower production cycles in an experimental validation study of DRIS and CND diagnostic norms for cut-rose flower crops ($p < 0.001$; $n = 24$).

Fig. 3. Correlation between the fraction of cut rose flower stems grade 40 s (> 40 and < 70 cm) and foliar zinc status expressed as leaf Zn concentration (A), DRIS Zn index (I_{Zn} , B) and CND Zn index (I_{Zn} , C) after two flower production cycles in an experimental validation study of DRIS and CND diagnostic norms for cut-rose flower crops ($p < 0.001$; $n = 24$).

correlation is nevertheless observed between the global nutrient balance indices of DRIS and CND with flower yields over the two flowering cycles ([Fig. 2](#page-7-0)). In agreement with the principles of DRIS and CND ([Parent, 2011;](#page-8-4) [Wadt and De Novais, 1999](#page-9-4)), reductions in the value of these indices correlated with higher flower yields. This means that the progressive corrective iterations made to the experimental fertilization treatments improved rose foliar nutrient balances, and effectively increase flower yield productivities towards the maximum ceiling of 120–130 flowers/ m^2 year. This was the flower productivity ceiling used to generate the DRIS and CND norms ([Franco-Hermida et al.,](#page-8-10) [2013\)](#page-8-10) being experimentally validated in the present study.

The progressive corrective iterations made to the fertilization treatments ([Table 1\)](#page-1-0), using DRIS diagnosis as a guide, made significant improvements to the elemental indices of most nutrients by the end of the second flowering cycle [\(Table 3\)](#page-3-0). However, the indices for Fe (I_{Fe}) and Mn (I_{Mn}), while reduced compared to their values at the start of this experimental validation study, still remained outside, and on opposite sides, of the NBI m and CND m r^2 boundaries. It is apparent that there is a strong antagonistic Fe:Mn interaction in greenhouse rose crops even when changing their concentration in supplied fertigation solutions. As we have already mentioned, at the soil level the degree of this relationship might be largely related to the inherently low levels of Mn found in the soils of the Bogota Plateau ([IGAC, 2000](#page-8-11)). On the other

hand, at the plant level, a common denominator for rose crops in the Bogota Plateau of Colombia, and actually across all rose growing regions in the American Continent, is the exclusive use of the rootstock 'Natal Briar' ([Cabrera, 2002](#page-8-12); [Cabrera and Solis-Perez, 2017\)](#page-8-6). Studies have pointed out that, compared to other traditional rootstocks, 'Natal Briar' confers a significantly different nutrient profile to the scions grafted on it [\(Cabrera, 2002\)](#page-8-12), and more detrimentally when exposed to stressful soil solution conditions like salinity [\(Cabrera et al., 2009\)](#page-8-7). The observations and diagnoses from the present study lend support to the anecdotal reports by rose growers in the Bogota Plateau who often face significant foliar interveinal chlorosis in the cultivar 'Freedom' used here, wrongly attributed to incipient Fe deficiencies that in turn were seldom corrected by increases in fertigation and foliar Fe supplies. Therefore, we contend that an immediate practical recommendation to seriously consider in commercial rose crops grafted on 'Natal Briar', is to sensibly increase Mn supplies while reducing Fe applications.

Whereas combined modifications to the supplies of Fe, Mn, Cu and Zn in the fertigation solutions applied during the second flowering cycle significantly improved the length of harvested rose stems ([Table 4](#page-5-1)), the leaf Zn status, expressed in concentration units and DRIs and CND indices, was the element that produced the best single correlations with stem lengths [\(Fig. 3\)](#page-7-1). While improvements in the overall plant micronutrient balance are plausibly involved in this response, Zn in particular

plays a physiologically significant role in the metabolism of auxins, mainly indole-acetic acid, which are determinant in cellular elongation ([Johnson and Uriu, 1989](#page-8-25); [Marschner, 1995](#page-8-18); [Swietlik, 2002\)](#page-9-6) and linked to apical dominace and stem length in roses (Calatayud et al., 2008; [Morisot et al., 1998](#page-8-24)). Increases in foliar Zn towards 50 mg kg−¹ \cdot ¹, the upper FNR level proposed by [Ortega \(1997\)](#page-8-14), reduces the percentage of less desirable shorter grade 40 rose stems ([Fig. 3](#page-7-1)A). Predictably, this foliar Zn concentration corresponds to elemental I_{Zn} indices approaching zero ([Figs. 3B](#page-7-1) and 3C), and contained within the boundaries of the mean nutrient balance indices NBIm and CNDm r^2 ([Table 3\)](#page-3-0).

4. Concluding remarks

The experimental results from this study effectively validate the DRIS and CND norms previously generated for greenhouse rose crops ([Franco-Hermida et al., 2013](#page-8-10)). Compared to conventional FRN and SNR methodologies, the integrative nutrient diagnostic capacity of DRIS and CND significantly enhance the ability to identify those elements with the most probability of crop response. This DRIS and CND diagnoses also help to determine the relative magnitude of the corrections needed on fertilization programs, to effectively balance crop nutrient status and produce positive yield responses.

Results from this study support the notion that the intrinsic consideration of relationships between all elements in DRIS and CND ([Parent, 2011](#page-8-4); [Wadt and De Novais, 1999\)](#page-9-4) significantly enhance the identification of their interactions and imbalances (e.g. antagonisms and synergies), particularly when yields or plant growth parameters are used as the response criterion. There are proposals that nutrient interactions are among the most important factors affecting crop yields ([Rietra et al., 2017\)](#page-8-23), before, if ever, nutrient disorders can be expressed visually during a growth crop cycle, a phenomenon often referred to as hidden hunger [\(Barker and Pilbeam, 2007;](#page-8-26) [Mills and Jones, 1996](#page-8-15)). As an example, the strong Fe:Mn interaction observed for roses in this study has been incorrectly diagnosed and addressed in the Bogota Plateau as a simple incipient or severe Fe deficiency when using conventional SNR and FNR methods (according to anecdotal references from growers and horticultural consultants). Application of integrative DRIS and CND methods has underlined the significance of this Fe:Mn interaction, modulated by changes in the supply and balance of micronutrients (Fe, Mn, Zn, Cu), the inherently low Mn levels in the soils of the region, and a major role of the dominant rose rootstock ('Natal Briar') in use. The present study supports the contention that the use of DRIS and CND methods, to guide the introduction of sensible (gradual and iterative) changes to nutrient supplies in fertilization programs, offer a higher probability of enhancing fertilizer use efficiency and lead to positive rose crop yield responses.

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CRediT authorship contribution statement

John J. Franco-Hermida: Data curation, Formal analysis, Investigation, Methodology, Validation, Writing - original draft. María F. Quintero-Castellanos: Formal analysis, Validation, Writing - review & editing. Ana I. Guzmán: Writing - original draft. Miguel Guzmán: Conceptualization, Formal analysis, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing. Raul I. Cabrera: Conceptualization, Methodology, Project administration, Supervision, Validation, Visualization, Writing - original draft, Writing review & editing.

Declaration of Competing Interest

John J. Franco-Hermida was employed by GR Chía, S.A. while conducting this research on their premises. However, he worked on this study on his own time, aside from his formal job duties, and GR Chía, S.A. did not play any role in design of the study, data collection and analysis, decision to publish, or preparation/revision of the manuscript. There are no patents, products in development or marketed products to declare.

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