Review

Methods for Nutrient Diagnosis of Fruit Trees Early in the Growing Season by using Simultaneous Multi-Element Analysis

Kaori Matsuoka*.,**

Institute of Fruit Tree and Tea Science, National Agriculture and Food Research Organization, Tsukuba 305-8605, Japan

Fruit tree nutrition is one of the most important factors in terms of growth and productivity. Measurement of nutritional requirements is an important aspect of nutrient management because nutritional disorders reduce yield and fruit quality. This review explores how nutrient imbalances affect the yield and fruit quality of fruit trees, and presents methods for diagnosing fruit tree nutritional disorders in order to correct nutritional deficiencies. In orchards, differences between soil sampling sites and locations of fruit tree roots when the root system is deep and unevenly distributed make it difficult to obtain representative soil samples from which to measure the forms of nutrients available to the trees. The delayed response of fruit trees to fertilizer applications compared with annual crops makes it difficult to determine their nutritional status through soil analysis immediately after application. In addition to soil analysis, plant tissue analysis is used to determine nutritional status and fruit tree nutritional requirements. In particular, earlier analysis in the growing season could allow sufficient time to correct any deficiencies before harvest. A recent approach that relies on analysis of the ionome, which is defined as the entire mineral nutrient and trace element complement in an organism, through simultaneous quantitative measurement enables comprehensive evaluation of multi-element composition. This approach could be especially effective as major decreases in yield and fruit quality are often caused by the interaction of several elements. Until recently, studies of fruit tree nutritional disorders have focused on particular nutrients, not multiple elements. Therefore, the application of ionomic analysis is a promising approach to elucidate multi-element interactions for accurate diagnosis of nutritional disorders.

Key Words: agriculture practice, ionomics, root distribution, soil analysis, spatial variability.

1. Introduction: Yield and Fruit Quality of Fruit Trees as Influenced by Nutritional Disorders

Among fruit tree nutritional disorders, Fe deficiency chlorosis is a problem in calcareous and alkaline soils in many European orchards (Álvarez-Fernández et al., 2003). Fe deficiency in peach trees reduces yield from approximately 50–60 t·ha\(^{-1}\) to 12–15 t·ha\(^{-1}\), and the fruits are poorly colored and small, although the nutritional value is slightly improved and firmness is unaffected (Álvarez-Fernández et al., 2003). Chloride stress in citrus with a leaf Cl content of > 0.7% (dry matter) reduces shoot growth and fruit yield, and causes “scorching”, “firing”, or defoliation of leaves (Raveh, 2005). Zn deficiency is widespread in many citrus orchards in Pakistan, particularly in calcareous and alkaline soils, and reduces fruit yield (Rashid and Ryan, 2004; Razzaq et al., 2013). In an apple cultivar grown in newly reclaimed soils and alluvial soils in Egypt, micronutrient deficiency limited growth (Nofal and Khalifa, 2002). The nutrient shortage in these soils in most cases is related to their alkalinity, clay content, and low organic matter, and Fe may be a limiting factor for apple growth at both locations (Nofal and Khalifa, 2002). Excessive use of acidifying fertilizers is associated with Mn toxicity, which causes defoliation, internal bark necrosis, and greenish spot disorder in satsuma mandarin, apple, and Japanese persimmon trees in Japan (Aoba, 1986), and apple trees in Canada (Fisher et al., 1977). Fertilizers containing high levels of N and K are associated with mild Zn deficiency symptoms in the leaves of orange trees in acidic deep sandy soil (Reuther and Smith, 1950). Such fruit tree nutritional disorders that often result from the inherent characteristics of soils and inappropriate fertilizer management cause major decreases in yield and fruit quality.

How can we prevent such nutritional disorders? One
way is to measure nutritional status to get an accurate diagnosis. In general, soil tests have limited value when applied to trees grown in orchards because the root system is deep and unevenly distributed, making it difficult to obtain a representative soil sample (Pestana et al., 2003). In addition, regional differences in soil fertility and plant nutritional problems explain inconsistent responses of orchards to fertilization (Srivastava and Singh, 2006). In some cases, the application of fertilizers to soil is not effective, as demonstrated for Mn, Zn, and Fe in high-pH soils with high concentrations of free CaCO$_3$ (Dordas, 2008). Because leaf analysis indicates actual uptake, it is considered to indicate the current nutritional status of fruit trees as an alternative to soil analysis (Stiles and Reid, 1991). However, standard leaf analysis has several limitations, notably that it is performed very late in the growing season (Johnson et al., 2006; Uçgun and Gezgin, 2017). Analysis early in the season has been proposed as an alternative by many researchers.

Plant nutritional disorders do not always arise from a particular nutrient, but are often caused by several elements through competition between ions with similar physicochemical properties (valency and ion diameter), such as the alkaline cations K$^+$, Rb$^+$, Cs$^+$, and Na$^+$, or between the Group II divalent cations Ca$^{2+}$, Sr$^{2+}$, and Ba$^{2+}$ in the rhizosphere, for entry into an ion channel protein or for binding to a carrier protein in plant roots (White, 2012). At high external concentrations, an accompanying ion that is taken up relatively slowly can reduce the uptake of an oppositely charged ion that is transported at a faster rate: SO$_4^{2-}$ depresses K$^+$ uptake and Ca$^{2+}$ depresses Cl$^-$ uptake from single-salt solutions (White, 2012). In fertilizer practices in orchards, application of non-Cl sources of K may adversely affect plant uptake of other cations, including Ca, and especially Mg, by tart cherry trees (Callan and Westcott, 1996). High rates of KCl (Cl source) fertilization resulted in Cl toxicity, suppressing P uptake and increasing Mn uptake by tart cherry trees (Callan and Westcott, 1996). In contrast to K, increasing the rate of N fertilization when growth was constrained by K deficiency increased leaf N and Mn and decreased leaf P and B (Neilsen et al., 2004). Similarly, long-term application of N fertilizer altered apple tree nutrition in fruits and leaves of not only N, but also other nutrients including K and Mn (and B and Zn in fruits) for which K fertilizer had been applied long term (Matsuoka et al., 2019). The above findings suggest that one nutrient interferes with others, and these nutritional imbalances can cause fruit tree nutritional disorders.

Several mineral nutrients can influence yield and fruit quality. The optimum concentrations of N, P, K, Ca, Mg, Cu, and Zn in mandarin leaves were very similar to those in high-yield orchards (Srivastava and Singh, 2006). N, K, P, Ca, and B are most often correlated with apple fruit quality and disorders (Fallahi et al., 2010), and N, K, Ca, and Mn are correlated more often than other nutrients with apple fruit quality parameters, such as fruit weight, fruit dry weight, color at harvest, and soluble solids concentration at harvest and storage (Fallahi and Simons, 1996). These reports demonstrate that fruit tree nutritional disorders and the concomitant major decreases in yield and fruit quality are caused by several nutrients.

The term ‘ionome’ was first defined to include all the mineral nutrients and trace elements found in an organism (Lahner et al., 2003). The idea of plant ionomics began with the mixing of metabolomics and mineral nutrition and was first suggested by Robinson and Pauling in the late 60s and early 70s (Singh et al., 2013). Ionomics is the study of the ionome (Fleet et al., 2011). Ionomics involves the simultaneous measurement of the elemental composition of an organism and its changes in response to environmental, physiological, or genetic modifications (Fleet et al., 2011). The application of the ionome is a promising approach to clarify multi-element interactions, as it has been argued that mineral biology, including the nutritional status of plants, should be examined as a system (Fleet et al., 2011). Ionomics is improving day by day, together with other systems biology approaches, i.e., metabolomics, proteomics, and transcriptomics (Fleet et al., 2011; Singh et al., 2013). The use of ionomics in plant biology, physiology, and genetics has been described in several reviews (e.g., Baxter, 2009; Fleet et al., 2011; Salt, 2004; Salt et al., 2008; Singh et al., 2013).

The objective of this review is to present methods of fruit tree nutrient diagnosis that could be used early in the growing season to correct nutrient levels early, as well as to assess the roles of different nutrients in fruit tree nutritional disorders. The spatial and temporal variations in tree root distribution and their relations with locations of soil sampling sites are discussed. These include methods for the diagnosis of fruit tree nutritional disorders early in the growing season as alternatives to conventional leaf analysis and the application of ionomics to studies of fruit tree nutrition in order to reveal multi-element interactions for accurate diagnosis of nutritional disorders.

2. Spatial Variability in Soil Properties and Root Distribution

1) Locations of soil sampling sites and tree roots

Soil sampling practices used in orchards are summarized in Table 1. In practice, collecting soil samples from depths of 15 to 90 cm (Table 1) tends to match the depths of the majority of fruit tree roots. Approximately 77% of all roots of nine-year-old grapevines on several rootstock cultivars were found at depths of 0–60 cm (Southey, 1992), and 67%–83% of the total dry weight of mature avocado tree roots was confirmed at the same depths (Salazar-Garcia and Cortés-Flores, 1986). However, sampling of the root-zone soil is difficult, espe-
| Reference | Sampling depth (cm) | Sampling sites | Other suggestions |
|-----------|-------------------|---------------|------------------|
| Editorial Board of Methods for Analysis of Soil Environment (1997) (Japan) | 0–20 and 20–40 cm | 2 or 3 sampling points at 30 cm within the rim of the tree crown | • Select 5 or 6 representative trees in an orchard  
• Sample at 20–40 cm to assess the root-zone soils on account of the deep distribution of tree roots  
• Each sample should be a composite of subsamples taken from each sampling depth |
| Sekiya (2002) (Japan) | About 0–20 and 30–50 cm | If soil samples are collected from a soil profile, the profile should be dug between rows of representative trees, or at 30 cm within the rim of the tree crown if canopies meet between rows | As described by the Editorial Board of Methods for Analysis of Soil Environment (1997), except as described here |
| Dow et al. (1991) (USA) | • In established orchards, to 91 cm (3 feet) where fertilizer was applied  
• At monitoring sites, to 91 cm (3 feet) | • In established orchards, usually at least halfway inside the drip line to trunk  
• At monitoring sites, 4 sides of each tree | • Before planting, take 0–30 cm (0–12 inches) samples for pH, organic matter, salts, P, K, B, and Zn, and 0–91 cm (0–3 feet) samples for NO₃⁻N  
• In established orchards, soils within 61 cm (2 feet) of the trunk can be frequently problems |
| Hue et al. (2004) (USA) | Top 20 cm (8 inches) plus a separate sample for the 20–61 cm (8–24 inches) zone | Between tree rows | |
| Iowa State University Extension and Outreach (2014) (USA) | Soil cores to 30.5 cm (12 inches) | Between tree rows | |
| Anderson et al. (2010) and Fery et al. (2018) (USA) | Surface down to about 15–20 cm (6–8 inches) or to the depth of tillage | Between tree rows | • Areas that have been fertilized or limed should not be sampled until at least 8 weeks after application  
• At bearing stage, soil sampling is generally required in late winter or early spring |
| Debnath and Pachauri (2014) (India) | 0–30, 30–60, and 60–90 cm as trees are deep rooted in nature | Between tree rows | |
| TNAU Agritech Portal, Agriculture (2019) (India) | Soil profile samples at 0–30, 30–60, and 60–90 cm | | |
| Bureau of Soils and Water Management, Department of Agriculture, Philippines (2019) (Philippines) | Below 25–30 cm | Soil sample taken below the rim of the tree crown | |
| Incitec Pivot Fertilisers (2019) (Australia) | Zone of maximum root growth and fertilization, extending from about 30 cm from base of the tree to up to 30 cm beyond the drip line | Where under-tree sprinklers are used to apply water and fertilizer, sample cores should be taken from the wetted zone, where most of the feeding roots grow | |

* TNAU Agritech Portal, Agriculture. 2019. Analytical techniques for soil testing. <http://agritech.tnau.ac.in/agriculture/agri_soil_sampling.html> (accessed October 24, 2019).
* Bureau of Soils and Water Management, Department of Agriculture, Republic of the Philippines. 2019. How to collect soil sample for analysis. Brochures, How tos. <http://www.bswm.da.gov.ph/Downloads/Brochures> (accessed October 24, 2019).
* Incitec Pivot Fertilisers. 2019. Horticulture soil sampling, procedure. Nutrient advantage. <https://www.hort360.com.au/wordpress/uploads/Soil/Chemistry/Horticulture%20Soil%20Sampling%20Procedure.pdf> (accessed October 24, 2019).
cially in orchards, because the deep root distribution of orchard trees cannot be examined without excavation, and this changes both spatially and temporally, as well as with irrigation application method (Tanasescu and Paltineanu, 2004). In contrast to field sampling, collecting root-zone soil is more effective where roots can be restricted, e.g., in pots and root-restriction culture with soil mounding as the roots are shallow, have high density, and are evenly distributed (Fujiwara, 1994; Kanehara, 2012). These features make it easier to obtain representative root-zone soil.

2) Spatial variability of soil properties and root distribution

Within a 0.8-ha apple orchard, soil NO$_3$-N, available Fe, available Zn, and available Cu at 0–30 cm had higher spatial variability (coefficient of variation [CV], 52.94%–70.59%) than texture, pH, cation exchange capacity, and organic matter (CV, 4.81%–16.15%) (Aggelopoulou et al., 2011). A similar difference was found at 30–60 cm, although available Fe, available Zn, and available Cu had moderate variability and available P and available B had high variability (Aggelopoulou et al., 2011). These results show that soil properties can vary considerably within the orchard.

The spatial variability of soil properties interacts with the variability of tree root distribution within orchards. Medium- to large-sized root density and total root density vary much more (CV, 109%–193%) than soil respiration, surface soil temperature, soil water content, soil C concentration, soil total and mineral N concentrations, fine root density, and fine root N concentration (CV, 8%–66%) on average over 0–40 cm (except for surface soil temperature at 10 cm and surface soil water content over 0–10 cm) (Ceccon et al., 2011). Such variability in root distribution both horizontally and vertically has been reported in mature avocado trees growing in two soils with different textures (sandy loam and clay) (Salazar-García and Cortés-Flores, 1986) and in satsuma mandarin trees grown on lysimeters in different soils (Tertiary clayey soil, Tertiary gravelly soil, or volcanic ash soil) of different depths (30, 60, or 90 cm) (Komamura and Sekiya, 1985). The size and location of grapevine roots of grown in a saline soil depend more on the soil characteristics than on the rootstock (Southey, 1992). The root system of mature avocado trees was more developed both horizontally and vertically in a sandy soil than in a clayey soil; there were almost four times more roots in the sandy soil as in the clayey soil, and the majority of the roots were distributed at 0–20 cm in the sandy soil (47%), but at 20–40 cm in the clayey soil (34%) (Salazar-García and Cortés-Flores, 1986). The root systems of satsuma mandarin trees were dispersed more widely and more deeply in clayey soil than in gravelly soil and even in volcanic ash soil (Komamura and Sekiya, 1985). The root density near the root trunk was greater when the available soil depth was deeper (60 or 90 cm) than shallower (30 cm) in all soil types, and the density of roots growing from the root trunk decreased in the order of gravelly soil > volcanic ash soil > clayey soil (Komamura and Sekiya, 1985). Overall, the above findings suggest that the tree root distribution can vary both spatially and temporally depending on the soil characteristics, especially physical properties, making it difficult to obtain a representative soil sample in the root zone.

3. Methods for Diagnosis of Fruit Tree Nutritional Disorders

1) Tree tissue analysis and interpretation

The nutrient status of fruit trees is commonly diagnosed by leaf analysis. The analysis of leaf samples taken in early to mid-summer, i.e., between 60 and 70 days after petal fall (Stiles and Reid, 1991), or at 120 days after full bloom (DAFB) (Sanz and Montañés, 1995) has been conventional practice for assessing fruit tree nutritional status worldwide. This timing of sampling in early to mid-summer was originally proposed because most nutrient levels remain fairly stable (Johnson et al., 2006; Stiles and Reid, 1991). However, Stiles and Reid (1991) pointed out that leaf samples collected much earlier tend to contain higher concentrations of N and K and lower Ca, and samples collected appreciably later tend to have lower N and K and higher Ca. In addition, an improvement in leaf sampling techniques is required because leaf tissue composition is dynamically influenced by leaf age, stage of growth, and also position of leaf sampled (Stiles and Reid, 1991; Walworth and Sumner, 1987). Conventional leaf analysis has several limitations. One is that the sampling date recommended for fruit trees is late in the growing season, generally very close to harvest (Pestana et al., 2004), by which time any nutrient input would be unlikely to increase yield in the current growing season (Sanz and Montañés, 1995). Approaches to overcome these limitations include leaf analysis in the early growing season, leaf blade and petiole analysis (in grapevines), and the analysis of flowers, dormant shoots, bark, and xylem sap as alternatives to conventional leaf analysis, as summarized in Table 2.

1) Leaf analysis

Analysis of leaf samples collected in the early growing season, particularly at 28 DAFB, can determine the nutrient status of N, P, K, Ca, Mg, Zn, Mn, and B (but not Fe or Cu) in apple trees (Uçgun and Gezgin, 2017) (Table 2). Rubio-Covarrubias et al. (2009) examined which of the N forms in ‘Fantasia’ nectarine leaves are better indicators of the N status of the trees; stable N indicators (total N and chlorophyll SPAD) in the leaves sampled in July over three years could be used to diagnose N deficiency, whereas soluble N compounds (NH$_4$-N and NO$_3$-N) could be used to diagnose N excess under high N supply, so the combination could be
| Tissue         | Stage                                      | Suggestion or usefulness                                                                 | Reference                                                                 |
|---------------|--------------------------------------------|------------------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Leaf          | Petal whitening                            | Stable N indicators (total N and chlorophyll SPAD) could be used to diagnose N deficiency   | Rubiera-Covarrubias et al. (2009); Ugan and Geggia (2017)                   |
|               | Full bloom (flowering)                     | Higher accuracy in determination of N compared to petiole analysis                         | Dominguez et al. (2014); Simonov et al. (2017)                             |
|               | 28 DAFB                                    | Loss variability and higher reproducibility compared to petiole analysis                   |                                                                          |
|               | 90 DAFB                                    |                                                                                           |                                                                          |
| Leaf blade    |                                            |                                            |                                                                          |
|               |                                            |                                            |                                                                          |
| Leaf petiole  |                                            |                                            |                                                                          |
|               |                                            |                                            |                                                                          |
| Flower        |                                            |                                            |                                                                          |
|               |                                            |                                            |                                                                          |
| Dormant shoot |                                            |                                            |                                                                          |
|               |                                            |                                            |                                                                          |

Table 2. Diagnosis of fruit tree nutrient status by tissue analysis in the early growing season.

DAFB: days after full bloom.

Elements show predictable nutrients.

Cultivar and species of fruit tree are indicated in parentheses.
used for N diagnosis over a broad range of N supply rates.

Diagnostic methods have been assessed in grapevines to determine whether leaf blade or petiole nutrient concentrations better reflect the nutritional status of the vines, and which sampling period results in the most reliable data. Dominguez et al. (2015) concluded that leaf blades are preferable to petioles for the diagnosis of N, P, K, Ca, and Mg in ‘Graciano’ grapevines at both flowering and veraison, owing to lower variability and higher reproducibility, but they showed that Fe, Mn, Zn, Cu, and B in leaf blades and petioles varied differently among vineyards, so it was difficult to determine which was the best tissue for diagnosis. Schreiner and Scagel (2017) showed that N concentrations in ‘Pinot noir’ grapevine leaf blades at flowering and veraison have a stronger relationship with productivity and must N nutrient concentrations at harvest than petioles, and are less variable and more stable than petiole concentrations. The advantage of leaf blade analysis also agrees with the report that ‘Tempranillo’ grapevine leaf petioles are less sensitive than blades at detecting deficiencies or excesses of N, P, K, Ca, Mg, Zn, and Mn at veraison, although petioles were better for detecting Fe and B deficiencies or excesses at flowering and veraison (Romero et al., 2014).

(2) Flower analysis

Flowers are short-lived and so are exposed for less time than leaves to metabolic changes and management practices. As a result, they have a lower risk of contamination by chemicals, pests, or diseases (Montañés Millán et al., 1997) (Table 2). As flowers in many fruit tree species appear well before any leaf material is present, this makes it possible to detect nutritional disorders very early in the season (Sanz and Montañés, 1995) and to prognose abnormal nutrition before symptoms appear (Montañés Millán et al., 1997). Flower analysis seems to be reliable at predicting macronutrient (N and P) and micronutrient (Cu, Zn, and Mn) levels in ‘Arbequina’ olive, as concentrations at the petal whitening stage were significantly correlated with the contents in leaves taken later, at the stone hardening stage, which coincides with the standard date for leaf sampling (Ben Khelil et al., 2010). Flower analysis could also determine the concentration of Fe for the prognosis of Fe deficiency of pear and peach trees in soils with a high pH and high total and active lime contents as an alternative to the analysis of leaf samples taken at 60 and 120 DAFB from pear trees and at 60 DAFB from peach trees (Sanz and Montañés, 1995). Fe chlorosis in ‘Valencia late’ orange trees in a calcareous soil was predicted from the Mg:Zn ratio in flowers: a ratio of < 100 indicated that the trees would develop Fe chlorosis, while a ratio of > 200 indicated that leaves would remain green (Pestana et al., 2004). The K:Zn ratio in peach flowers could also be used, along with the flower Fe concentration, for prognosis of Fe chlorosis in peach trees, because a regression model including the flower K:Zn ratio explained as much as 27% of the variation in leaf chlorophyll concentration at 120 DAFB across five crop seasons, whereas flower Fe concentration explained 6% of the variation of chlorophyll concentrations at 120 DAFB across five crop seasons (Igartua et al., 2000). These findings show that flower analysis enables the detection and correction of deficiencies before fruit set, giving sufficient time for nutrient applications to improve yield and fruit quality (Pestana et al., 2004).

(3) Analysis of other tissues

Analysis of other tissues as indicators of fruit tree nutrient status has also been proposed (Table 2).

Dormant shoot analysis of ‘Zee lady’ peach and ‘Grand pearl’ nectarine trees offers promise as a tool for the early fine-tuning of the fertility programs in orchards (Johnson et al., 2006). The analysis of dormant shoots sampled in January or February could determine the nutritional status of N, P, B, and Zn in the trees, and levels of P, B, and Zn related in part to the parameters of tree nutrition and productivity (Johnson et al., 2006).

Bark analysis allows the early prognosis of Fe chlorosis in peach trees, at least a month earlier than flower analysis; there were significant correlations between bark Fe concentration and leaf Fe concentration or SPAD values (Karagiannidis et al., 2008).

Xylem sap analysis has been used for many years to monitor the mineral uptake status of plants (Osonubi et al., 1988; Stark et al., 1985). Early-season xylem sap analysis of kiwifruit between budbreak and leafburst showed potential as a pre-season guide to Mn and B status, but not Zn deficiency (Clark et al., 1986). Raveh (2005) monitored the Cl concentration in leaves, fruit, stem-xylem sap, and roots and demonstrated that leaf Cl content, which is the traditional way of assessing citrus Cl status, indicated the current level of Cl toxicity in the trees, but xylem sap and root analysis better indicated the current Cl uptake status. The author concluded that the most useful tools for assessing potential Cl stress in citrus trees were the combination of xylem sap and leaf analyses (Raveh, 2005).

2) Ionomic analysis (simultaneous multi-element analysis)

Ionomics can simultaneously measure the elemental composition of organisms and their changes in response to physiological stimuli, developmental state, genetic changes, and environment (Salt et al., 2008; Singh et al., 2013). Ionomics requires the application of high-throughput elemental analysis technologies, such as inductively coupled plasma mass spectrometry (ICP-MS), quadrupole inductively coupled plasma mass spectrometry (ICP-QMS), or inductively coupled plasma optical emission spectroscopy (ICP-OES) (Table 3). It has the ability to detect changes in the ionome composition in relation to alterations in a plant’s physiology (Singh...
Table 3. Ionomic analysis (simultaneous multi-element analysis) studies of fruit trees in relation to the species, and physiological and environmental factors.

| Reference                        | Sample                                      | Purpose of analysis                                                                 | Element or oxide                  | Extraction                                                                 | Separation and detection          |
|----------------------------------|---------------------------------------------|-------------------------------------------------------------------------------------|-----------------------------------|----------------------------------------------------------------------------|-----------------------------------|
| Shibuya et al. (2015)            | Apple and Japanese pear (fruit and leaf)    | Differentiation between species and plant organs                                    | B, Na, Mg, Al, P, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Mo, Cd, and Cs (19 elements) | HNO₃                              | ICP-MS²                           |
| Geana et al. (2013)              | Wine and soil where the vines grow          | Geographical origin of wine                                                         | Be, V, Cr, Mn, Co, Ni, Cu, Zn, Rb, Sr, Ag, and Pb (12 elements) | HNO₃ (wine) H₂O₃, HF, and HCl (soil) | ICP-MS                           |
| Pepi et al. (2016)               | Grape berries (juice residue and solid residue) and soil where the vines grow | Geographical origin of fruit and the geochemical characterization according to soil management | Li, B, Na, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Rb, Sr, Y, Zr, Nb, Cd, Ba, Pb, La, Ce, Nd, Th, Na₂O, MgO, Al₂O₃, SiO₂, P₂O₅, K₂O, CaO, TiO₂, MnO, and Fe₂O₃ (30 elements and 10 oxides) | H₂O₂                                | ICP-MS (plant) X-ray fluorescence (soil) |
| Pepi et al. (2018)               | Grape (leaf) and soil where the vines grow  | Geographical origin of wine                                                         | Li, B, Na, Mg, Al, P, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Rb, Sr, Y, Zr, Nb, Cd, Ba, Sn, Te, Ba, Pb, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, U, Na₂O, MgO, Al₂O₃, SiO₂, P₂O₅, K₂O, CaO, TiO₂, MnO, and Fe₂O₃ (45 elements and 10 oxides) | HNO₃ (wine) and 37% H₂O₂ (plant)     | No extraction (soil) IC-P-MS (plant) X-ray fluorescence (soil) |
| Sofo et al. (2013)               | Grape (fruit)                               | Changes in fruit skin induced by irrigation water and light                        | Al, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Zr, Nb, Mo, Ru, Pd, Ag, Cd, In, Sn, Sb, Te, Cs, Ba, Hf, W, Re, Os, Ir, Pt, Hg, Ti, Pb, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Er, Sm, Yb, and Lu (59 elements) | HNO₃ and H₂O₂                       | ICP-QMS²                          |
| Lang et al. (2018)               | Apple (leaf, stem, and root)                | Effects of exogenous dopamine under drought stress                                 | B, N, Mg, Al, P, S, K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, As, Fe, Mo, Cd, and Pb (18 elements) | H₂SO₄ and HClO₄ (≥30%) for N, P, and K | No extraction for any elements (soil solution) IC-P-MS for the other 15 elements |
| Matsuoka et al. (2018)           | Blueberry (bush) and root-zone soil solution| Changes induced by soil type and soil treatments (fertilization or acidification)   | N, Na, Mg, Al, P, K, Ca, Mn, Fe, Ni, Cu, Zn, Rb, and Cs (13 elements) | HNO₃ for all elements except N (plant) | No extraction for N measured by CNS analyzer and H₂SO₄ for Kjeldahl N |
| Matsuoka et al. (2019)           | Apple (fruit and leaf) and soil where the trees grow | Changes induced by long-term application of N fertilizer                           | B, N, Mg, Al, K, Ca, Mn, Fe, Ni, Cu, and Zn (11 elements) | HNO₃ for all elements except N (plant) | ICP-MS for 10 elements (plant and soil) NC analyzer (plant) and colorimetric methods (soil) for N |
| Parent et al. (2013a)            | Lowbush blueberry, cloudberry, kiwifruit, guava, orange, mango, apple, and cranberry (leaf) | To present an approach based on a novel nutrient balance concept to classify plant ionomes | N, Mg, P, K, and Ca (5 elements) | HNO₃ and HClO₄ for all elements except N (plant) and CO₂ for all elements except N (plant) | ICP-OES², AA spectrophotometry, and a colorimetric method for Mg, P, K, and Ca |
| Parent et al. (2013b)            | Mango (leaf)                                | To present an unbiased statistical approach for plant nutrient diagnosis using the balance concept | B, N, Mg, P, S, K, Ca, Mn, Fe, Cu, and Zn (11 elements) | HNO₃ and HClO₄ for all elements except N H₂SO₄ for N | ICP-OES for 10 elements Micro-Kjeldahl digestion for N |

² Inductively coupled plasma mass spectrometry (ICP-MS).
³ Quadrupole inductively coupled plasma mass spectrometry (ICP-QMS).
⁴ Inductively coupled plasma optical emission spectroscopy (ICP-OES).
et al., 2013), gene function (functional genomics), and physiological status (Salt et al., 2008), the geographical origins of rice (Li et al., 2012; Qian et al., 2019) and of wine, honey, olive oil, coffee, cheese, fruits, vegetables, and spices (Danezis et al., 2016), as well as the effects of past cropping with an arbuscular mycorrhizal host plant and manure application on soybean seeds (Sha et al., 2012).

The ionomics of fruit trees, especially in relation to the species as well as to physiological and environmental factors, are summarized in Table 3. Several important studies have demonstrated its utility. Ionomic analysis has been used to compare element concentrations between species and between plant organs of horticultural crops, including apple and Japanese pear (e.g., Shibuya et al., 2015). Ionomic analysis has become important for determining the authenticity of a wine’s origin. The ionomic signature of Romanian wines depends on the geochemistry of the soil in which the grapevines are grown (Geana et al., 2013). Ionomic signatures classify grape berries by rare earth elements according to their geographical origin, because these elements are not greatly affected by agricultural practices or environmental conditions and depend very little on grapevine rootstocks (Pii et al., 2017). Nonetheless, a full 34-component ionomic signature of the grape berries did not fully allow the discrimination of their geographical origin, most likely owing to heterogeneity in the vineyards and the limited number of samples analyzed (Pii et al., 2017). In addition, geochemical characterization of both major and trace elements could be useful in developing fingerprints of vines according to soil management (cover cropping, soil tillage, or irrigation levels) and geographical origin (Pepi et al., 2016).

Ionomic composition in grape berries is also affected by water and light regimes (Table 3). Differences in irrigation management and in exposure of berries to light result in quantitative changes in metals and metalloids in the skin, one of the most important mineral sinks of developing fingerprints of vines according to soil management (Pii et al., 2017). Ionomic composition in grape berries is also affected by water and light regimes (Table 3). Differences in irrigation management and in exposure of berries to light result in quantitative changes in metals and metalloids in the skin, one of the most important mineral sinks of developing fingerprints of vines according to soil management (Pii et al., 2017). Ionomic composition in grape berries is also affected by water and light regimes (Table 3). Differences in irrigation management and in exposure of berries to light result in quantitative changes in metals and metalloids in the skin, one of the most important mineral sinks of developing fingerprints of vines according to soil management (Pii et al., 2017).

The use of ionomic analysis in nutritional studies and nutritional diagnosis of fruit trees is also still limited, but there are several reports that demonstrate its utility (Table 3). Ionomes of 13 elements in blueberry bushes and the root-zone soil solution were influenced by soil type and soil treatments (N and K fertilization and acidification); N, P, K, Mn, Cu, and Zn were significantly positively correlated in terms of the concentrations in the soil solution and the content in the blueberry bushes across all soil types and soil treatments, but those of Na, Mg, Al, Ca, Fe, Rb, and Cs were not (Matsuoka et al., 2018). Long-term application of N fertilizer also affected the ionomic signature of the fruits and leaves of ‘Jonathan’ apple trees; it altered tree nutrition with not only N, but also K and Mn, owing to the fertilizer-induced changes in nutrient availability in the subsoil (Matsuoka et al., 2019). Parent et al. (2013a) proposed a novel approach to the nutrient balance concept that is based on growth-limiting nutrient concentrations, supported by the “Law of minimum” illustrated by Liebig’s barrel, and classified wild or domesticated species by using leaf ionomes of fruit species’ data sets. The authors further analyzed leaf ionomes composed of 11 elements (B, N, Mg, P, S, K, Ca, Mn, Fe, Cu, and Zn) in mango and could classify the orchard’s productivity; however, the [P|N,S] and [Mn|Cu,Zn] leaf balances appeared to limit mango yields in Brazil (Parent et al., 2013b). In further studies, the use of ionomics and data analysis could be developed to diagnose fruit tree nutrition.

4. Conclusions and Perspectives

This review covers two main issues that need to be resolved in the future (Fig. 1). Firstly, analyses of fruit tree tissues early in the growing season could overcome the limitations of conventional leaf mineral analysis in which samples are collected during early to midsummer; early analysis would allow sufficient time before harvest to correct nutritional disorders in the current growing season. However, further research is needed to determine the appropriate timing of sampling and tissues suitable for accurate analysis. Secondly, the growth and productivity of fruit trees are often considered to be affected by multiple elements, not just one. Therefore, the ionomic approach is useful to reveal the role of different nutrients in fruit tree nutritional disorders, how changes in ionome composition (multi-elemental composition) respond to a given agricultural practice and climate conditions, and how ionomic signatures could be used to determine better cultivation conditions for higher yield and fruit quality. By combining fruit tree tissues collected early in the growing season, the ionomic approach could become a useful tool for the early diagnosis of fruit tree nutritional disorders (Fig. 1).

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