Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Foliar nutrient imbalance and its association with european apple canker incidence

Leandro Hahn

leandrohahn@epagri.sc.gov.br

Santa Catarina Agricultural Research and Rural Extension Company (Epagri)

Thyana Lays Brancher

Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina

Cláudio Ogoshi

Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina

Léon-Étienne Parent

Université Laval

Caetano Albuquerque

California State University

Gustavo Brunetto

University of Santa Maria (UFSM)

Research Article

Keywords: Neonectria ditissima, canker lesion, tissue nutrient composition, centered log ratio (clr), weighted log ratio (wlr), nutrient balance

Posted Date: October 8th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11036353/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Foliar nutrient imbalance and its association with european apple canker incidence

Leandro Hahn[a]*, Thyana Lays Brancher[b], Cláudio Ogoshi[b], Léon-Étienne Parent[c], Caetano Albuquerque[d], Gustavo Brunetto[e]

*corresponding author. Email: leandrohahn@epagri.sc.gov.br

[a]Urussanga Research Center, Santa Catarina Agricultural Research and Rural Extension Company (Epagri), SC-446, n. 6, Postcode 88840-000, Urussanga, Santa Catarina, Brazil; [b]Caçador Research Center, Santa Catarina Agricultural Research and Rural Extension Company (Epagri); [c]Department of Soils and Agro-Food Engineering, Université Laval, Québec, Canada; [d]Department of Biology and Chemistry, California State University, Monterey Bay, Seaside, California; [e]Soil Science Department of Federal University of Santa Maria (UFSM), Santa Maria 97105-900, Brazil.

The study aimed to relate foliar tissue nutrients to the incidence of canker in two orchards of southern Brazil. The incidence of European canker was evaluated on 282 trees across two commercial orchards located in the municipality of Água Doce, Santa Catarina state, southern Brazil. Foliar tissue and soil samples were collected to determine nutrient composition. Plant tissue nutrients were expressed using three distinct mathematical approaches: raw concentrations, centered log ratios (*clr*), and weighted log ratios (*wlr*). Dual log ratios were weighted by gain ratios using machine learning algorithms to assess their relative importance. The *wlr* expression showed more significant differences in nutrient levels than concentration or clr expressions. The infected trees indicated higher *wlr* levels of Mn ($p < 0.10$), Cu ($p < 0.001$) and C-H-O ($p < 0.05$) and less Zn ($p < 0.01$) compared to healthy trees. Differences in Mn, Cu and Zn were not significant using the *clr* expression, supporting the need to consider the importance of dual ratios regarding the incidence of canker. In conclusion, weighted log-ratio analysis provides a refined paradigm for plant nutrition diagnosis, demonstrating that enhancing apple tree resilience against European canker relies on the precise management of interdependent foliar nutrient balances rather than independent elemental concentrations.

Keywords: *Neonectria ditissima;* canker lesion; tissue nutrient composition; centered log ratio (*clr*); weighted log ratio (*wlr*); nutrient balance.

Introduction

European apple canker is a highly destructive fungal disease caused by the wood pathogen *Neonectria ditissima*. The infection appears on trunks, branches, twigs and fruits and can lead to death of organs including fruit rot. The incidence of canker has been related to mild and humid climatic conditions, soil conditions, actively growing trees (< 5 years old) and the genetic background of the apple cultivar (Weber & Børve, 2021; Xu et al., 2022; Karlstrom, 2024).

Treatments against apple canker include pruning of infected branches, fungicide sprays (Weber & Børve, 2021), antagonistic microbes (Bozorov et al., 2024) and uprooting the tree in extreme scenarios (Weber & Børve, 2021). Cultural practices can partially mitigate the disease spread caused by European Canker, and while varieties with increased resistance would be the most effective solution to limit disease spread, none are currently available (Karlstrom, 2024).

A promising solution to alleviate the impact of European Canker in orchards is to have adequately fertilized trees to improve their immune system, thus enhancing resistance to biotic factors (Flors et al., 2024). Indeed, most essential elements such as N, K, P, Mn, Zn, B, Cl and Si are involved in plant resistance to pests (Chaboussou, 2004; Dordas, 2008). Plant immunity could thus be increased through appropriate fertilization (Chaboussou, 2004). Nitrogen fertilization doses above official recommendations in apple orchards increases plant susceptibility to canker due to vigorous vegetative growth and high tissue N content (Weber and Børve, 2021), and excessive tissue N levels weakens plant structural and physiological defense mechanisms against pests (Martinez et al., 2021; Harteveld et al., 2022). However, severity of canker was also found to be inversely related to leaf K fertilization and tissue concentration (Peng et al., 2016).

The interactive nutrient balance between essential elements can be diagnosed using tools of Compositional Data Analysis (Parent & Dafir, 1992) and machine learning methods (Yamane et al., 2022). Treating nutrients as independent variables may compromise the accurate interpretation of plant nutritional status (Baxter, 2009). While recent studies have analyzed individual nutrient thresholds or traditional compositional data analysis (*clr*) in plant pathology, they assign equal weight to all nutrient pairs regardless of their diagnostic relevance. This study advances the field by integrating machine learning-derived gain ratios to weight dual log-ratios (*wlr*), isolating key nutri-

tional interactions associated with *Neonectria ditissima* susceptibility. We hypothesized that the incidence of apple canker was related to nutrients in leaf tissue in two orchards of southern Brazil.

Material and Methods

Experimental design

The two commercial apple (*Malus domestica*) orchards used in this experiment were located in the municipality of Água Doce (Figure 1), in the central Santa Catarina state, Brazil (26.759232 S and 51.32800W for Orchard-1 and 26.758571 S and 51.543452 W for Orchard-2) were evaluated for the incidence of canker. There were alternating rows of varieties 'Maxi Gala' and 'Fuji Suprema' grafted on 'Marubakaido' with 'M9' as inter-stock. There were 21 canker-free trees in total. The trees showing canker lesions to various degrees represented 92.6% of the population.

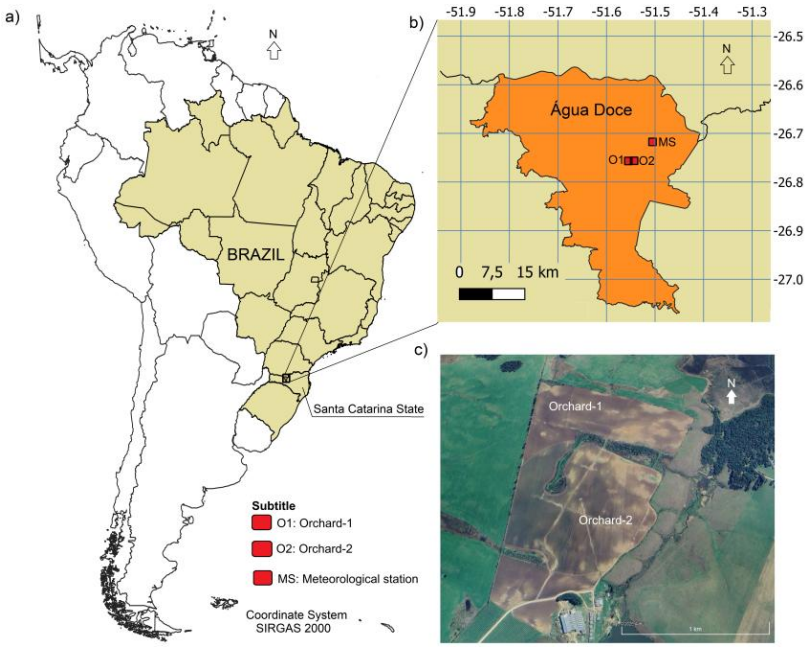


Figure 1. The geographic location of the Água Doce municipality (b), Santa Catarina state, south of Brazil (a), where the two orchards (c) and the meteorological station are located. Map created by QGis software version 3.34.3.

The orchards were established in 2010 at a density of 2500 trees ha^{-1}, spaced 1 m on the row and 4 m between rows. The trees were trained with a central leader (tall spindle). There was no N and P granular fertilization nor foliar fertilization during the whole survey. The K was surface-applied under the canopy as potassium chloride at a rate of 40 kg K ha^{-1}. The pest management program followed guidelines of the Integrated Production for Apples using fungicides (mancozeb, difeconazol, captan, fluazinam, fluxapyroxade, pyraclostrobina, mainly) acaricides (abamectin,mainly) and insecticides (phosmet, acetamiprid, spinetoram, etofenprox, fenitrotion, lufenuron, methoxyfenozide, novaluron and malathion, mainly) (Sezerino, 2018).

Sixty georeferenced points were collected in 2018 and 51 in 2019, each point consisting of two trees. Only rows of 'Maxi Gala' grafted on 'Marubakaido' with 'M9' as inter-stock were evaluated by sampling two plants every 13 plants to determine the number of lesions, sample leaves for mineral analysis and collect soil.

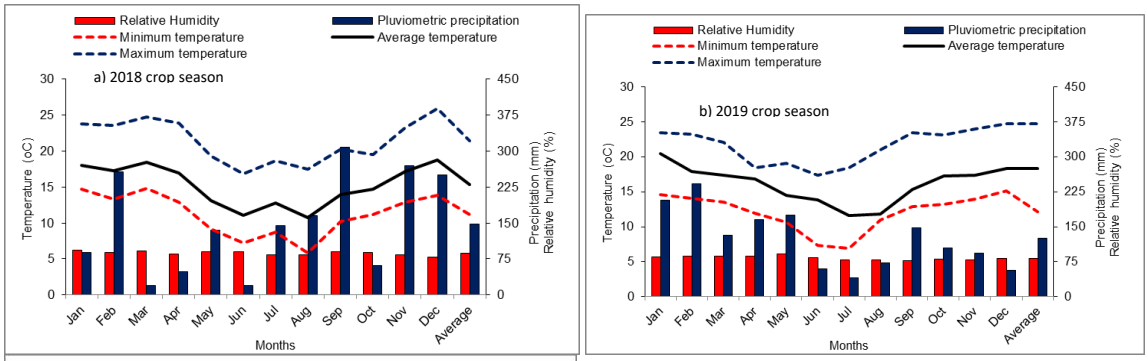
The incidence of European canker was evaluated on two trees as number of lesions per tree. The assessments were carried out during the last week of January, corresponding to the pre-harvest period in the Southern Hemisphere, when apple trees were at the fruit development stage (BBCH 79). After each production cycle, all branches showing canker (at least one lesion) were pruned during the months of July and August then removed from the orchard.

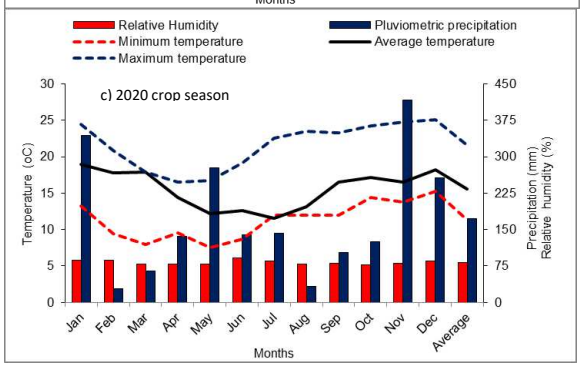
In the first orchard, the incidence of canker was evaluated in 2018, 2019 and 2020 and related to soil and tissue nutritional status characterized in 2018. In the second orchard, canker was evaluated in 2019 and 2020 and related to soil and tissue analyses made in 2019. This timeline accounts for the chronic nature of *Neonectria ditissima* infections, where the baseline nutritional balance establishes a long-term physiological

predisposition to wood pathogen colonization, combined with a prolonged incubation period before full symptomatic expression in subsequent seasons (Weber & Børve, 2021). Additionally, orchard management and fertilization practices remained consistent over the monitoring period, maintaining the baseline nutritional trends stable across seasons. Hence, a total of 111 individual trees were monitored (60 trees in the first orchard and 51 trees in the second orchard). Over the multi-year study period, this resulted in 60 × 3 longitudinal evaluations in the first orchard and 51 × 2 longitudinal evaluations in the second orchard, compiling a total dataset of 282 time-series observations. From 2018 to 2019 and 2019 to 2020, the number of injuries varied, generally decreasing due to earlier pruning.

Soil and climate

The soil was classified as a Typic Hapludalf (Soil Survey Staff, 2014). The soil was well drained and showed no problem of waterlogging. Slope inclination was < 5% with an east-west orientation. The regional climate was classified as humid subtropical (Cfb) (Alvarez et al. 2013), characterized by mild temperatures and rainfall with variation throughout the year. Monthly climate conditions are presented in Figure 2 and were obtained daily from the EPAGRI meteorological station closest 6 km to the orchards (26.717417 S and 51.505317 W). The rainiest year was 2020 followed by 2018. The year 2018 showed the highest air humidity. The year 2019 was the driest.

126 

127 

128 Figure 2. Monthly climatic conditions during the apple crop cycle in the experimental
129 area in 2018 (a), 2019 (b) and 2020 (c) crop seasons, in southern Brazil.

130

131 ***Soil and tissue analysis***

132 Soil and leaves were collected during the last week of January (CQFS-RS/SC,
133 2016). Twenty-five fully expanded healthy leaves per tree were sampled for a total of 50
134 leaves per two trees, taken from the middle third of healthy, active current-year growing
135 shoots (free of visible canker symptoms). Tissue and soil analyses were performed ac-
136 cording to Tedesco et al. (1995). The leaves were gently cleaned with distilled water and
137 oven-dried at 65 °C until constant weight. Leaves were ground in a Wiley mill, sieved <
138 0.05 mm, and stored in a paper bag.

139 Eight soil samples were taken in the 0–20 cm soil layer within the canopy projec-
140 tion and composited. Soil analysis was performed according to Tedesco et al. (1995).
141 This sampling depth (0–20 cm) aligns with the official regional guidelines for orchard
142 nutrient management in Southern Brazil (CQFS-RS/SC, 2016), capturing the zone with

the highest density of active nutrient-absorbing roots and where lime and fertilizer applications are highly concentrated. The nutrient extraction method was Mehlich1. Soil pH was measured in water (1:1 volume ratio). Clay content was measured by sedimentation. Soil organic matter was determined using the Walkley-Black procedure. Phosphorus was quantified by colorimetry. Exchangeable acidity was assessed from the SMP buffer pH and cations were quantified by atomic absorption spectrophotometry (Tedesco et al. (1995). Cation exchange capacity (CEC) was computed as the sum of exchangeable cations (Ca, Mg, K) and the SMP-exchangeable acidity on a molar basis. Base saturation was computed as the sum of exchangeable cations (Ca, Mg, K) divided by CEC. The descriptive statistics for canker indices, along with soil and tissue nutritional attributes, are summarized in Tables 1 and 2.

Statistical analysis

Foliar nutrient data are compositional by nature, meaning that the elements are parts of a whole and their concentrations are strictly interdependent; an increase in one nutrient naturally forces a mathematical decrease in the percentage of others due to the closure capacity of the tissue (100% or 1000 g kg^{-1}). To resolve this redundancy and eliminate the bias of scale, raw concentrations were transformed into log-ratios. The centered log-ratio (*clr*) transformation achieves this by rationing each nutrient concentration against the geometric mean of all elements in the sample, shifting the data from a restricted space to an open, continuous scale suitable for standard statistical analysis. Subsequently, the weighted log-ratio (*wlr*) approach refines this by prioritizing specific nutrient pairs based on their predictive power and relevance to the target condition (European canker incidence), avoiding the equal-weight assumption of traditional *clr* transformations.

Centered log ratio (clr) data transformation

Dual ratios are thought to reflect interactions between nutrients (Sumner, 1978; Wilkinson et al., 2000). There are several know dual interactions (Sumner, 1978; Wilkinson et al., 2000; Courbet et al., 2019) and several undocumented ones in the literature. The dual log ratios were integrated into centered log ratio (*clr*) variables computed as follows (Parent & Dafir, 1992):

$$clr_i = ln(x_i/g[x] = ln(x_i) - g[x])$$

Where $g[x]$ is the geometric mean across D components including x_i nutrients and the filling value x_D to close the simplex to the unit of measurement, and

$$g(x) = (x_1 x_2 \dots x_D)^{\frac{1}{D}}$$

The clr variable is the average of the $D \times (D-1)$ dual ratios computable from a D-parts composition, as follows (Greenacre et al., 2023):

$$ln\left(\frac{x_i}{g(x)}\right) = \frac{1}{D}\left[ln\left(\frac{x_i}{N}\right) + ln\left(\frac{x_i}{P}\right) + ln\left(\frac{x_i}{K}\right) + ln\left(\frac{x_i}{Ca}\right) + ln\left(\frac{x_i}{Mg}\right) + ln\left(\frac{x_i}{S}\right) + ln\left(\frac{x_i}{B}\right) + ln\left(\frac{x_i}{Cu}\right) + ln\left(\frac{x_i}{Zn}\right) + ln\left(\frac{x_i}{Mn}\right) + ln\left(\frac{x_i}{Fe}\right) + \dots + ln\left(\frac{x_i}{x_D}\right)\right]$$

Where x_i is the i^{th} quantified concentration and xD is the filling value between measurement unit and the sum of quantified elements. The *clr* values are distributed in the real space required to run statistical analyses (Aitchison, 1986).

The *clr* differences between two equal-length compositional vectors at local scale return nutrient indices as follows:

$$I_{x_i} = clr_{x_i} - clr_{x_i}^*$$

Where clr_{x_i} is the *clr* value of nutrient x_i for an unhealthy tree, and $clr_{x_i}^*$ is the *clr* value for a healthy tree of healthy specimens taken as reference.

Nutrient indices used for diagnostic purposes are computed as follows (Parent & Dafir, 1992):

$$I_{x_i} = \frac{\left(clr_{x_i} - clr^*_{x_i}\right)}{SD^*_{x_i}}$$

Where $SD^*_{x_i}$ is the standard deviation of the mean $clr^*_{x_i}$ value taken as reference. The *clr* index is thus distributed like a t-value.

Weighted log ratio (wlr)

While the *clr* expression assigns an equal weight ($\frac{1}{D}$) to all dual log ratios, the importance of each dual log ratio were reported to vary with the species, growing conditions and the growth stage (Kenworthy, 1967). Dual log ratios can be weighted using coefficients computed as gain ratios to measure of their importance (Parent, 2025; Srivastava et al., 2025). A high gain ratio means that the dual log ratio is important for canker as documented in the database.

The weighted log ratio (*wlr*) is a *clr* expression whereby each dual log ratio is weighted by the corresponding gain ratio φ_j regarding the target variable, as follows:

$$wlr_{x_i} = \frac{1}{D}\sum_{j=1}^{D} \varphi_j \, ln\left(\frac{x_i}{x_j}\right), i \neq j$$

Where x_i represents nutrient *I*, x_j refers to other elements, and φ_j is the gain ratio. The gain ratio was obtained using the widget "Rank" in Orange Data Mining vs. 3.37 (Lubljana, Slovenia), trees with or without lesion categorial target variable and the 55 log ratios as features. This allows assigning a coefficient of importance to every dual log ratio. If all $\varphi_j = 1$, $wlr_{x_i} = clr_{x_i}$. Computing *wlr*, x_i is maintained at numerator as of DRIS indices (Walworth & Sumner, 1987). If x_i is at denominator in the dual log ratio, it is moved at numerator by multiplying the dual log ratio by -1, i.e., $ln\left(\frac{x_j}{x_i}\right) = -ln\left(\frac{x_i}{x_j}\right)$.

Nutrient levels of healthy and canker-infected trees were compared using t-tests with unequal variance. Although this binary classification collapses the quantitative gradient of canker counts per tree, it establishes a robust diagnostic threshold focused strictly on disease onset and initial physiological predisposition. Categorizing trees based on presence or absence of symptoms is a standard requirement for calibrating compositional nutrient diagnosis norms, ensuring a clear operational baseline to distinguish balanced (healthy) from imbalanced (infected) nutritional profiles (Parent & Dafir, 1992). To diagnose a composition, the *wlr* nutrient index of component x_i was computed as follows:

$$I_{x_i} = \frac{\left(wlr_{x_i} - wlr^*_{x_i}\right)}{sd^*_{x_i}}$$

Where $sd^*_{x_i}$ is the standard deviation about the mean $wlr^*_{x_i}$ value of healthy specimens taken as reference.

Results

Characterization of orchard fertility, tissue nutrients, and canker baseline

The comprehensive baseline database detailing soil chemical attributes (such as pH, organic matter, and available macro- and micronutrients) and cumulative canker incidence across the monitored orchards is fully presented in Table 1 and 2. Soil nutrient levels reflected typical regional variations, establishing the chemical background used to compute the directional relationships with plant tissue composition.

Table 1. Variation in canker indices and soil variables in the apple canker database of southern Brazil.

Variable	Orchard-1			Orchard-2			Differences
	Min.	Median	Max.	Min.	Median	Max.	t-test

pH_{water}	4.7	5.7	7.2	4.9	5.6	6.4	ns
			%				
Clay	31	40.5	53	28	35	43	*
Organic matter	3.1	6.7	7.5	2.9	3.5	8.3	*
Base saturation	48.6	79.3	93.9	48.0	78.1	91.5	ns
			$cmol_c\ dm^{-3}$				
Ca test	7.0	13.2	15.9	42.3	65.4	77.4	*
Mg test	1.0	1.7	5.7	4.4	9.2	19.8	ns
$H^{+}+Al^{3+}$ test	1.3	4.2	9.7	1.5	4.0	11.4	ns
Cation exchange capacity	16.0	19.7	23.1	14.6	18.3	24.3	*
			$mg\ dm^{-3}$				
P test	4.7	39.3	98.3	9.7	26.9	215	ns
K test	108.0	294.0	428.0	52.0	208.0	312.0	*
Zn test	6.5	14.9	20.6	6.5	14.9	18.5	ns
Cu test	1.4	3	4.7	1.4	2.9	4.7	ns
Mn test	2.6	9.4	27.4	2.6	10.2	27.4	*
Fe test	20.1	40.9	85.4	20.1	39.7	83.5	ns

Significant differences between Orchard-1 and Orchard-2 according to Student's t-test ($p < 0.05$) are indicated with an asterisk (*). Non-significant differences are indicated as "ns".

Table 2. Descriptive statistics of foliar nutritional attributes and European canker indices across the monitored apple orchards.

Variable	Orchard-1			Orchard-2			Differences
	Min.	Median	Max.	Min.	Median	Max.	t-test
			Canker index				
2018	0	0.9	10.5	-	-	-	NA
2019	0	4.3	19.5	1.0	8.0	25.0	*
2020	0	4.0	9.0	3.0	7.0	15.5	*
			Tissue analysis				
			$g\ kg^{-1}$				
N	17.0	21.0	28.0	11.0	16.0	25.0	*
P	1.3	2.0	5.9	1.6	2.2	4.9	ns
K	14.0	21.0	26.0	16.0	23.0	30.0	*

Ca	8.0	16.0	23.0	8.3	11.2	18.3	*
Mg	1.8	2.8	4.5	1.9	2.9	4.2	ns
Fe	22.3	53.3	91.6	7.2	25.4	50.2	*
Mn	81.1	184.4	345.9	102.6	160.2	289.4	*
Zn	174.6	348.9	2502.0	16.9	31.6	51.6	*
Cu	1.3	2.6	5.9	5.3	8.0	9.8	*
B	37.5	51.5	86.3	16.6	28.3	50.7	*

Significant differences between Orchard-1 and Orchard-2 according to Student's t-test ($p < 0.05$) are indicated with an asterisk (*). Non-significant differences are indicated as "ns". "NA" means "not applicable", used when the statistical test could not be performed due to insufficient data.

Gain ratios

The gain ratios varied from 0.001 to 0.022 (Table 3). This wide range demonstrates that nutritional relationships do not affect European canker development uniformly, highlighting that specific nutrient balances exert a much stronger regulatory effect on the tree's defensive framework than others. This variation indicates that the differential importance of each dual log-ratio regarding the incidence of canker must be considered in the diagnostic model. Consequently, the gain ratios were applied as weighting coefficients to their corresponding dual log-ratios to compute weighted log-ratio (*wlr*) variables, mathematically prioritizing the key physiological imbalances while filtering out non-influential background nutritional noise. The *wlr* expression removed the distortions inherent to raw concentration values and traditional *clr* variables, hence providing a more accurate and biologically meaningful assessment of nutrient imbalance in canker-infected trees.

Table 3. Diagnostic importance (gain ratio weights) of specific dual nutrient log-ratios in differentiating healthy from European canker-infected apple trees

Log	Gain	Log	Gain	Log	Gain	Log	Gain	Log	Gain

ratio	ratio	ratio	ratio	ratio	ratio	ratio	ratio	ratio	ratio
N/P	0.009	P/Ca	0.005	K/Mn	0.008	Ca/xD	0.002	Fe/xD	0.011
N/K	0.011	P/Ma	0.008	K/Zn	0.018	Mg/Fe	0.010	Mn/Zn	0.017
N/Ca	0.003	P/Fe	0.009	K/Cu	0.016	Mg/Mn	0.003	Mn/Cu	0.011
N/Mg	0.009	P/Mn	0.003	K/B	0.018	Mg/Zn	0.017	Mn/B	0.004
N/Fe	0.006	P/Zn	0.019	K/xD	0.011	Mg/Cu	0.016	Mn/xD	0.003
N/Mn	0.004	P/Cu	0.012	Ca/Mg	0.005	Mg/B	0.012	Zn/Cu	0.017
N/Zn	0.017	P/B	0.014	Ca/Fe	0.005	Mg/xD	0.001	Zn/B	0.022
N/Cu	0.016	P/xD	0.005	Ca/Mn	0.002	Fe/Mn	0.005	Zn/xD	0.017
N/B	0.005	K/Ca	0.002	Ca/Zn	0.017	Fe/Zn	0.016	Cu/B	0.017
N/xD	0.022	K/Mg	0.001	Ca/Cu	0.018	Fe/Cu	0.022	Cu/xD	0.016
P/K	0.004	K/Fe	0.012	Ca/B	0.004	Fe/B	0.005	B/xD	0.016

xD = filling value between measurement unit and the sum of quantified elements

Nutrient status of healthy and infected trees

Statistics of nutrient levels in the 21 trees that remained completely free of canker lesions across all multi-year assessments are presented in Table 4. There were five significant differences between the mean concentrations of healthy and infected specimens. In comparison, there were eight significant differences using the *clr* expression and nine significant differences and one trend ($p \leq 0.10$) using the *wlr* expression.

Comparing raw concentrations revealed that infected trees exhibited significantly lower levels of N (10.7% decrease; $p < 0.001$), Fe (24.6% decrease; $p < 0.01$), and B (12.0% decrease; $p < 0.001$), along with a 66.7% increase in Cu ($p < 0.001$) relative to healthy controls (Table 4). However, raw concentrations failed to capture critical micronutrient interactions. The *wlr* diagnosis successfully resolved these balances, highlighting clear statistically significant deviations in infected trees: Copper (Cu) showed the most severe excess in infected trees, with a positive *wlr* index nearly 5.0-fold higher

than the healthy baseline ($p < 0.001$). Zinc (Zn) exhibited the most critical deficit, dropping to a negative *wlr* index 2.3-fold lower than healthy trees ($p < 0.001$). Manganese (Mn) displayed a significant accumulation trend, reaching a positive *wlr* index 1.8-fold greater than healthy specimens ($p < 0.001$). Filling value (xD) increased significantly in infected plants, yielding a *wlr* index 2.7-fold higher than controls ($p < 0.001$), indicating a relative accumulation of unquantified structural components (C, H, O). Unlike raw concentrations or *clr* expressions—which showed non-significant differences for Mn ($p > 0.10$)—the *wlr* method specifically isolated Cu, Zn and Mn imbalances as the primary nutritional drivers associated with canker occurrence.

Table 4. Statistics of nutrient compositions (Mean±SD) of the 21 healthy trees and 261 infected trees.

Element	Concentration		*clr*		*wlr*	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
	---------- g kg^{-1} -------		---------- unitless ----------			
N	21.5±2.3	19.2***±3.3	2.776±0.135	2.780ns±0.131	0.0024±0.00027	0.0024ns±0.00022
P	2.2±1.0	2.3ns±0.8	0.437±0.342	0.638*±0.359	0.0021±0.000359	0.0026***±0.00064
K	21.1±1.9	21.3ns±3.2	2.756±0.115	2.890***±0.262	0.0062±0.00031	0.0066***±0.00059
Ca	16.0±3.9	14.6ns±3.5	2.458±0.246	2.495ns±0.177	0.0041±0.00023	0.0043***±0.00021
Mg	2.9±0.6	2.9ns±0.5	0.767±0.212	0.879*±0.260	0.0032±0.00020	0.0034***±0.00043
Fe	0.057±0.019	0.043**±0.021	-3.213±0.330	-3.450**±0.467	-0.0024±0.00039	-0.0026*±0.00072
Mn	0.198±.061	0.187ns±0.055	-1.952±0.311	-1.876ns±0.256	-0.0003±0.00019	0.00006***±0.00032
Zn	0.457±0.357	0.320ns±0.416	-1.228±0.500	-2.051***±1.195	-0.0021±0.0020	-0.0068***±0.00399
Cu	0.003±0.001	0.005***±0.003	-6.073±0.342	-5.649***±0.719	-0.0176±0.00047	-0.0153***±0.00196

B	0.050±0.006	0.044***±0.013	-3.281±0.120	-3.342*±0.219	-0.0063±0.00040	-0.0062†±0.00039
xD	935.5±2.9	939.0***±5.7	6.554±0.060	6.685***±0.180	0.0109±0.00022	0.01150***±0.00065

ns, †, *, **, ***: difference between healthy and infected specimens non-significant and significant at the 0.10, 0.05, 0.01, and 0.001 levels, respectively. *Clr*: centered log ratios; *wlr:* weighted log ratios.

Nutrient indices were computed only using the *clr* and *wlr* expressions that are additive as compositional variables (Figure 3). The *clr* expression (Figure 3a) showed a single significant index, xD, that indicated an accumulation of C, H, O and other unquantified elements relative to quantified elements in the infected specimens. Hence, there was no clear indication of nutrient imbalance in infected specimens. In contrast, the *wlr* (Figure 3b) expression provided evidence that Cu, xD and, to a lesser extent, Mn were in relative excess and that Zn was in relative shortage in the average infected specimens.

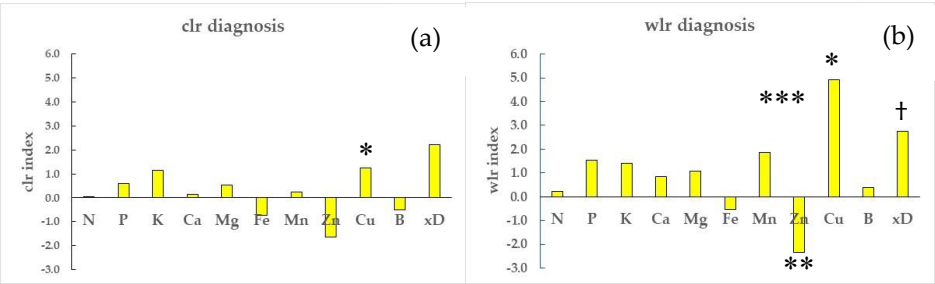


Figure 3. Centered log ratios (*Clr*) (a) and weighted log ratios (*wlr)* (b) diagnosis of the mean values for the infected specimens using the mean and standard deviation of healthy specimens. †, *, **, ***: significant at the 0.10, 0.05, 0.01 and 0.001 levels, respectively.

Discussion

This study demonstrated that evaluating nutrients as an interdependent compositional system, rather than as independent variables, provides a highly accurate diagnostic framework for European apple canker susceptibility. Our model successfully isolated specific multi-elemental imbalances that predispose apple trees to *Neonectria ditissima* infection, leveraging the gain ratio as a biological weight to identify key diagnostic pairs. Furthermore, the establishment of stable nutritional norms from trees that remained completely symptom-free over multiple seasons confirms that orchard longevity and immunity are tightly linked to homeostatic nutrient ratios, particularly involving structural and metabolic balancing elements.

Nutrient diagnosis

Several tissue nutrients, especially K, showed some connection with the incidence of canker (Peng et al. (2016). The critical leaf K concentration was reported to be 10 g K kg^{-1} at high apple yield in Canada (Parent & Granger, 1989) compared with 13 g K kg^{-1} to resist apple canker in China (Peng et al., 2016). Excessive K levels impact other key nutrients (Parent & Granger, 1989; Peng et al., 2016; Kuzin & Solovchenko, 2021) including those involved in plant resistance to diseases like N, P, Ca, Mn, Zn, B, Cl and Si (Dordas, 2008; Martinez et al., 2021). The Si plays a central role in pest and disease control (Deshmukh et al., 2017; Coskun et al., 2018; Frew et al., 2018).

Plant availability of Al, Fe and Si increases in acid soils (McKeague & Cline, 1963; Schaller et al., 2025; Zheng et al., 2021). The Al can be toxic at pH < 5.5 (Rout et al., 2001; Vardar & Unal, 2007), potentially weakening plant defense mechanisms. In the two orchards, 37% of soil analyses showed pH values less than 5.5. The Al^{3+} can be detoxified in the vacuoles of root cells depending on plant species (Alarcón-Poblete et al., 2018). However, tissue Al, S and Si as well as soil Si and S were not analyzed. Plant

defense against pests requires balanced nutrition (Dordas, 2008). Diatomaceous earth (Sandhya et al., 2018) and the alkaline silicate slag (Savant et al., 1999), Ca silicate (Paim et al., 2003), and potassium silicate (USDA, 2003; USEPA, 2007) can supply Si. Gypsum can tackle Al and supply S (Sumner, 1993).

Results of the *wlr* diagnosis (Figure 3b) showed that Mn, Cu, and Zn levels differed in the leaves of healthy and canker-infected trees. This divergence occurs because *Neonectria ditissima* targets the vascular system of the host, where wood necrosis physically restricts xylem and phloem transport, disrupting the upward translocation of essential micronutrients to the foliage. Additionally, the localized modulation of Mn, Cu, and Zn reflects the host's activation of enzymatic defense mechanisms, as these microelements are essential cofactors for superoxide dismutase (SOD) and are heavily recruited during oxidative stress responses, lignification, and compartmentalization processes at infection sites (Tadayon & Sadeghi, 2023; Mertoğlu et al., 2024). Apple orchards of the 'Golden Delicious' cultivar exhibiting a high fire blight (*Erwinia amylovora* Burr.) severity index were also characterized by reduced leaf Zn and elevated leaf Mn concentrations, consistent with the findings observed in our study (Tadayon & Sadeghi, 2023). Pathogens can affect nutrient uptake, utilization, and translocation, ultimately impacting the relationship between leaf nutrient balances and the incidence of canker. This can occur through various mechanisms, including direct consumption of plant nutrients, interference with nutrient transport, and modification of plant metabolism (Huber et al., 2012). To make adequate fertilization decisions to mitigate the incidence of canker using foliar diagnosis, plant nutrition and pest control should be disentangled.

Methodological limitations and future perspectives

There were only 21 healthy specimens among 282 observations in the database. The database should be expanded to include more variables and more observations. An infected specimen can also be compared to a healthy one under otherwise comparable conditions (Munson & Nelson, 1990) by difference between two equal-length compositions to compute the Euclidean distance separating them and display the *clr* (Aitchison, 1986) or *wlr* (Parent, 2025) differences on a histogram.

While the compositional weight-based model (*wlr*) successfully identified key nutritional balances associated with European canker, certain limitations in our current database must be addressed to enhance its predictive robustness. First, our soil and tissue nutritional baseline was captured during a single specific phenological window (late January, pre-harvest). Because nutrient translocation and *Neonectria ditissima* infection dynamics fluctuate throughout the season—particularly during high-risk windows like leaf fall and pruning—incorporating multi-temporal sampling is critical to capture seasonal ionic shifts. Second, our model currently relies on a binary classification (healthy vs. infected), which collapses the quantitative gradient of disease severity (canker counts per tree). Expanding the database to include continuous disease severity scales will allow machine learning algorithms to transition from basic susceptibility screening to predicting aggressive symptom progression. Lastly, incorporating multi-year climate data and a wider range of commercial cultivars will prevent regional overfitting, ensuring these compositional norms can be universally applied as reliable diagnostic tools in diverse apple-producing regions.

In parallel with expanding environmental and genetic variables, future database updates must also broaden the chemical spectrum of the ionome; specifically, tissue Al, S, and Si should be quantified to measure elemental balances that are currently overlooked

but mechanically linked to soil toxicity mitigation, metabolic defense, and cell wall reinforcement. Soil S and Si availability should be determined and calibrated using the Mehlich-1 method (Vergutz et al., 2015) or other acidic extraction methods (Snyder, 2001; Wang et al., 2009). However, the solubility of monosilicic acid in soils is reduced as the amount and crystallinity of Al oxides diminishes (Jones & Handreck, 1963). Addressing more predictors will require more observations, hence a much larger and diversified database to document numerous site-specific features to increase ML model's capacity to generalize.

Conclusion

This study confirms the hypothesis that European apple canker incidence is directly associated with leaf tissue nutrient imbalances when evaluated as an interdependent compositional system. By replacing unweighted diagnostic tools with machine learning-weighted log-ratios (*wlr*), we demonstrated that disease susceptibility is driven by specific microelemental disruptions rather than individual nutrient deficiencies.

Mechanistically, the marked surplus of foliar Cu and Mn paired with a severe deficit in Zn in infected trees reflects both vascular disruption caused by *Neonectria ditissima* wood necrosis and the intense recruitment of microelemental cofactors (such as superoxide dismutase) for oxidative stress defense and tissue lignification. Furthermore, the relative accumulation of the structural filling fraction (xD) illustrates a metabolic shift toward unquantified carbon-rich compounds in response to pathogenesis. In conclusion, enhancing apple tree resilience to European canker depends on maintaining balanced dual-nutrient ratios—specifically balancing Zn uptake while managing Cu and Mn levels—rather than applying isolated fertilizations. Future diagnostic frameworks

must expand the ionomic spectrum to include Si, S and Al to account for soil acidity constraints and structural defense mechanisms.

Author contributions

CRediT: Conceptualization: Leandro Hahn (Lead); Cláudio Ogoshi (Equal). **Data curation**: Leandro Hahn (Lead); Cláudio Ogoshi (Equal). **Formal analysis**: Leandro Hahn (Lead); Léon-Étienne Parent (Equal). **Funding acquisition**: Leandro Hahn (Lead); Cláudio Ogoshi (Equal). **Investigation**: Leandro Hahn (Lead); Cláudio Ogoshi (Equal); **Methodology:** Leandro Hahn (Lead); Cláudio Ogoshi (Equal); Thyana Lays Brancher (Equal); **Project administration**: Leandro Hahn (Lead); Cláudio Ogoshi (Equal); **Validation**: Leandro Hahn (Lead); Cláudio Ogoshi (Equal); Léon-Étienne Parent (Equal). **Visualization**: Leandro Hahn (Lead); Cláudio Ogoshi (Equal); Léon-Étienne Parent (Equal); Caetano Albuquerque (Equal); Gustavo Brunetto (Equal). **Writing—original draft**: Léon-Étienne Parent (Lead). **Writing—review and editing:** Leandro Hahn (Lead); Cláudio Ogoshi (Equal); Thyana Lays Brancher (Equal); Léon-Étienne Parent (Equal); Caetano Albuquerque (Equal); Gustavo Brunetto (Equal).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported in part by Research and Innovation Support Foundation of the State of Santa Catarina-FAPESC (2023TR000346 and 2025TR001736)/ The National Council for Scientific and Technological Development-CNPq, project #2023TR000346.

ORCID

Leandro Hahn: https://orcid.org/0000-0002-1796-9761

Thyana Lays Brancher: https://orcid.org/0000-0003-3337-6314

Cláudio Ogoshi: https://orcid.org/0000-0002-8629-602X

Léon-Étienne Parent: https://orcid.org/0000-0002-4384-4495

Caetano Albuquerque: https://orcid.org/0000-0001-6222-3996

Gustavo Brunetto: https://orcid.org/0000-0002-3174-9992

Declaration of generative AI use

The authors report generative AI was not used in their research or preparation of this manuscript.

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

Aitchison J (1986) The statistical analysis of compositional data. Chapman and Hall, London

Alarcón-Poblete E, Inostroza-Blancheteau C, Alberdi M, et al (2018) Molecular regulation of aluminum resistance and sulfur nutrition during root growth. Planta 247:27–39. https://doi.org/10.1007/s00425-017-2805-6

Alvares CA, Stape JL, Sentelhas PC, et al (2013) Köppen's climate classification map for Brazil. Meteorol Z 22:711–728. https://doi.org/10.1127/0941-2948/2013/0507

Baxter I (2009) Ionomics: studying the social network of mineral nutrients. Curr Opin Plant Biol 12:381–386. https://doi.org/10.1016/j.pbi.2009.05.002

Baxter I (2015) Should we treat the ionome as a combination of individual elements, or should we be deriving novel combined traits? J Exp Bot 66:2127–2131. https://doi.org/10.1093/jxb/erv040

Bozorov TA, Toshmatov ZO, Kahar G, et al (2024) Uncovering the antifungal activities of wild apple-associated bacteria against two canker-causing fungi, *Cytospora mali* and *C. parasitica*. Sci Rep 14:6307. https://doi.org/10.1038/s41598-024-56969-4

Chaboussou F (2004) Healthy crops: a new agricultural revolution. Jon Carpenter Publishing, Charlbury

Coskun D, Deshmukh R, Sonah H, et al (2019) The controversies of silicon's role in plant biology. New Phytol 221:67–85. https://doi.org/10.1111/nph.15343

Courbet G, Gallardo K, Vigani G, et al (2019) Disentangling the complexity and diversity of crosstalk between sulfur and other mineral nutrients in cultivated plants. J Exp Bot 70:4183–4196. https://doi.org/10.1093/jxb/erz214

CQFS-RS/SC (2016) Manual de adubação e de calagem para os Estados do Rio Grande do Sul e de Santa Catarina. Comissão de Química e Fertilidade do Solo - RS/SC, Sociedade Brasileira de Ciência do Solo, Porto Alegre

Deshmukh R, Ma JF, Bélanger RR (2017) Editorial: role of silicon in plants. Front Plant Sci 8:1858. https://doi.org/10.3389/fpls.2017.01858

Dordas C (2008) Role of nutrients in controlling plant diseases in sustainable agriculture: a review. Agron Sustain Dev 28:33–46. https://doi.org/10.1051/agro:2007051

Flors V, Kyndt T, Mauch-Mani B, et al (2024) Enabling sustainable crop protection with induced resistance in plants. Front Sci 2:1407410. https://doi.org/10.3389/fsci.2024.1407410

Frew A, Weston LA, Reynolds OL, Gurr GM (2018) The role of silicon in plant biology: a paradigm shift in research approach. Ann Bot 121:1265–1273. https://doi.org/10.1093/aob/mcy009

Harteveld D, Kerkhof E, van der Sluis B, et al (2022) Balancing nitrogen fertilization in apples: boosting plant growth or European canker? In: Marinov M, Zvezdomir J, Lennox C, et al (eds) Pome fruit diseases: proceedings of the 12th meeting at Plovdiv (Bulgaria), pp 53–56

Huber D, Römheld V, Weinmann M (2012) Relationship between nutrition, plant diseases and pests. In: Marschner P (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Academic Press, Cambridge, pp 283–298. https://doi.org/10.1016/B978-0-12-384905-2.00010-8

Jones LHP, Handreck KA (1963) Effects of iron & aluminum oxides on silica in solution in soils. Nature 198:852–853

Karlstrom UA (2024) Understanding the genetic basis of resistance to European canker in apple. PhD Thesis, University of Reading, Reading. https://doi.org/10.48683/1926.00116087

Kenworthy AL (1967) Plant analysis and interpretation of analysis for horticultural crops. In: Stelly M, Hamilton H (eds) Soil testing and plant analysis, Part II. Soil Science Society of America, Madison, pp 59–75

Kuzin A, Solovchenko A (2021) Essential role of potassium in apple and its implications for management of orchard fertilization. Plants 10:2624. https://doi.org/10.3390/plants10122624

Martinez DA, Loening UE, Graham MC, Gathorne-Hardy A (2021) When the medicine feeds the problem: do nitrogen fertilisers and pesticides enhance the nutritional quality of crops for their pests and pathogens? Front Sustain Food Syst 5:701310. https://doi.org/10.3389/fsufs.2021.701310

McKeague JA, Cline MG (1963) Silica in soil solutions: the form and concentration of dissolved silica in aqueous extracts of some soils. Can J Soil Sci 43:70–82

Mertoğlu K, Evrenosoğlu Y, Akkurt E, et al (2024) Mineral composition modulates *Erwinia amylovora* resistance in pear based on path analysis. Eur J Plant Pathol 169:593–599. https://doi.org/10.1007/s10658-024-02858-5

Munson RD, Nelson WL (1990) Principles and practices in plant analysis. In: Westerman RL (ed) Soil testing and plant analysis, 3rd edn. Soil Science Society of America Book Series, Madison, pp 359–387

Paim LA, Carvalho R, Melo WDC, Furtini Neto AE (2003) Effect of silicon and phosphorus on the availability of soil heavy metals by Mehlich-1 extractor. Ciênc Agrotec 27:780–787. https://doi.org/10.1590/S1413-70542003000400006

Parent LE (2025) Compositional and machine learning tools to model plant nutrition: overview and perspectives. Horticulturae 11:161. https://doi.org/10.3390/horticulturae11020161

Parent LE, Dafir M (1992) A theoretical concept of compositional nutrient diagnosis. J Am Soc Hortic Sci 117:239–242. https://doi.org/10.21273/JASHS.117.2.239

Parent LE, Granger RL (1989) Derivation of DRIS norms from a high-density apple orchard established in Quebec Appalachians. J Am Soc Hortic Sci 114:915–919. https://doi.org/10.21273/JASHS.114.6.915

Peng HX, Wei XY, Xiao YX, et al (2016) Management of Valsa canker on apple with adjustments to potassium nutrition. Plant Dis 100:884–889. https://doi.org/10.1094/PDIS-09-15-0970-RE

Quaggio JA, van Raij B, Malavolta E (1985) Alternative use of the SMP-buffer solution to determine lime requirement of soils. Commun Soil Sci Plant Anal 16:245–260. https://doi.org/10.1080/00103628509367600

Rout GR, Samantaray S, Das P (2001) Aluminium toxicity in plants: a review. Agronomie 21:3–21. https://doi.org/10.1051/agro:2001105

Sandhya K, Prakash NB, Meunier JD (2018) Diatomaceous earth as source of silicon on the growth and yield of rice in contrasted soils of Southern India. J Soil Sci Plant Nutr 18:344–360. https://doi.org/10.4067/S0718-95162018005001201

Savant NK, Korndörfer GH, Datnoff LE, Snyder GH (1999) Silicon nutrition and sugarcane production: a review. J Plant Nutr 22:1853–1903. https://doi.org/10.1080/01904169909365761

Schaller J, Kleber M, Puppe D, et al (2025) The importance of reactive silica for maintaining soil health. Plant Soil. https://doi.org/10.1007/s11104-025-07299-5

Sezerino AA (org) (2018) Sistema de produção para a cultura da macieira em Santa Catarina. Epagri, Florianópolis

Snyder GH (2001) Methods for silicon analysis in plants, soils, and fertilizers. Stud Plant Sci 8:185–196. https://doi.org/10.1016/S0928-3420(01)80015-X

Soares JC, Santos CS, Carvalho SM, et al (2019) Preserving the nutritional quality of crop plants under a changing climate: importance and strategies. Plant Soil 443:1–26. https://doi.org/10.1007/s11104-019-04229-0

Soil Survey Staff (2014) Keys to soil taxonomy, 12th edn. USDA-NRCS, Washington, DC

Srivastava AK, Huchche AD, Parent LE, et al (2025) Mineral nutrition of *Citrus* across the mandarin belts of India. Horticulturae 11:254. https://doi.org/10.3390/horticulturae11030254

Sumner ME (1987) Interpretation of nutrient ratios in plant tissue. Commun Soil Sci Plant Anal 9:335–345

Sumner ME (1993) Gypsum and acid soils: the world scene. Adv Agron 51:1–32

Tadayon MS, Sadeghi S (2023) Relationships between the incidence of fire blight in apple orchards and plant nutritional imbalance indices. J Hortic Sci Biotechnol 98:394–407. https://doi.org/10.1080/14620316.2022.2147100

Tedesco MJ, Gianello C, Bissani CA, et al (1995) Análise de solo, plantas e outros materiais, 2ª edn. Universidade Federal do Rio Grande do Sul, Porto Alegre

USDA (2003) Potassium silicate for use in crop production. National Organic Program, University of California Sustainable Agriculture Research and Education Program (UC SAREP), Davis

USEPA (2007) Potassium silicate: biopesticides registration action document (PC Code 072606). Office of Pesticide Programs, United States Environmental Protection Agency, Washington, DC

Vardar F, Unal M (2007) Aluminum toxicity and resistance in higher plants. Adv Mol Biol 1:1–12

Vergutz L, Novais RF, Melo LCA (2015) Loss of extraction capacity of Mehlich-1 and monocalcium phosphate as a variable of remaining P and its relationship to critical levels of soil phosphorus and sulfur. Rev Bras Cienc Solo 39:1079–1087. https://doi.org/10.1590/01000683rbcs20140551

Walworth JL, Sumner ME (1987) The Diagnosis and Recommendation Integrated System (DRIS). Adv Soil Sci 6:149–188. https://doi.org/10.1007/978-1-4612-4682-4

569 Wang JJ, Dodla SK, Henderson RE (2004) Soil silicon extractability with seven select-
570 ed extractants in relation to colorimetric and ICP determination. Soil Sci 169:861–870

571 Weber RWA, Børve J (2021) Infection biology as the basis of integrated control of ap-
572 ple canker (*Neonectria ditissima*) in Northern Europe. CABI Agric Biosci 2:1–16.
573 https://doi.org/10.1186/s43170-021-00024-z

574 Wilkinson SR, Grunes DL, Sumner ME (2000) Nutrient interactions in soil and plant
575 nutrition. In: Sumner ME (ed) Handbook of soil science. CRC Press, Boca Raton

576 Xu X, Passey T, Robinson-Boyer L, et al (2022) Development of European apple can-
577 ker on different cultivars in relation to planting time at three sites in the UK. Front Hor-
578 tic 1:995776. https://doi.org/10.3389/fhort.2022.995776

579 Yamane DR, Parent S-É, Natale W, et al (2022) Site-specific nutrient diagnosis of or-
580 ange groves. Horticulturae 8:1126. https://doi.org/10.3390/horticulturae8121126

581 Zheng X, Yan X, Qin G, et al (2021) Soil acidification and phosphorus enrichment en-
582 hanced silicon mobility in a Hydragric Anthrosol. J Soils Sediments 21:3107–3116.
583 https://doi.org/10.1007/s11368-021-03017-7

584