

Variation in the Nutrient Contents of Leaves, Bark, and Wood of Persian Oak Trees (*Quercus brantii*) Affected by Decline

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Tree decline is a physiological phenomenon resulting from climatic disturbances that involves damage to forest ecosystems. This study examined the effects of tree decline on nutrient concentrations in the leaves, bark, and wood of Persian oak (*Quercus brantii*) trees. Trees were categorized by decline severity (healthy, slight, moderate, and severe decline). Leaves were collected from the middle and outer parts of the crowns. Bark and wood samples were taken at breast height (1.3 m). The contents of Mg, Ca, P, Fe, K, and Na were analyzed by atomic absorption spectrophotometry and flame photometry. As decline severity increased, the concentrations of Mg, Ca, P, Fe, K, and Na in the foliage increased. However, the P and K in the bark and the P in the wood were lower in trees in the higher decline classes. Moreover, nutrient contents in the tissues examined varied across the different decline severities. The variations may have been due to defense mechanisms of the trees enhancing tolerance against induced stress. The results suggested that nutrient stoichiometry can reflect uptake in forest ecosystems and plant-environmental stress relationships.

Keywords: Nutrient elements; Stoichiometry; Decline index; Persian oak; Plant tissues

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INTRODUCTION

The semi-arid Zagros Forest in the west of Iran is dominated by oak and covers an area of 5 million hectares, representing 40% of the total Iranian forest area. Persian oak (*Quercus brantii* L.) is the most common tree species in the forest. This species is sensitive to changing environmental factors (Talebi *et al.* 2014). In recent years, these forests have been damaged by various natural factors, such as climate effects and pathogens. Drought is an important factor that causes mortality and decline in forest trees (Hosseini 2012). Lack of expected precipitation for a decade and global warming have resulted in drought, which, along with overgrazing, understory farming, and land use change, has encouraged outbreaks of destructive pests and diseases and the death of large numbers of oak trees in the Zagros forests. The phenomenon of decline has been reported in many provinces of the Zagros Region, especially in Ilam (Babaie-Kafaie 2004).

Comparing the quantities of nutrient elements in different tissues of healthy and declining trees is a reliable method of differentiating weakened from healthy trees

(Niinemets 2010). During severe climatic or environmental disturbance, tree responses lead to changes in concentrations of many elements in different oak tissues (Hosseini 2017). In an oak stand affected by drought stress, trees vary in the damage sustained. The main factors that affect the response of oak to decline phenomena are tree physiology, nutrient availability and uptake, and tree vigor. As trees in older age classes are more susceptible to weakness and decline than younger trees, recovery is inhibited in the aftermath of pest attacks, pathogen attacks, or abiotic stresses, such as drought (Clatterbuck and Kauffman 2005).

Plant nutrition has direct and indirect roles in determining disease resistance (Daroub and Snyder 2007). Many diseases alter the nutritional balance in plants, and symptoms caused by abiotic and/or biotic factors are often difficult to differentiate (Römheld 2012). The response of trees to external factors can indicate changes in the nutrient contents of the affected organs. Therefore, it is useful to examine the response of forest ecosystems to environmental changes and stresses in terms of alterations in essential nutrients. Trees that are subject to varied stresses lose vigor, which can result in death (Lukac *et al.* 2010). Stress causes a change in water transfer (Silva *et al.* 2009), gas exchange and photosynthetic efficiency (Pagter *et al.* 2005), metabolism of carbohydrates, proteins, and amino acids, and accumulation of stress-related organic compounds in the plant (Šircelj *et al.* 2005). The consequent reduction in nutrient flow from the roots and leaves to other tissues impairs growth (Burke 2007).

Onset of decline symptoms can be induced by drought and severe water deficiency, severe winters or cold springs, and the intensification of the effects of decay-causing fungi that follow (Lakzian *et al.* 2013). The effects of nutrient deficiencies, principally phosphorous (P), on hydraulic architecture, water relations, and stomatal behavior have been reported (Bucci *et al.* 2006; Samuelson *et al.* 2008; Ward *et al.* 2008). Macronutrients are generally considered the largest limiters of plant growth (Lebauer and Treseder 2008; Vitousek *et al.* 2010; Harpole *et al.* 2011). A study of sugar maple (*Acer saccharum* Marsh.) in the northeastern USA found that, on sites with low Mg, K and P, plus high Al and Mn concentrations in the soil, the trees exhibited symptoms of decline (Schaberg *et al.* 2006; Long *et al.* 2009; Bal *et al.* 2015). In addition, Ca deficiency was frequently observed in sugar maple foliage and soil in declining stands. Tavakolinekou *et al.* (2008) reported high amounts of Na in healthy *Cupressus arizonica* but low amounts of K in trees with severe drought stress. Nutrients, such as Na, P, K, Zn, and Fe did not vary in relation to dieback.

Measurement of the mineral and organic compounds in tree tissues provides information on the relationship between mineral uptake and vigor (Zarin Kafsh 2001). There has yet to be specific research on the nutritional contents of Persian oak in western Iran and the relationship between nutrition and decline in this species. Assessing the quantities of nutrients in tissues of *Q. brantii* that exhibit different severities of decline enables the identification of resistant individuals, which can be utilized to produce planting stock and detect the initial stages of decline in conservation programmes. Due to the increasing occurrence of decline in the Zagros forests, the work described here aimed to investigate variations in nutrients in the leaves, stem sapwood and bark of Persian oak trees that were exhibiting various decline symptoms. The results provided information on the uptake and the quantities of nutrients in the trees and their relationships with development decline.

EXPERIMENTAL

Study Area

The Gachan forest area, which has a high prevalence of oak decline, was selected for this study. This 6.6-hectare forest has an elevation of 1821 m, average precipitation of 585.2 mm, is on a slope of approximately 35%, and is located to the northeast of Ilam city at 33°38'55" N; 46°30'37" E (Fig. 1). The main tree species present include *Quercus brantii* (Persian oak), *Pistacia atlantica*, *Amygdalus scoparia*, *Acer monspessulanum*, and *Crataegus aronica*. The first occurrence of decline as a consequence of drought in Zagros forest was reported in 2002. Symptoms on declining trees is noticeable in the foliage, usually with an initial deterioration of leaves turning pale green and yellow (Pourhashemi and Sadeghi 2021).

Sampling Method

The leaves, bark, and stem sapwood of healthy and declining oaks in different classes of decline were sampled in mid-June as the leaves matured. The selected trees were mature, natural regeneration, with diameters at breast height ranging between 40 and 50 cm. The forest area sampled had uniform conditions (habitat, altitude, and topography). All trees were in the same biosocial classes. A minimum of 3 trees were selected for sampling from each level of decline. Selected trees were classified based on severity of crown dieback (class of defoliation) as follows: healthy trees (up to 5%); slight (5% to 33%); moderate (34% to 66%); or dead (100% defoliation) (Kabrick *et al.* 2008). Leaves were sampled from the middle and outer parts of the crowns (except for the final class) with telescopic secateurs; the inner bark (phloem) and sapwood were taken at breast height.

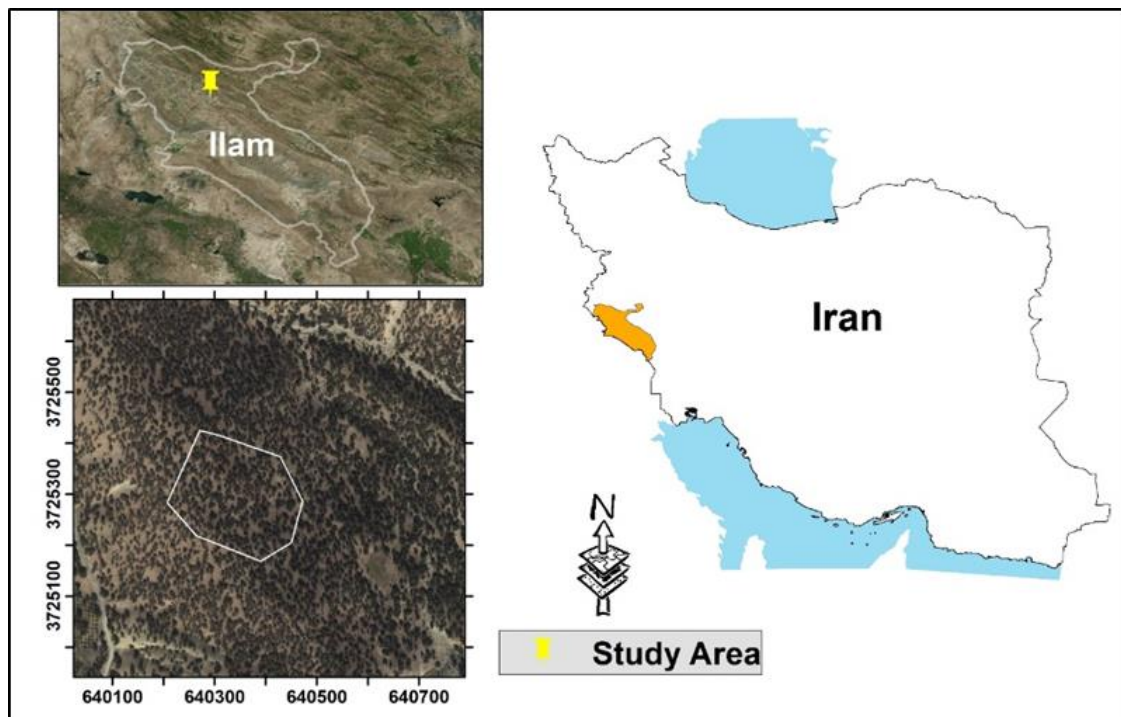


Fig. 1. Location of Gachan forest in Ilam

Sample Preparation for Nutrient Analyses

To remove dust and other attached particles, sampled leaves were gently washed in distilled water before drying in an oven at 60 ± 3 °C for approximately 48 h. Dried samples were milled and passed through a sieve with a mesh of 35 to produce uniform particles of equal size. Five (± 0.001) g dried powder was ashed in an electric oven (Shimifan F.47, Tehran, Iran) at 500 °C for 5 h. To measure Mg, Ca, P, Fe, K, and Na with a dry digestion method, the ash was combined with 20 mL of HCl (1 N), passed through Whatman® 42 filter paper (Whatman Inc., Piscataway, NJ, USA), and the volume adjusted to 100 mL (Miles *et al.* 2001). Thereafter, the following devices were used to measure the content of each element: Fe and Mg were measured *via* atomic absorption (Analytikjena NOVAA 400P, Jena, Germany); P was measured *via* spectrophotometry (BT1500 Autoanalyser, Biotechnica Instruments S.p.A, Rome, Italy); Ca, Na, and K were measured *via* flame photometry (Jen way 850S, Staffordshire, England).

Statistical Analysis

The normality and homogeneity of the data were evaluated using Shapiro-Wilk and Levene's tests, respectively. Data were subjected to one-way analysis of variance (ANOVA) and means compared using the Tukey test at a significance level of 0.01%. Comparisons of the mean concentrations of nutrient elements (Mg, Ca, P, Fe, K, and Na) in the leaves, bark and sapwood of oaks in the different decline classes were made using the Tukey test. The data were analyzed using IBM SPSS ver. 24., Chicago, IL, USA.

RESULTS AND DISCUSSION

Quantities of nutrients found in leaf, bark, and sapwood samples of *Q. brantii* based on classes of decline are shown in Table 1. There was no significant effect of decline class on Mg or K contents in leaves. However, there were significant differences in Ca, P, Fe, and Na ($P < 0.01$) between decline classes. Concentrations of Mg, Ca, P, Fe, K, and Na in the bark of sampled trees differed significantly ($P < 0.01$). The effects of decline on Mg, Ca, Fe, and Na concentrations were significant in the sapwood ($P < 0.01$), but no significant differences were observed among K or P. The highest and lowest differences in nutrient elements in leaves were Ca and Mg, respectively. In bark, the highest and lowest differences in nutrient elements were Fe and Ca, respectively. In the sapwood, Mg and K had the highest and lowest differences in nutrient elements, respectively.

Nutrients in Leaves

No significant differences were observed among the Mg, P, or K concentrations in the leaves (Table 2). However, quantities of Ca, Fe, and Na differed significantly between decline classes. Generally, concentrations of Mg, Ca, P, Fe, and Na increased as tree decline increased ($P < 0.01$).

Nutrients in Bark

Compared to healthy trees, no significant differences were detected between the concentrations of Mg in the inner bark tissues of trees with decline severities of 30% and 70%. However, a significant difference ($\alpha = 0.004$) was observed for trees with severe decline (100%). In most of the tissues sampled, concentrations of Ca were significantly higher in declined than healthy trees ($P < 0.01$).

Table 1. ANOVA Results of Nutrient Contents of *Quercus brantii* Leaves, Inner Bark, and Wood in Relation to Decline Class

Mean Square								
Tissue	Source of Variation	Df	Mg (ppm)	Ca (ppm)	P (ppm)	Fe (ppm)	K (ppm)	Na (ppm)
Leaf	Decline	2	15.44 ^{ns}	10.33 ^{**}	19.33 ^{**}	0.24 ^{**}	1314.1 ^{ns}	8.5 ^{**}
	Coefficient of variation	-	5.81	20.9	12.2	9.92	7.48	11.94
	Error	4	15.78	180.1	14.33	0.01	829.4	0.52
	Significance level	-	0.45	0.001	0.01	0.007	0.31	0.01
Bark	Decline	3	19.97 ^{**}	3000 ^{**}	11.55 ^{**}	0.14 ^{**}	60.75 ^{**}	41.23 ^{**}
	Coefficient of variation	-	29.48	9.72	24.85	35.85	21.03	19.43
	Error	6	12.13	75.42	0.29	0.005	301.75	1.78
	Significance level	-	0.003	0.000	0.000	0.001	0.002	0.001
Wood	Decline	3	259.4 ^{**}	501.6 ^{**}	6.1 ^{ns}	0.07 ^{**}	267.9 ^{ns}	72.44 ^{**}
	Coefficient of variation	-	56.73	20.96	18.71	22.93	8.48	28.89
	Error	6	7.29	216.8	2.89	0.003	165.9	3.81
	Significance level	-	0.0	0.001	0.2	0.001	0.28	0.002

** : P < 0.01; ns: non-significant

Table 2. Comparison of Different Elements (Means \pm SE) in the Leaves, Bark and Wood of Persian Oak Trees in Different Classes of Decline Based on Tukey Test

Tree Organ	DCD	Mg (ppm)	Ca (ppm)	P (ppm)	Fe (ppm)	K (ppm)	Na (ppm)
Leaf	Healthy	69.7 $\pm 1.15^a$	192.3 $\pm 0.57^c$	66.3 $\pm 2.08^a$	2.4 $\pm 0^b$	369.3 $\pm 1.15^a$	12.0 $\pm 0.45^b$
	30%	68.7 $\pm 7.02^a$	254.3 $\pm 10.06^b$	55.0 $\pm 3.6^b$	2.8 $\pm 0.2^a$	408.3 $\pm 20.84^a$	13.8 $\pm 1.34^{ab}$
	70%	73.0 $\pm 1^a$	309.7 $\pm 25.92^a$	70.7 $\pm 5.5^a$	3.0 $\pm 0.06^a$	402.0 $\pm 41.32^a$	15.3 $\pm 0.37^a$
Bark	Healthy	24.30 $\pm 1.52^b$	258.3 $\pm 3.51^b$	10 $\pm 0.11^a$	0.5 $\pm 0.01^b$	219.7 $\pm 0.57^a$	20.9 $\pm 1^a$
	30%	17.70 $\pm .057^b$	320.3 $\pm 13.31^a$	5.3 $\pm 0.57^c$	0.5 $\pm 0.02^b$	217.3 $\pm 14.29^a$	14.6 $\pm 1.53^b$
	70%	26.70 $\pm 1.57^b$	324.3 $\pm 8.08^a$	8.0 $\pm 1^b$	0.5 $\pm 0.02^b$	239.7 $\pm 23.54^a$	15.3 $\pm 1.62^b$
	100%	37.00 $\pm 6.24^a$	319.7 $\pm 10.06^a$	6.7 $\pm 0.57^{bc}$	1.0 $\pm 0.14^a$	137.7 $\pm 13.5^b$	21.8 $\pm 0.66^a$
Sapwood	Healthy	6.2 $\pm 0.26^b$	161.3 ^{bc} ± 0.57	11.0 ^a ± 0.17	0.5 ^b ± 0	173.0 ^a ± 2.64	9.9 $\pm 3.2^b$
	30%	8.7 $\pm 2.08^b$	149.7 $\pm 1.52^c$	9.3 $\pm 1.52^a$	0.6 $\pm 0.05^b$	195 $\pm 9.84^a$	19.9 $\pm 0.7^a$
	70%	22.0 $\pm 4.6^a$	194.0 $\pm 26.51^b$	11.0 $\pm 1.73^a$	0.8 $\pm 0.08^a$	179.3 $\pm 20^a$	20.3 ^a ± 0.89
	100%	24.7 ^a ± 2.08	241.0 ^a ± 13.22	8.0 ^a ± 2	0.9 ^a ± 0.02	186.3 ^a ± 20.59	15.0 ^a ± 0.85

Note: The superscript letters in each column for each tissue indicate significant differences (P > 0.01) between the means of the nutrients; DCD: Classes of decline; SE: Standard error

There were significant differences between P concentrations in bark tissues among all decline classes, but no specific pattern was discernable. Trees in the healthy, 30% decline and 70% decline classes differed significantly in Fe concentrations compared to those in the 100% decline class ($P < 0.01$). Fe concentrations were highest in trees in the 100% decline class (Table 2).

Similar to the differences in Fe concentrations, there was a significant difference between K concentrations in trees with severe decline (100%) and those in other decline classes, and healthy trees ($P < 0.01$). Otherwise, variations in K concentration showed no specific pattern. There was no significant difference between the concentrations of Na in healthy compared with 100% declined trees (Table 2).

Nutrients in Sapwood

Concentrations of Mg, Ca, Fe, and Na did not vary significantly across different decline classes (Table 2). Mg and Fe concentrations were significantly higher in the sapwood of trees in the 70% and 100% decline classes than in other classes ($P > 0.01$). In addition, the Ca and Na concentrations were significantly higher in the 100% decline class than in other decline classes ($P < 0.01$).

Magnesium is of great importance in chlorophyll and is, therefore, crucial to photosynthesis (Jones and Huber 2007). The relationship between Mg and plant diseases and disorders may be related to its indirect effects on general plant health or its direct impacts on plant growth due to its specific physiological roles (Cakmak 2013).

Analysis of *Q. brantii* in various stages of decline showed that the amount of Mg in the leaf, bark, and wood tissues increased as stress increased. Drought reduces Mg absorption, which increases Mg deficiency and the resultant symptoms. Grabřová and Martinková (2001) showed that, during the growth periods of Norway spruce (*Picea abies*), drought caused a larger reduction in N and Mg contents than in P and K contents under the same conditions. In addition, drought resulted in reduced Mg uptake in both the roots and shoots of *Spartina alterniflora* (Brown *et al.* 2006).

In all tissues tested in this study, Ca increased as decline severity increased. In plants, Ca is involved in environmental stress mitigation (Pessarakli *et al.* 2015). Calcium regulates cell metabolism and is a major component in cell wall structure (Akinci and Simsek 2004). Calcium deficiency may result in increased spread of disease-causing agents in infected plants (Hawkesford *et al.* 2012), which is partly due to reductions in the physical resistance of plant tissues to penetration by fungal hyphae. The activities of cell wall degrading enzymes in plant pathogenic fungi and bacteria decrease in the presence of excess Ca (Rahman and Punja 2007).

Quantities of Ca in leaves of declining trees were higher than in healthy trees, which may be due to antagonism between Ca and K; in cases of K deficiency, more Ca is absorbed by the foliage (Gransee and Fuhrs 2013). Further, Ca absorption is highly dependent on transpiration, which is limited in drought stress conditions (Setayeshmehr and Ganjali 2013). Singh and Singh (2004) examined soil nutrient mobility and uptake by *Dalbergia sissoo* seedlings grown under different irrigation regimens, showing that Ca concentrations increased considerably in the leaves and roots under severe drought. Hu and Schmidhalter (2005) reported that, in plants under drought conditions, P and K absorption decreased considerably, whereas that of Ca decreased slightly. Analysis of the long-term effects of drought in a Mediterranean evergreen (*Quercus ilex*-dominated) forest suggested that drought leads to decreases in Ca concentrations in the above-ground biomass, which was attributed to a reduction in transpiration flux (Sardans *et al.* 2008).

Phosphorus is an essential mineral nutrient with a key role in conserving and transferring energy in living organisms, and is required in relatively large quantities to maintain plant growth (Amtmann and Blatt 2009). As decline severity increased, the amount of P in the oak leaves increased. This change may mitigate the impact of stress by reducing the transpiration rate in leaves *via* controlling the opening and closing of stomata (Hosseini 2017). In stressed *Quercus ilex*, the quantity of P increased in leaves and decreased in wood and root tissues, which suggests that P was actively transported from the stems to the leaves (Sardans and Peñuelas 2007). High concentrations of P in response to induced stress are associated with increased water use efficiency (Diaz-Pereira and Roldán 2000) and elevated stress defense responses (Egilla *et al.* 2005). Increases in P in leaves of declining oak trees indicated that the plants attempted to actively transport P from the soil to the crowns and leaves of affected trees to improve water consumption efficiency and protect against crown dieback (Hosseini 2017). In wood and bark samples, increased decline severity led to decreased P contents. This reduction in P contents may have also been due to disruption of water-soil relations and the response of trees to stress (Hosseini 2017).

The roles of Fe in plant growth and development include nitrogen fixation, photosynthesis, the electron transfer system (Bennett 1993), chlorophyll synthesis, and thylakoid function (Imsande 1998). In addition, Fe is involved in defensive reactions to stress (Nikolic and Römheld 1999). Increased Fe content was associated with increased decline severity in the 3 sampled tissues of *Q. brantii*. Alizadeh *et al.* (2008) showed that Fe played an active role in defense against some diseases, but it was not as effective as other elements, such as P, Mn, Cu and Zn. Competition between plants and pathogens for Fe is a factor in the development of disease, as some pathogens require high amounts of Fe in plant cells (Kieu *et al.* 2012). Iron uptake increases under stress conditions; therefore, elevated Fe uptake is a potential indicator of plant infection (Martins *et al.* 2003).

Potassium has a critical role in plant defense against pathogens and pests through its influence on cuticle thickness and bark structure (Amtmann *et al.* 2008). A reduction in K availability during bark formation can result in tree weakening and decline (Ahmad *et al.* 2009). Potassium is involved in metabolism *via* the regulation of enzyme activities and is a key element regulating stomatal opening and closing (Hawkesford *et al.* 2012). In this study, increased decline severity was associated with higher K concentrations in the leaf and sapwood of *Q. brantii*. In contrast, K concentrations in bark decreased as decline severity increased. Changes in the K contents of declining trees relative to healthy trees may contribute to weakening of trees. High K concentrations can have detrimental effects, such as reducing the absorption of Mg and Ca (Hawkesford *et al.* 2012). Mahouachi (2007) found that banana plants exhibited reduced K contents under drought conditions. In addition, in leaves of water-stressed *Olea europaea* L. trees, water was a major determinant in the availability of mineral nutrients (including K) from the soil, absorption by plants, and translocation from roots to the shoots regardless of overall nutritional status (Restrepo-Diaz *et al.* 2008). Arquero *et al.* (2006) showed that K-deficient olive trees under water stress regulated stomatal closure. In addition to its positive impact on tolerance to abiotic stresses, Anshütz *et al.* (2014) showed that K plays a crucial role in signaling the presence of stress factors, such as drought.

Increased decline severities in *Q. brantii* led to accumulation of increasing quantities of Na in leaf, bark and sapwood tissues. Quantifying changes in nutrient concentrations in plant tissues is an effective method of evaluating the stress tolerance of plants, and Na is a particularly important indicator (Bremner and Mulvaney 1982). Absorbing greater

amounts of Na enables plants to maintain osmotic balance more effectively by increasing water absorption (Munns and James 2003). Higher levels of stress lead to increased accumulation of Na in plants. For example, Battie-Laclau *et al.* (2013, 2014) suggested that in *Eucalyptus grandis*, Na plays a role in the structural and physiological adjustments to drought and improves osmoregulatory and photosynthetic functions.

CONCLUSIONS

1. This work indicated that the decline of *Quercus brantii* affects the absorption of nutrients in leaf, bark, and sapwood tissues.
2. The stress response of *Q. brantii* led to changes in the quantities of nutrients absorbed in different tissues. The changes varied across tissue types.
3. Tree decline reduced mass flow-dependent mineral nutrient uptake and subsequent translocation of nutrients from the roots to the shoots. In addition to impaired plant development and reduced vigor, problems with mineral nutrition occurred as secondary effects of tree decline.
4. Improved understanding of the effects of decline on plant nutrition is useful for developing strategies to minimize the damage caused by decline.

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