Foliar nutrient status of a natural fir forest in Greece

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ABSTRACT: The foliar nutrient status was examined in a degraded Greek fir (*Abies cephalonica* Loud.) forest in Mount Parnitha near Athens, Greece. The examination lied in comparing the foliar concentrations of Ca, Mg, K, N, P, Fe, Mn, Zn and Cu with the critical values referring to conifers and the elemental percentages with regard to N of the forest with the corresponding percentages of a healthy Bulgarian fir (*Abies borisii regis*) stand, the closest relative of the Greek fir. It was found that the needles of the Greek fir had significantly lower concentrations from the N and P critical values. Significant differences were found for the Ca/N, Mg/N, P/N, Fe/N and Mn/N percentages. Significant correlations for the Greek fir were found between needle weight and foliar N as well as between needle weight and the percentages Ca/N and Fe/N. It is highly probable that N and P in the Greek fir are in short supply.

Keywords: critical values; nutrient/N percentages

The elemental composition of plant tissues is important for plant physiology, plant nutrition, fertilization, ecology and environmental protection. Foliage analysis is a common method for assessing the nutrient status of trees, whereas soil analysis is often hindered by the heterogeneity of forest soils (Schleppi et al. 2000). Critical values are considered concentration thresholds below which nutrient deficiencies may occur. In forestry, the critical value is conventionally defined as the foliar concentration at which yield attains 90% of the possible maximum (Ulrich, Hills 1967). Morrison (1974) quoted tables with critical values for a plenty of conifer species. However, the critical concentration approach has undergone criticism on the grounds that the chemical elements in plant tissues are not independent of each other (Richards, Bevege 1972). For this reason, concentration ratios are also used, especially those between N and other nutrients (Linder 1995; Tessier, Raynal 2003). The great value of using nutrient ratios for a particular species is that they can be used irrespective of the place and development stage (Bangroo et al. 2010). Ingestad (1970) used nutrient percentages based on n = 100 for forest seedlings and found that they varied insignificantly with the age of plants. The diagnosis and recommendation integrated system (DRIS) also uses nutrient ratios but requires a great number of trees (Gregoire, Fisher 2004). The vector analysis approach to fertilizer assessment developed by Timmer and Stone (1978) has been gaining acceptance recently. However, it often happens in practice that for natural forests there is no previous data on critical values and the application of fertilizers is difficult to carry out due to the remoteness of the area, to the old age of trees (growth is difficult to monitor), and to the need of fast response to forest services because a natural disaster took place and reforestations must start immediately. In such cases the results of foliage analysis can be compared with those from literature and nutrient ratios with those of the same species or a very close one.

The species silver fir (*Abies alba* Mill), Greek fir (*Abies cephalonica* Loud.) and their hybrid Bulgarian fir (*Abies borisii regis*) cover an extensive area of northern, central and southeastern Europe. All of these species are very sensitive to environmental changes. In 2000 the percentage of moderately to severely damaged trees (crown defoliation) of silver fir in Croatia reached 70% (Potočic et al. 2005). In Greece a great number of fir trees (*Abies cephalonica* and *Abies borisii regis*) died in the years 1988–1989. This phenomenon was attributed to drought and insect attacks (Markalas 1992). The national park of Parnitha, 40 km to Athens, also had thousands of dead trees of Greek fir in the late eighties. In 2007 a tremendous wildfire wiped out two thirds of the Parnitha fir forest. Reforestations started immediately and results are encouraging. However, as the soils of the area are not very suitable for demanding species like fir, the growth of seedlings must be monitored. In this respect knowledge of the foliar nutrient status of grown up fir trees could be a guide to future fertilizations.

The objectives of this work that took place in the fir forest of Parnitha were:

– to compare the needle concentrations in Greek fir with the critical values reported in literature for conifers,
– to compare the ratios of nutrients to N with the corresponding ratios of Bulgarian fir trees of a healthy stand. It was decided to use the Ingestad percentages,
– to find correlations of needle weight and defoliation with nutrient concentrations and Ingestad percentages.

MATERIAL AND METHODS

Site description and sampling

The study was conducted in Mount Parnitha situated 40 km north of Athens, the capital of Greece. The sampling sites were located in the zone of Greek fir (*Abies cephalonica*) at an altitude of approximately 800 m. The average rain height for the years 2002 and 2003 was found 1,231 mm (Michopoulos et al. 2007).

The soils of this area are derived from flysch and hard limestone and classified as Eutric Regosols (FAO-UNESCO 1988). The soils are shallow to moderately deep (25–50 cm) and stony, especially those derived from hard limestone. The values of average soil chemical parameters and details of chemical analysis were quoted by Michopoulos et al. (2004).

The flora comprises species of Greek fir, Kermes oak (*Quercus coccifera*), Phylirea (*Phylirea latifolia*), Holm oak (*Quercus ilex*), Strawberry tree (*Arbutus unedo*), junipers (*Juniperus* sp.) and thorns (*Crataegus* sp.).

In the total forest area of 2,500 ha, a sub-area of 1,250 × 1,250 m was selected to monitor the forest health. This selection was done on the basis of criteria of covering the main categories of soil parent material. Within this sub-area a grid of 30 experimental plots was installed, each covering an area of 0.1 ha. Thirteen plots were selected at random for foliage and soil sampling. In each of these plots, four trees of Greek fir, dominant or codominant, bearing no symptoms of fungi or insect attacks, were selected at random for foliage sampling. In total, 52 trees, 80 years old or older, were selected for foliage sampling.

Site of the healthy fir stand

The experimental plot was established at Mount Timfristos in central Greece at an altitude of 1,170 m. The forest vegetation is the Bulgarian fir (*Abies borisii regis*) having an average age of 100 years. The soils are classified as Humic Alisols (FAO-UNESCO 1988) and were developed on a sandy flysch parent material. The soils are deep (depth > 50 cm) and support a stand of very good quality. The average rain height in 2002 and 2003 was 1,572 mm (Michopoulos et al. 2007).

Assessment of defoliation

An evaluation of the percent defoliation exhibited by the foliage of each tree in both sites was done from the ground. The crown condition assessment was carried out by experienced personnel according to the instructions of the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests 2013). Ratings were based on 5% increments between 0 and 100%. The crown assessment for the healthy fir stand refers to the five trees from which needle samples were collected and that took place every year since 1995. The last assessment was done in 2007.

Foliage sampling

Foliage was collected in December of the years 1999 and 2007. Four branches, in the four orientations on each tree, were selected from the upper crown growing in full sun by pole pruners. Dust was removed with a brush and the tree branches were dried at a temperature of 80°C for 24 h and current year needles were removed. From the four branches of each sampled tree equal quantities of current year needles were mixed and formed a composite sample. So, in total, 52 composite needle samples were formed. Oven dried needle weight was measured from each composite sample and expressed as 1,000 needle weight. Subsequently, all needles from each composite sample were ground in a mill equipped with a stainless steel screen.

Tree needles from the Bulgarian fir are collected systematically in the same way as above in the framework of a European project. Five dominant trees were selected in 1995 and ever since, every two years in December, needles were collected and formed a composite sample. The last collection took place in 2007. In total there were seven collections (seven composite needle samples). The needles were ground as mentioned above.

Needle chemical analysis

Oven dried needles were ground and digested in a mixture of $HNO_3/HClO_4$ (2:1). Concentrations of Ca, Mg, Fe, Zn, Mn and Cu in the digests were determined by atomic absorption spectrometry and those of K^+ by flame emission spectrometry. For the Ca and Mg determination a solution of $LaCl_2$ was used as a releasing agent, whereas for the K determination a solution of $CsCl_2$ was used as an ionization buffer. Nitrogen concentrations were measured by the Kjeldahl distillation method. For the P determination one g of ground needle material was ignited at 500°C for eight hours. The ash was dissolved in 5 ml of 20% HNO_3. The P concentrations were measured colorimetrically by the ammonium molybdate method in a U/V spectrophotometer at a wavelength of 660 nm.

Elemental concentrations in plant tissues are expressed on an oven-dry basis (80°C).

Quality assurance and quality control in chemical analysis

Reference plant material was used throughout the needle chemical analysis. In general, the accuracy and precision of the analysis were quite good. The recovery of the real value of the reference material was close to 90% and coefficient of variation for replicates was always less than five.

Table 1. Significant Pearson's correlation coefficients for needle weight in Greek fir

	N	Ca/N	Fe/N
Needle weight	0.350*	–0.375**	–0.309*

*, **indicates statistically significant difference at the probability levels 0.05, 0.01

Data handling and statistical analysis

The concentrations of all metals in the needles of the fir in Parnitha and the healthy stand were converted to percentages with regard to N concentrations. The concentrations and the percentages of elements were approximately normally distributed (bell-shaped histograms) so there was no need for transformation.

Pearson's correlation coefficients were used to find possible associations between nutrient concentrations, Ingestad percentages and defoliation percentages as well as nutrient concentrations, Ingestad percentages and needle weight.

The statistical tests used were: one tailed *t*-test to compare the element concentrations in Greek fir with the critical values and two tailed *t*-test to compare the elemental percentages in Parnitha and the healthy Bulgarian fir stand. The critical values were derived from literature and more specifically from Morrison (1974), Joslin et al. (1994) and Thelin et al. (1998). All these sources refer to nutrient concentrations of current year needles of spruce and pine species.

RESULTS

The average defoliation of the fir trees in Parnitha was found to be 27.2%, which is rather high compared to the defoliation of the five sampled trees of the healthy fir stand, which ranged from 5 to 10% in the period 1997–2007. No correlation was found

Table 2. Elemental concentrations of current year needles in the forest of Parnitha, in *Abies borisii regis* stand and critical concentrations derived from literature

		Ca	Mg	K	N	P	Fe	Mn	Cu	Zn
		($mg·g^{-1}$)					($mg·kg^{-1}$)			
Parnitha forest	concentration	8.31	1.38	7.71	11.6	0.968	48.5	126	3.33	31.8
	CV	(39.2)	(26.4)	(17.3)	(9.8)	(28.1)	(29.8)	(122)	(18.5)	(37.4)
Abies borisii regis stand	concentration	9.57	1.31	8.22	12.5	1.310	86.4	320	3.78	32.3
	CV	(23.6)	(17.8)	(18.8)	(6.1)	(17.1)	(40.2)	(36.4)	(24.7)	(41.4)
Critical concentration		1.7	0.6	3	12	1.4	50	25	3	14

CV – coefficient of variation (%), means were derived from 52 measurements for *Abies cephalonica* and 7 for the *Abies borisii regis*

Table 3. Critical ratios (percentages of N concentrations) of elements in the needles of *Abies cephalonica*, *Abies borisii regis*

		Ca/N	Mg/N	K/N	P/N	Fe/N	Mn/N	Cu/N	Zn/N
Abies cephalonica	concentration	73,2	12,0*	67,1	8,38***	0,42***	1,14***	0,029	0,28
	CV	(44,6)[a]	(27,7)	(18,8)	(26,1)	(34,6)	(130)	(17,6)	(39,2)
Abies borisii regis	concentration	77,1	10,6	66,2	10,5	0,69	2,57	0,030	0,26
	CV	(24,3)[a]	(18,4)	(19,7)	(16,5)	(38,1)	(36,7)	(23,7)	(43,1)

CV – coefficient of variation (%); *, **, ***indicates statistically significant difference at the probability levels 0.05, 0.01, and 0.001, respectively for a ratio in the same column of Table 3; means were derived from 52 measurements for *Abies cephalonica* and 7 for *Abies borisii regis*

for the Greek fir between defoliation percentages and either nutrient concentration or Ingestad percentages. The average needle weight of 1,000 current year Greek fir needles was found to amount to 7.84 g. Significant correlations were found between needle weight and N concentrations, Ca/N and Fe/N percentages (Table 1).

Table 2 contains the data on the Greek fir, Bulgarian fir and the critical values. The *t*-test was used to compare elemental concentrations of current year needles in the forest of Parnitha (first row) and critical concentrations derived from literature (third row). The results showed that only the N ($P < 0.05$) and P ($P < 0.001$) concentrations were below the critical values derived from other conifer species.

When the Ingestad percentages of the Greek fir were compared with those of the healthy Bulgarian fir stand, statistical differences were found for the percentages Ca/N, Mg/N, P/N, Fe/N and Mn/N (Table 3).

DISCUSSION

The absence of the correlation between defoliation percentages and either nutrient concentration or Ingestad percentages for the Greek fir means that other factors are more critical to defoliation. Audley et al. (1998) found that foliar N and P contents were negatively correlated with defoliation percentages in a red spruce stand in West Virginia. However, the majority of the trees of that red spruce stand were healthy and the defoliation percentages did not exceed 10%. In our work the adverse nature of calcareous soils can be a critical factor. Ewald (2005) found that the crown transparency of Norway spruce in the Bavarian part of the northern Calcareous Alps increased towards shallow calcareous soils.

The significant correlations found between needle weight and N concentrations, Ca/N and Fe/N percentages in the Greek fir are a useful finding as the mean needle weight is considered a parameter for the measurement of a foliar response to fertilization because it correlates well with other parameters such as shoot growth, height increment and fibre production (Timmer, Stone 1978). The fact that N is the denominator in the last two relationships together with the positive correlation of N with needle weight can lead to the first suspicion that N can be a growth limiting factor.

The critical values for nutrients in this work have been derived from data referring to other conifers but not to fir species. For this reason the comparisons with the found concentrations in the Greek fir needles should be considered with caution. Still, they can serve as an indication of deficiencies and can be of avail in combination with the Ingestad percentages.

As Table 2 shows, apart from N, P is probably an element in short supply. Before final conclusions are drawn, the Ingestad percentages have to be examined. In all significant cases (Table 3) found in the comparison of the Ingestad percentages between the Greek and Bulgarian fir, the N element was present. Also in our work, the N content in needles was found below the critical value and the needle weight was correlated significantly and positively with the N content. So the first conclusion can be that N is a limiting factor. Phosphorus was implicated in the critical levels and in the percentage P/N. Also it is a nutrient that can be considered to limit growth. The other elements Ca, Fe and Mn are also presented in Tables 2 and 3. However, all these elements are related with N (Ingestad percentages). As Sumner (1978) argued, the ratio of nutrients or Ingestad percentage is the quotient of a numerator and a denominator and thus a mere statement of their relative proportions and does not give any information about the actual magnitudes of either element. In our case, the concentrations of Ca, Mg, and Mn may or may not be at their optimum levels as N is probably in deficiency. So no definite assessments for Ca, Mg and Mn can be done.

In conclusion, priority should be given to N and P. During reforestation fir seedlings will be fertilized but they are usually left on their own when they become young trees. It is important to check for imbalances at that stage and if possible to apply fertilization until they achieve a robust growth. The critical values and the Ingestad percentages are the first indications of nutrient deficiencies but the final answer will be given by the response of plants to the fertilizer applications.

Acknowledgements

The author expresses his sincere thanks to Dr. P. Tsopelas, coordinator of the project that financed this research, Dr. A. Economou and Mrs. Voulala for their help with field sampling as well as Mrs. S. Kassioti and C. Mitropoulou for their contribution to the analytical work.

References

Audley D.E., Skelly J.M., Mccrmick L.H, Jackson W.A. (1998): Crown condition and nutrient status of red spruce (*Picea Rubens* Sarg.) in West Virginia. Water, Air and Soil Pollution, ***102***:177–199.

Bangroo S.A., Bhat M.I., Ali T., Bhat M.A., Wani M.A. (2010): Diagnosis and recommendation integrated system (DRIS): A review. International Journal of Current Research, ***10***: 84–97.

Ewald J. (2005): Ecological background of crown condition, growth and nutritional status of *Picea abies* (L.) Karst in the Bavarian Alps. European Journal of Forest Research, ***124***: 9–18.

FAO-UNESCO (1988): Soil Map of the World. Rome, FAO-UNESCO: 119.

Gregoire N., Fisher R.F. (2004): Nutritional diagnoses in loblolly pine (*Pinus taeda* L.) established stands using three different approaches. Forest Ecology and Management, ***203***: 195–208.

Ingestad T. (1970): A definition of optimum requirements in birch seedlings. I. Physiologia Plantarum, ***23***: 1127–1138.

Joslin J.D., Wolfe M.H. (1994): Foliar deficiencies of mature southern Appalachian red spruce determined from fertilizer trials. Soil Science Society of American Journal, ***58***: 1572–1579.

ICP-Forests (2013): ICP Forests Manual. Available at http://icp-forests.net/page/icp-forests-manual

Linder S. (1995): Foliar analyses for detecting and correcting nutrient imbalances in Norway spruce. Ecological Bulletins, ***44***: 178–190.

Markalas S. (1992): Site and stand factors related in mortality rate in a fir forest after a combined incidence of drought and insect attack. Forest Ecology and Management, ***47***: 367–374.

Michopoulos P., Economou A., Nikolis N. (2004): Soil extractable manganese and uptake in a natural fir stand grown on calcareous soils. Communications in Soil Science and Plant Analysis, ***35***: 233–241.

Michopoulos P., Baloutsos G., Economou A., Voulala M., Bourletsikas A. (2007): Bulk and throughfall deposition chemistry in three different forest ecosystems. Fresenius Environmental Bulletin, ***16***: 91–98.

Morrison I.K. (1974): Mineral Nutrition of Conifers with Special Reference to Nutrient Status Interpretation: A Review of Literature. Ottawa, Canadian Forestry Service: 74.

Potočić N., Ćosić T., Pilaš I. (2005): The influence of climate and soil properties on calcium nutrition and vitality of silver fir (*Abies alba* Mill.). Environmental Pollution, ***137***: 596–602.

Richards B.N., Bevege D.I. (1972): Principles and practices of foliar analysis as a basis for crop-logging in pine plantations: I. Basic considerations. Plant and Soil, ***36***: 109–119.

Schleppi P., Tobler L., Bucher J.B, Wyttenbach A. (2000): Multivariate interpretation of the foliar chemical composition of Norway spruce (*Picea abies*). Plant and Soil, ***219***: 251–262.

Sumner M.E. (1978): Interpretation of nutrient ratios in plant tissue. Communications in Soil Science and Plant Analysis, ***9***: 335–345.

Thelin G., Rosengren-Brinck U., Nihlgård B., Barkman A. (1998): Trends in needle and soil chemistry of Norway spruce and Scots pine stands in South Sweden 1985–1994. Environmental Pollution, ***99***: 149–158.

Tessier J.K., Raynal D.J. (2003): Use of nitrogen to phosphorus ratios in plant tissue as an indicator of nutrient limitation and nitrogen saturation. Journal of Applied Ecology, ***40***: 523–534.

Timmer V.R., Stone E.L. (1978): Comparative foliar analysis of young balsam fir fertilized with N, P, and lime. Soil Science Society of American Journal, ***42***: 125–130

Ulrich A., Hills F.J. (1967): Principles and practices of plant analysis. In: Hardy G.W., Ripie D., George A.G., Morse M.D. (eds): Soil Testing and Plant Analysis. Madison, Soil Science of America: 1–24.

Received for publication September 11, 2011
Accepted after corrections May 17, 2012

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