

Critical Nutrient Concentrations and DRIS Norms for *Pinus patula*

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Abstract: *Pinus patula* is one of the most planted wood conifer species worldwide; however, no foliar nutrient standards exist for this species up to date. The objective of the present study was to generate and verify two sets of foliar nutrient standards for nearly ten-year-old *P. patula* trees: critical nutrient concentrations and DRIS norms. Nutrients studied were N, P, K, Ca, Mg, Fe, Cu, Zn, Mn, and B. The reference standards were verified experimentally by installing two fertilization trials; one of them located in Huayacocotla, state of Veracruz and the other one in Aquixtla, state of Puebla, Mexico. Nutrient status of each fertilization trial was correctly predicted by critical nutrient values and DRIS as well. Both standards were able to detect the secondary growth-limiting nutrient deficiency in the Huayacocotla trial, where the primary limitation for growth was scarcity of solar radiation within tree crowns. The limiting nutrient in both experimental trials was K.

Keywords: plant nutrition; chemical fertilization; nutrient diagnosis; forest plantation; foliar nutrients

1. Introduction

Use of chemical fertilizers in intensively managed forest plantations is a key factor to increase productivity of commercial species such as *Pinus patula* Schiede ex Schlechtendal & Chamisso, particularly when it is combined with management practices that decrease inter and intraspecific competition for above and belowground resources [1]. Choice of the appropriate type of fertilizer, dose, and application method require knowledge of the stand nutrient status, since each site has its own soil and climate properties, and nutrient requirements vary among tree species [2,3]. However, implementation of nutrient diagnosis procedures to determine the nutrient status of forest plantations generally requires knowledge of nutrient standards for the nutrients and species being managed. At present, there are few studies on nutrition of *P. patula* [4] that can provide some light on nutrient critical levels in foliage; however, in a strict sense, no nutrient standards are available for this species in the literature.

Among the most used nutrient diagnosis methods in forest plantations, foliar nutrient critical concentrations and DRIS (Diagnosis and recommendation Integrated System, [5]) are included. Foliar critical concentration of a nutrient is the concentration below which, plant growth is limited by that nutrient. When concentration of a particular nutrient in a plant tissue is above the critical concentration, positive responses of plants after addition of such nutrient might not occur [6]. On the other hand, DRIS is a nutrient diagnosis procedure that takes into account the plant internal nutrient balance among the various nutrients. This procedure is based on the theory that plant nutrient status

41 varies less when plants reach their potential growth rate [7]. Although DRIS has been used much
42 more extensively in agricultural species, it has also been used in forest ones [8,9,10,3].

43 Because of its rapid growth rate, good wood quality, and extension of the area planted especially
44 in the southern hemisphere, *P. patula* is an outstanding conifer species [11]. Its wood is used to make
45 highly resistant products (fence posts, railroad ties, beams, and packing boxes, among others) and
46 aesthetic interior and exterior finishes as well. Because of its wood fiber characteristics, it has also
47 been used for manufacture of paper [12,13].

48 The high biomass accumulation rate of *P. patula*, necessarily implies that its demand for nutrients
49 is also high, as compared with that of slow-growing conifer species. This is why, the sustainability of
50 high productivity rates of *P. patula* plantations, generally requires the integration of fertilization
51 programs to the silvicultural system. Nonetheless, the definition of a fertilization program needs
52 information about the nutritional standards for the species. The aim of the present study was to
53 generate and verify two types of nutrient standards: critical concentrations and DRIS norms for
54 nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe),
55 Zinc (Zn), manganese (Mn), and boron (B) in *P. patula* saplings.
56

57 2. Materials and Methods

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59 Data for derivation of critical concentrations and DRIS norms were obtained from four
60 municipalities of the state of Puebla, Mexico (Ahuazotepec, Aquixtla, Chignahuapan and Zacatlán),
61 four municipalities of the state of Hidalgo, Mexico (Acaxochitlán, Agua Blanca, Metepec and
62 Zacualtipán), and one municipality of the state of Veracruz (Huayacocotla). Among other geographic
63 areas in Mexico, *P. patula* is native to these sites. By September and October 2011, a trip was carried
64 out all over the mentioned area to select 50 *P. patula* trees 15 to 17 cm in diameter at breast height
65 (DBH). Geographical location and DBH data were recorded for each tree. Additionally, a foliar
66 sample was obtained from each of the trees, by following the protocol indicated by [1]. In October
67 2012, the DBH was measured again in order to determine the annual increments in DBH (IDBH).
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69

69 Foliar samples were processed in the Soil Fertility Laboratory of Colegio de Postgraduados,
70 Mexico. Foliar nutrients determined were nitrogen (N), phosphorus (P), potassium (K), calcium (Ca),
71 magnesium (Mg), copper (Cu), iron (Fe), Zinc (Zn), manganese (Mn), and boron (B). N was
72 determined by the semi-micro-Kjeldahl method [14]. The remaining nutrients were determined by
73 digesting the material with a mixture of nitric and perchloric acids (1:2 at 210 °C [15]. P was quantified
74 colorimetrically, by the molibdivanadate method, while the remaining nutrients were determined by
75 atomic absorption spectrophotometry [16]. With foliar nutrient concentrations and IDBH data for
76 each tree, a database was generated, where critical concentrations and DRIS norms were developed
77 from.
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79 To determine the critical concentrations and DRIS norms, the database was sorted by IDBH, and
80 divided into two sub-populations: low and high yielding. The high yield sub-population included 16
81 % of the observations in the database. This proportion is close to the one suggested by [9,17]. Critical
82 concentrations and DRIS norms were developed exclusively from the high yield sub-population.
83

84 Verification of the critical concentrations and DRIS norms

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86 Verification of the nutritional standards generated was done by using two fertilization
87 experiments; one located at Huayacocotla, Veracruz and the other at Aquixtla, Puebla, Mexico. The
88 Huayacocotla plantation (20° 27' 19.59" N, 98° 29' 30.59" W; 2409 m above sea level) is located at Ejido
89 Palo Bendito, where climate is cold temperate with the rainy season during the summer time. Mean
90 annual temperature is between 16 and 18 °C and annual precipitation varies from 600 to 1200 mm.
91 Main soil type is acid andosol [18]. Dominant vegetation types at Palo Bendito are pine forest (mainly
92 *Pinus montezumae* Lamb., *P. pseudostrobus* Lndl. and *P. Leiophylla* Schl. et Cham., [18]) and pine-oak
93 forest [19]. Among the broad-leaved tree species are *Alnus arguta* (Schltdl.) Spach. [19] and *Quercus*
94 *laurina* Humb. et Bonpl. The Aquixtla study site is at 19° 44' 27.7" N y 98 ° 00' 8.7" W, with elevation
95 being 2840 m above sea level. Soils are moderately deep with sandy loam texture [20] and vegetation
96 type is pine forest.

97 In the Huayacocotla study site, a fertilization experiment with N, P, and K was established in
98 2011. The experiment was a factorial (3X3X2) set of treatments established under a complete
99 randomized design. Factors tested were N, P, and K with three levels (doses) for N (0, 150, and 300 g
100 urea per tree) and P (0, 35, and 70 g triple superphosphate per tree) and two levels for K (0 and 25 g
101 potassium sulfate per tree). Treatments were replicated ten times and the experimental unit was a
102 tree 18 ± 3 cm in DBH.

103 Tree spacing in the plantation was 2.30 X 3.0 m and fertilizers were applied broadcast within the
104 drip zone of the selected trees. After the application of the treatments, DBH was measured every six
105 months. In October 2012, three trees were randomly chosen from each treatment and a foliage sample
106 was obtained from each of them. Foliar samples were sent to the laboratory for N, P, and K
107 determination. Foliar samples were collected from the highest third of tree crowns, as recommended
108 by [1-21]. Chemical analysis procedures were the same described above for foliar samples used to
109 develop critical concentrations and DRIS norms.

110 At Aquixtla, Puebla, the experiment was a complete randomized one with four treatments and
111 three replicates per treatment. The experimental unit was a tree, approximately 15 years old. The
112 treatments tested were fertilization with: 1) nitrogen (250 g urea per tree), 2) phosphorus as triple
113 superphosphate (240 g TSP per tree), and 3) potassium (140 g potassium sulfate per tree), and 4) no
114 fertilization. Treatments were applied on September 19, 2012. In February 2014, one foliar sample per
115 replicate was collected to determine N, P, and K concentrations, by using the above mentioned
116 laboratory methods. In September 2014, the annual IDBH was evaluated.

117

118 Statistical analyses

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120 Data sorting and generation of sub-populations for development of critical concentrations and
121 DRIS norms were carried out by using EXCEL ver. 2007. Data from the experiments for verification
122 of critical concentrations and DRIS norms were processed by analysis of variance [22] according to
123 the model:

124

$$125 Y_{ijk} = \mu + N_i + P_j + K_k + NP_{ij} + NK_{ik} + PK_{jk} + NPK_{ijk} + \epsilon_{ijk} \quad (1)$$

126

127 Where:

128 Y_{ijk} : response to treatment with the levels i, j, k of the factors tested; μ : general mean; N_i : effect of
 129 nitrogen; P_j : effect of phosphorus; K_k : effect of potassium, and ε_{ijk} : random error.

130 DRIS computations were carried out by using the software NUTRIDRIS (Colegio de
 131 Postgraduados), recommended by [23].

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133

134 3. Results

135

136 Critical nutrient concentrations (Table 1) and DRIS norms (Table 3) for *P. patula* saplings were
 137 generated in order to help silviculturists to study the nutrient status of saplings of this species and
 138 decide, in a particular situation, what fertilization treatments to apply.

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141 3.1. Critical nutrient concentrations for *P. patula* saplings

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143 The critical nutrient concentrations obtained indicate that the nutrients most highly required by
 144 *P. patula*, during its sapling stage, are nitrogen and potassium (Table 1). However, K critical
 145 concentration is only high in absolute terms. In fact, when related to nutrients such as N, it is really
 146 quite low (high foliar N/K ratio, Table 3), which agrees with the finding by [24] for the case of *P. patula*
 147 during the nursery stage. Among micronutrients, Mn seems to be the most required followed by Fe.
 148 Although essential, the nutrient required in the lowest concentrations is Cu.

149

150 Table 1. Preliminary leaf critical concentrations (CC) for *Pinus patula* saplings.

	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn	B
	%					ppm				
CC	1.49	0.13	0.63	0.33	0.14	118.69	2.14	30.60	187.47	11.25

151

152 Regarding yields of the sub-populations derived from the database, trees included in the high-
 153 yielding sub-population showed higher increment of diameter at breast height (IDBH) than those
 154 from the low yield sub-population (Table 2). The higher IDBH in the high yield sub-population
 155 suggests that the corresponding trees probably grew under better climate, soil, and management
 156 conditions than the trees from the low yield sub-population.

157

158 Table 2. Comparison of diameter increment at breast height (IDBH) between subpopulations.

Subpopulation	N	Mean IDBH	Pr>F	Pr>t
High yielding	7	9.60	0.015	0.0001
Low yielding	43	6.00		

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160

161 3.2. DRIS norms for *P. patula* saplings

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163 The set of DRIS standards produced in the present study is composed of 45 nutrient ratios with
 164 a balanced contribution of each of the nutrients to the whole set (Table 3). The DRIS norm set is
 165 conformed by the means and variation coefficients of the nutrient ratios from the high-yielding sub-
 166 population.

167 It is worth noticing that derivation of the macronutrient/micronutrient ratios was done by using
 168 % (of dry matter weight) to express concentration of macronutrients and ppm for micronutrients.

169

170 Table 3. DRIS norms for *Pinus patula* saplings ten years of age.

Nutrient ratio	Mean	C.V.	Nutrient ratio	Mean	C.V.
N/P	11.400	18.9	K/B	0.059	25.0
N/K	2.383	14.1	Ca/Mg	2.458	24.2
N/Ca	4.998	32.6	Ca/Fe	0.003	49.4
N/Mg	12.332	43.9	Ca/Cu	0.491	122.5
N/Fe	0.015	42.6	Ca/Zn	0.012	35.6
N/Cu	1.710	107.6	Ca/Mn	0.001	39.2
N/Zn	0.055	40.5	Ca/B	0.030	40.1
N/Mn	0.006	51.9	Mg/Fe	0.001	53.0
N/B	0.138	23.6	Mg/Cu	0.219	133.5
P/K	0.211	12.9	Mg/Zn	0.005	52.5
P/Ca	0.442	32.4	Mg/Mn	0.001	31.8
P/Mg	1.062	32.8	Mg/B	0.013	44.1
P/Fe	0.001	43.5	Fe/Cu	181.976	151.1
P/Cu	0.164	112.6	Fe/Zn	4.621	52.2
P/Zn	0.005	45.0	Fe/Mn	0.442	52.1
P/Mn	0.001	33.3	Fe/B	11.034	48.1
P/B	0.012	26.4	Cu/Zn	0.059	74.5
K/Ca	2.120	35.9	Cu/Mn	0.010	99.6
K/Mg	5.152	41.1	Cu/B	0.203	76.1
K/Fe	0.006	39.0	Zn/Mn	0.130	72.0
K/Cu	0.793	115.9	Zn/B	2.829	40.5
K/Zn	0.024	43.3	Mn/B	26.923	49.7
K/Mn	0.003	41.2			

171

172 Table 3 shows that nutrient ratios involving copper generally exhibit high coefficients of
 173 variation, thus indicating that Cu is probably highly variable within the *P. patula* foliage.

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175

176 3.3. Verification of critical concentrations

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178 Table 4 shows the process for the verification of the critical concentrations using the fertilization
 179 experiment installed in Huayacocotla, Veracruz, Mexico. N, P, and K concentrations in the control
 180 treatment were 1.79, 0.16, and 0.52 %, respectively. When compared with the critical concentrations
 181 (Table 1), N and P concentrations resulted to be sufficient, while K concentration corresponded to the
 182 deficiency level; that is, foliar K concentration in the control trees (0N, 0P, 0K) is lower than the critical
 183 concentration.

184 Among the treatments applied in the fertilization experiment there is a treatment consisting of
 185 the application of K only. If the critical concentration set produced in the present study correctly
 186 predicts the nutrient status of *P. patula*, then fertilization with K, according to the "Liebig's Law of
 187 the Minimum", should result in an improvement of the response variable (IDBH). In fact, the
 188 treatment 0N, 0P, and 25K resulted in a slightly higher value for IDBH. Nonetheless, K continues to
 189 be the deficient nutrient in those trees. This means that application of the K treatment was adequate,
 190 even though the applied dose (25 g K₂SO₄ tree⁻¹) was insufficient to correct the deficiency detected in
 191 the treatment 0N, 0P, 0K. Unfortunately, the experiment included only two levels of K, and it was not
 192 possible to amend the K deficiency remaining after the application of K.

193

194 Table 4. Verification of the *Pinus patula* critical concentrations: Huayacocotla experiment.

Treatment*			Foliar concentration (%)			Nutrient status			IDBH (cm y ⁻¹)
N	P	K	N	P	K	N	P	K	
0	0	0	1.79	0.16	0.52	S	S	D	0.52
0	0	25	1.81	0.18	0.58	S	S	D	0.55
CC			1.49	0.13	0.63				

195 *Grams of fertilizer material per tree; S: sufficient; D: deficient; CC: Critical concentration

196

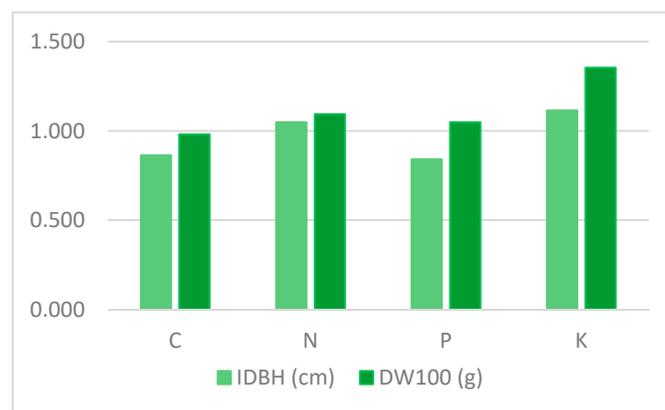
197 The IDBH augmented from 0.52 to 0.55 cm when a dose of 25 g K₂SO₄ per tree was applied.
 198 This means that the set of critical concentrations generated in the present study correctly predicted
 199 the K deficiency. Consequently, when this nutrient was applied, the trees positively responded by
 200 rising the IDBH.

201 It is worth stressing that the change in IDBH resulting from the application of the deficient
 202 nutrient (K) was quite slight (5.45 % of control). This is probably due to the high stand density in the
 203 experimental site. In fact, tree spacing in the plantation is 2.30 X 3.00 and, at present, tree heights
 204 are about 20 m. Under these conditions, incident solar radiation within tree crowns is likely to be the
 205 most limiting factor for growth because of mutual shading among crowns. If this effect is taking place
 206 in the experimental plantation, then the nutrient deficiencies could be just secondary limiting factors,
 207 whose amendment, according to the Liebig's law of the minimum, is not likely to result in spectacular
 208 responses in terms of growth [25,26].

209 It is worth noticing that the second treatment analyzed (Table 4) showed higher N and P
 210 concentrations than those of the control trees, even when neither N nor P were applied. This behavior
 211 could be the result of a random effect, but it could also be an effect of a higher N and P absorption
 212 brought about by a higher underground biomass resulting from the application of K.

213 As in the case of the Huayacocotla fertilization experiment, the one in Aquixtla, Puebla, Mexico
 214 shows a higher response in the trees that received K in comparison with the other treatments,
 215 including the control trees (no fertilization, Figure 1). This means that the limiting nutrient in the
 216 Aquixtla site probably is K. On the other hand, the comparison of concentrations of control trees with
 217 the species critical concentrations indicates that P and K are the deficient nutrients in the site (Table
 218 5). According to tree responses to application of nutrients (Figure 1), P is sufficient or maybe slightly
 219 deficient, since such response is only slightly higher than that of the control trees as judged by the
 220 dry weight of 100 needles (DW100). Accordingly, it is feasible to state that the set of critical
 221 concentrations determined in the present study, does correctly predict P deficiencies in *P. patula*.

222 In the case of K, there exists a total congruence between the diagnosis based on tree response to
 223 application of K (Figure 1) and the one derived from the critical concentrations generated in the
 224 present study (Table 5). This indicates that our critical concentration set correctly predicts the nutrient
 225 status of *P. patula* saplings and it allow us to detect the growth-limiting nutrient. Consequently, we
 226 fully recommend the use of the set of critical nutrient concentrations generated to diagnose the
 227 nutrient status and prescribe fertilization treatments on *P. patula* trees or stands.
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229
 230 Figure 1. IDBH and DW100 eight months after fertilization with C (control), N, P, and K at
 231 Aquixtla, Puebla, Mexico.

232

233 Table 5. Verification of the *Pinus patula* critical concentrations: Aquixtla experiment.

	Foliar concentration (%)			Diagnosis			IDBH (cm y ⁻¹)
	N	P	K	N	P	K	
Critical concentration	1.49	0.12	0.63				
Control	1.62	0.08	0.21	S	D	D	0.863
Treatment with K	1.62	0.09	0.19	S	D	D	1.115

234 S: sufficient; D: deficient; IDBH: Increment of diameter at breast height

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236 Besides helping detect the growth-limiting nutrients, Table 5 demonstrates that correction of the
 237 K deficiency improved the IDBH. This confirms the deficiency of K in the Aquixtla site and shows
 238 the goodness of the set of critical nutrient concentrations derived in the present study to determine
 239 the nutrient status and prescribe fertilization treatments in trees or stands of *Pinus patula*.

240

241 3.4. Verification of the DRIS norms

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243 According to the process for verification of the DRIS norms by using the Huayacocotla
 244 fertilization experiment (Table 6), the DRIS indices of the control trees indicate that they are deficient
 245 in K (negative indices, Table 6). This fact coincides with the diagnosis derived from the critical
 246 concentrations for the same site. The correction of this deficiency with the treatment 0N, 0P, 25K
 247 contributed to improve IDBH, meaning that prediction by the DRIS norm set was right. Nonetheless,
 248 the improvement of the IDBH was quite slight probably due to scarcity of solar radiation within tree
 249 crowns, as explained above.

250

251 Table 6. Verification of the *Pinus patula* DRIS norms: Huayacocotla experiment.

Treatment*			Foliar concentration (%)			DRIS index			IDBH (cm y ⁻¹)
N	P	K	N	P	K	N	P	K	
0	0	0	1.79	0.16	0.52	14.0	19.0	-33.0	0.52
0	0	25	1.81	0.18	0.58	7.5	22.1	-29.6	0.55

252 *grams of fertilizer material (urea for N, TSP for P, and potassium sulphate for K) per tree.

253

254

255 Even though the nutrient diagnosis methods tested suggest K deficiency in the Huayacocotla
 256 experimental plantation, the analysis of variance (Table 7) shows that IDBH after the application of
 257 the fertilization treatments was statistically the same ($P > 0.515$) in all treatments (including
 258 fertilization with K). The lack of significance of the effect of treatments is consistent with the low
 259 IDBH values obtained with the application of the deficient nutrient (K) as diagnosed by critical
 260 concentrations and DRIS.

261

262 Table 7. ANOVA for increment of diameter at breast height of *Pinus patula*: Huayacocotla trial.

Source of variation	DF	SS	MSE	F	P>F
Model	17	2.59	0.15	0.95	0.515
N	2	0.56	0.28	1.77	0.175
P	2	0.13	0.07	0.42	0.656
K	1	0.17	0.17	1.08	0.302
N*P	4	0.24	0.06	0.38	0.822
N*K	2	0.22	0.11	0.69	0.503
P*K	2	0.36	0.18	1.14	0.324
N*P*K	4	0.89	0.22	1.39	0.240
Error	118	18.85			

263 DF: Degrees of freedom; SS: Square sum; MSE: Mean square error.

264

265 DRIS norm set verification from the Aquixtla fertilization experiment (Table 8) indicates that K
 266 is the growth-limiting nutrient in this experimental site. This diagnosis agrees with the one obtained
 267 with the critical concentration set. In fact, according to Table 8, K was the growth-limiting nutrient in
 268 all treatments (negative indices) including even the treatment with K, which means that the K dosage

269 applied was insufficient to correct the K deficiency. The same table also shows that treatments with
 270 N, P, or K contributed to reduce the IDBH relative to control trees, being the treatment with K the
 271 one that reduced the least the IDBH. The DW100 also was reduced by the N and P treatments.
 272 However, application of K contributed to increase DW100 relative to control, thus confirming that K
 273 is the growth-limiting nutrient in the Aquixtla study site.

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Table 8. Verification of the *Pinus patula* DRIS norms: Aquixtla trial.

Fertilization treatment	Foliar concentration (%)			DRIS index			IDBH (cm y ⁻¹)	DW100 (g)
	N	P	K	N	P	K		
C	1.624	0.086	0.207	98.6	20.2	-118.8	0.861	3.222
N	1.670	0.084	0.210	102.2	15.6	-117.9	0.667	3.170
P	1.649	0.094	0.230	85.7	21.5	-107.2	0.733	3.146
K	1.618	0.089	0.194	104.1	29.3	-133.4	0.806	4.062

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278 4. Discussion

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290 In absolute terms, the critical K concentration for *P. patula* is high (0.63); however, when related
 291 to nutrients such as N, it is rather low (high foliar N/K ratio, Table 3), which agrees with the finding
 292 by [24] for the case of *P. patula* nursery seedlings.

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301 As compared with DRIS norms for conifer species such as *Abies religiosa* Schl. et Cham. [8], the
 302 N/K ratio for *P. patula* resulted too high (2.383 Vs. 1.779 for *P. patula* and *A. religiosa*, respectively),
 303 which can only be explained by a low K requirement by *P. patula*, since even the critical N
 304 concentration is lower in *P. patula* than in *A. religiosa* (1.49 Vs. 1.55, respectively). The N/P ratio (11.4)
 for *P. patula* is too high when compared with that reported for *Pinus radiata* D. Don (9.3 [30]). This
 indicates that P requirement by *P. patula* is probably lower than that of *P. radiata*. The differences in
 nutrient ratios among conifer species come from the differences in nutrient requirements among
 plant species, and suggest that we should develop particular DRIS norms for each tree species.

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Regarding the Huayacocotla experiment for verification of the critical concentrations generated
 in the present study, Table 4 shows that and improved IDBH was obtained when the deficient
 nutrient (K) was applied, thus indicating that our critical concentrations correctly predict tree nutrient
 status. Certainly, the improvement of the response variable was slight (5.45 % of control), thus

305 indicating that a factor other than K, primarily limited tree growth [25,26]. Solar radiation within tree
306 crowns was likely to be the above mentioned factor, since stand density (2.3 X 3.0 m) was too high as
307 related to tree height (about 20 m) during the experimental period.

308 Even with the masking effect of light limitation, our critical concentrations were able to find the
309 secondary limiting factor (K) which means that this critical concentration set is probably highly
310 efficient at determining *P. patula* nutrient status.

311 One additional reason for the limited tree-growth response to the application of the limiting
312 nutrient (K; Table 4) may be the low dose of K applied (25 g of K₂SO₄). If this was the case, such
313 behavior could be interpreted as a high sensitivity of our critical concentrations set to detect tree
314 nutrient status.

315 The Aquixtla experiment showed coincidence between diagnoses based on tree response
316 analyses and those derived from application of our critical concentrations. Both procedures indicated
317 that K was the limiting nutrient in that study site. Consequently, we fully recommend the use of the
318 set of critical nutrient concentrations generated to diagnose the nutrient status and prescribe
319 fertilization treatments on *P. patula* trees or stands.

320 Regarding DRIS, this diagnosis technique has been used mainly for nutrient diagnosis of
321 agricultural crops and fruit trees [31,32], and there are DRIS norms for many of such crops; however,
322 there exist DRIS norms only for the most important forest species such as teak [33] and some eucalypt
323 species [23] among other few ones. The scarcity of DRIS norms for forest species has limited the
324 number of studies using DRIS in forest tree species [8, 9]. The correct predictions by the DRIS norm
325 set generated in the present study suggest that such set can be used to predict the nutrient status of
326 any *P. patula* plantation approximately 10 years of age, taking into account that there are evidences
327 that nutrient balance within tree foliage may change with tree age [29,34].

328 As discussed before, the small responses to correction of deficiencies shown during the processes
329 of verification of both critical concentrations and DRIS norms are probably a reflection of the high
330 tree density in the Huayacocotla experimental plantation. High tree density is likely to be promoting
331 competition for light among tree crowns, so that this factor probably has become the main growth-
332 limiting factor. If this effect is occurring, then, according to the low of the minimum, responses to the
333 application of nutrients are expected to be low [25] as was the case in this study. Results from the
334 analysis of variance (Table 7) suggest that the nutrient diagnosis procedures tested in this research
335 work are able to detect the most growth-limiting nutrient even when another primary limiting factor
336 such as inter-crown-competition-generated light scarcity is present.

337 The Aquixtla experiment helped confirm that our DRIS norms correctly predict tree nutrient
338 status since they detected K deficiency, which is in full agreement with the finding by means of the
339 critical concentration set.

340 Based on the findings in the present study, we can state that the DRIS norms generated in the
341 present study, correctly predict the nutrient status of *P. patula* saplings, help detect the deficient
342 nutrient, and allow prescribe fertilization treatments that will eventually increase tree growth rates.
343

344 5. Conclusions

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346 A set of critical nutrient concentrations and one of DRIS norms, both for N, P, K, Ca, Mg, Fe, Cu,
347 Zn, Mn, and B in foliage of *Pinus patula* saplings were generated. The processes of verification of the

348 sets suggest that they correctly predict the nutrient status of *P. patula* saplings, even when sunlight
349 scarcity throughout tree crowns limits tree growth. This points out the power of the nutrient
350 standards generated to determine the limiting nutrient in any *P. patula* plantation about ten years old,
351 as well as their usefulness to help foresters increase productivity of patula pine plantations. The
352 nutrient diagnosis methods coincided to diagnose the growth-limiting nutrients in the Huayacocotla
353 plantation as well as in the Aquixtla one. K is the limiting nutrient in both experimental plantations.
354 Based on the nutrient diagnosis carried out we suggest to correct the K deficiency in the Huayacocotla
355 plantation by using a potassium sulphate dose higher than 25 g per tree, along with a thinning
356 treatment. This will allow us to redistribute the site resources (sunlight and nutrients). In the case of
357 the Aquixtla plantation we recommend to apply a potassium sulphate dose higher than 140 g per
358 tree. Diagnosis of the nutrient status of *P. patula* plantations by means of critical nutrient
359 concentrations and/or DRIS is useful to prescribe fertilization treatments that allow us to increase
360 yields.

361

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369

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374

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379 References

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