Critical Nutrient Concentrations and DRIS Norms for

**Pinus patula**

Agustina Sánchez-Parada 1, Miguel Ángel López-López 1,*, Armando Gómez-Guerrero 1 and
Marlín Pérez-Suárez 2

1 Colegio de Postgraduados, Mexico; lopezma@colpos.mx
2 Universidad Autónoma del Estado de México; marpersua@gmail.com
* Correspondence: lopezma@colpos.mx; Tel.: +52-595-952-0200

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

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

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

2. Materials and Methods

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

Foliar samples were processed in the Soil Fertility Laboratory of Colegio de Postgraduados, Mexico. Foliar nutrients determined were nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), copper (Cu), iron (Fe), Zinc (Zn), manganese (Mn), and boron (B). N was determined by the semi-micro-Kjeldahl method [14]. The remaining nutrients were determined by digesting the material with a mixture of nitric and perchloric acids (1:2 at 210 °C [15]. P was quantified colorimetrically, by the molibdovanadate method, while the remaining nutrients were determined by atomic absorption spectrophotometry [16]. With foliar nutrient concentrations and IDBH data for each tree, a database was generated, where critical concentrations and DRIS norms were developed from.

To determine the critical concentrations and DRIS norms, the database was sorted by IDBH, and divided into two sub-populations: low and high yielding. The high yield sub-population included 16 % of the observations in the database. This proportion is close to the one suggested by [9,17]. Critical concentrations and DRIS norms were developed exclusively from the high yield sub-population.
Verification of the critical concentrations and DRIS norms

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

In the Huayacocotla study site, a fertilization experiment with N, P, and K was established in 2011. The experiment was a factorial (3X3X2) set of treatments established under a complete randomized design. Factors tested were N, P, and K with three levels (doses) for N (0, 150, and 300 g urea per tree) and P (0, 35, and 70 g triple superphosphate per tree) and two levels for K (0 and 25 g potassium sulfate per tree). Treatments were replicated ten times and the experimental unit was a tree 18 ± 3 cm in DBH. Tree spacing in the plantation was 2.30 X 3.0 m and fertilizers were applied broadcast within the drip zone of the selected trees. After the application of the treatments, DBH was measured every six months. In October 2012, three trees were randomly chosen from each treatment and a foliage sample was obtained from each of them. Foliar samples were sent to the laboratory for N, P, and K determination. Foliar samples were collected from the highest third of tree crowns, as recommended by [1-21]. Chemical analysis procedures were the same described above for foliar samples used to develop critical concentrations and DRIS norms.

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

Statistical analyses

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

\[ Y_{ijkl} = \mu + N_i + P_j + K_k + NP_{ij} + NK_{ik} + PK_{jk} + NPK_{ijk} + \epsilon_{ijkl} \] (1)
Where:

- $Y_{ijk}$: response to treatment with the levels i, j, k of the factors tested;
- $\mu$: general mean;
- $N_i$: effect of nitrogen;
- $P_j$: effect of phosphorus;
- $K_k$: effect of potassium, and
- $\epsilon_{ijk}$: random error.

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

3. Results

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

3.1. Critical nutrient concentrations for $P.\ patula$ saplings

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

Table 1. Preliminary leaf critical concentrations (CC) for $Pinus\ patula$ saplings.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>1.49</td>
<td>0.13</td>
<td>0.63</td>
<td>0.33</td>
<td>0.14</td>
<td>118.69</td>
<td>2.14</td>
<td>30.60</td>
<td>187.47</td>
<td>11.25</td>
</tr>
</tbody>
</table>

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

Table 2. Comparison of diameter increment at breast height (IDBH) between sub-populations.

<table>
<thead>
<tr>
<th>Subpopulation</th>
<th>N</th>
<th>Mean IDBH</th>
<th>Pr&gt;F</th>
<th>Pr&gt;t</th>
</tr>
</thead>
<tbody>
<tr>
<td>High yielding</td>
<td>7</td>
<td>9.60</td>
<td>0.015</td>
<td>0.0001</td>
</tr>
<tr>
<td>Low yielding</td>
<td>43</td>
<td>6.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2. DRIS norms for *P. patula* saplings

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

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

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

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

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

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

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

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

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Foliar concentration (%)</th>
<th>Nutrient status</th>
<th>IDBH (cm y⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

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

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

It is worth noticing that the second treatment analyzed (Table 4) showed higher N and P concentrations than those of the control trees, even when neither N nor P were applied. This behavior could be the result of a random effect, but it could also be an effect of a higher N and P absorption brought about by a higher underground biomass resulting from the application of K.
As in the case of the Huayacocotla fertilization experiment, the one in Aquixtla, Puebla, Mexico shows a higher response in the trees that received K in comparison with the other treatments, including the control trees (no fertilization, Figure 1). This means that the limiting nutrient in the Aquixtla site probably is K. On the other hand, the comparison of concentrations of control trees with the species critical concentrations indicates that P and K are the deficient nutrients in the site (Table 5). According to tree responses to application of nutrients (Figure 1), P is sufficient or maybe slightly deficient, since such response is only slightly higher than that of the control trees as judged by the dry weight of 100 needles (DW100). Accordingly, it is feasible to state that the set of critical concentrations determined in the present study, does correctly predict P deficiencies in *P. patula*.

In the case of K, there exists a total congruence between the diagnosis based on tree response to application of K (Figure 1) and the one derived from the critical concentrations generated in the present study (Table 5). This indicates that our critical concentration set correctly predicts the nutrient status of *P. patula* saplings and it allow us to detect the growth-limiting nutrient. Consequently, we fully recommend the use of the set of critical nutrient concentrations generated to diagnose the nutrient status and prescribe fertilization treatments on *P. patula* trees or stands.

Figure 1. IDBH and DW100 eight months after fertilization with C (control), N, P, and K at Aquixtla, Puebla, Mexico.

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

<table>
<thead>
<tr>
<th>Foliar concentration (%)</th>
<th>Diagnosis</th>
<th>IDBH (cm y⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
</tr>
<tr>
<td>Critical concentration</td>
<td>1.49</td>
<td>0.12</td>
</tr>
<tr>
<td>Control</td>
<td>1.62</td>
<td>0.08</td>
</tr>
<tr>
<td>Treatment with K</td>
<td>1.62</td>
<td>0.09</td>
</tr>
</tbody>
</table>

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

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

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

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Foliar concentration (%)</th>
<th>DRIS index</th>
<th>IDBH (cm y⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>0</td>
<td>1.79</td>
<td>0.16</td>
<td>0.52</td>
</tr>
<tr>
<td>0</td>
<td>1.81</td>
<td>0.18</td>
<td>0.58</td>
</tr>
</tbody>
</table>

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

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

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

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

DRIS norm set verification from the Aquixtla fertilization experiment (Table 8) indicates that K is the growth-limiting nutrient in this experimental site. This diagnosis agrees with the one obtained with the critical concentration set. In fact, according to Table 8, K was the growth-limiting nutrient in all treatments (negative indices) including even the treatment with K, which means that the K dosage
applied was insufficient to correct the K deficiency. The same table also shows that treatments with N, P, or K contributed to reduce the IDBH relative to control trees, being the treatment with K the one that reduced the least the IDBH. The DW100 also was reduced by the N and P treatments. However, application of K contributed to increase DW100 relative to control, thus confirming that K is the growth-limiting nutrient in the Aquixtla study site.

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

<table>
<thead>
<tr>
<th>Fertilization treatment</th>
<th>Foliar concentration (%)</th>
<th>DRIS index</th>
<th>IDBH (cm y⁻¹)</th>
<th>DW100 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>K</td>
<td>N</td>
</tr>
<tr>
<td>C</td>
<td>1.624</td>
<td>0.086</td>
<td>0.207</td>
<td>98.6</td>
</tr>
<tr>
<td>N</td>
<td>1.670</td>
<td>0.084</td>
<td>0.210</td>
<td>102.2</td>
</tr>
<tr>
<td>P</td>
<td>1.649</td>
<td>0.094</td>
<td>0.230</td>
<td>85.7</td>
</tr>
<tr>
<td>K</td>
<td>1.618</td>
<td>0.089</td>
<td>0.194</td>
<td>104.1</td>
</tr>
</tbody>
</table>

4. Discussion

Critical foliar nutrient concentrations and DRIS norms were derived from a high-yielding *Pinus patula* sub-population. Since high yields can only occur in the absence of limiting factors (Low of the minimum), it is possible to assume that nutrient concentrations within tree foliage in such sub-population are nearly adequate [27]. Moreover, our results indicate that both nutrient diagnosis tools generated are highly efficient at identifying the nutrient limiting growth. Our critical N concentration for *P. patula* (Table 1) is slightly higher than that reported by [28]; however, P and K critical concentrations are lower than those found in the mentioned study. Nonetheless, in other study reported by the same authors [1], P critical concentration coincided with the one determined in the present study (0.13 %), confirming that such concentration corresponds to an adequate P status for *P. patula*.

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

As compared with DRIS norms for conifer species such as *Abies religiosa* Schl. et Cham. [8], the N/K ratio for *P. patula* resulted too high (2.383 Vs. 1.779 for *P. patula* and *A. religiosa*, respectively), which can only be explained by a low K requirement by *P. patula*, since even the critical N 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.

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

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

One additional reason for the limited tree-growth response to the application of the limiting
nutrient (K; Table 4) may be the low dose of K applied (25 g of K$_2$SO$_4$). If this was the case, such
behavior could be interpreted as a high sensitivity of our critical concentrations set to detect tree
nutrient status.

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

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

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

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

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

5. Conclusions

A set of critical nutrient concentrations and one of DRIS norms, both for N, P, K, Ca, Mg, Fe, Cu,
Zn, Mn, and B in foliage of *Pinus patula* saplings were generated. The processes of verification of the
sets suggest that they correctly predict the nutrient status of *P. patula* saplings, even when sunlight scarcity throughout tree crowns limits tree growth. This points out the power of the nutrient standards generated to determine the limiting nutrient in any *P. patula* plantation about ten years old, as well as their usefulness to help foresters increase productivity of patula pine plantations. The nutrient diagnosis methods coincided to diagnose the growth-limiting nutrients in the Huayacocotla plantation as well as in the Aquixtla one. K is the limiting nutrient in both experimental plantations. Based on the nutrient diagnosis carried out we suggest to correct the K deficiency in the Huayacocotla plantation by using a potassium sulphate dose higher than 25 g per tree, along with a thinning treatment. This will allow us to redistribute the site resources (sunlight and nutrients). In the case of the Aquixtla plantation we recommend to apply a potassium sulphate dose higher than 140 g per tree. Diagnosis of the nutrient status of *P. patula* plantations by means of critical nutrient concentrations and/or DRIS is useful to prescribe fertilization treatments that allow us to increase yields.

**Acknowledgements**

We are grateful with the foresters Salvador Castro Zavala and León Jorge Castaños Martínez because of the partial financial support to our study, which was part of the project “Diagnosis of the nutrient status and fertilization recommendations for *Pinus patula* in private plantations at Fracción Rancho Chichicaxtla and Conjunto Predial Forestal Aquixtla. We also acknowledge Mr. Víctor Badillo because of the facilitation to work at Ejido Palo Bendito, Huayacocotla, Veracruz.

**Author Contributions:** Mrs. Sánchez-Parada and Mr. López-López identified the problem and suggested the study. All authors discussed and conceived the methodologies to use. The experiments were conducted by Mrs. Sánchez-Parada and Mr. López-López. All authors contributed to data analyses. Mrs. Sánchez-Parada wrote the paper, which was improved by Drs. López-López, Gómez-Guerrero, and Pérez-Suárez.

**Conflicts of Interest:** The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

**References**


24. López, L.M.A. Estudio de nutrición de *Pinus patula* Schl. et Cham. en sistema hidropónico
   [Nutritional study of *Pinus patula* Schl. et Cham. in a soilless system]. Bachelor thesis.


   para diagnóstico nutrimental de especies forestales [Interpretation of vector analysis

27. Lázaro-Dzul, M.O.; Velázquez-Mendoza, J.; Vargas-Hernández, J.J.; Gómez-Guerrero, A.;
   Álvarez-Sánchez, M.E.; López-López, M.A. Fertilización con nitrógeno, fósforo y potasio en un
   latizal de *Pinus patula* Schl. et Cham. [Fertilization with nitrogen, phosphorus, and potassium in
   a sapling-stage stand of *Pinus patula* Schl. et Cham.]. *Rev. Chapingo Serie Cien. For. Amb.* 2012, 18,
   33-42.

28. Crous, J.W.; Morris, A.R.; Scholes, M.C. Effect of phosphorus and potassium fertilizer on tree
   growth and dry timber production of *Pinus patula* on gabbro-derived soils in Swaziland. *Southern

29. López, L.M.A. Evaluación nutrimental de *Abies religiosa* en el Desierto de los Leones, D. F.

30. Truman, R.; Lambert, M.J. The use of DRIS indices to determine the balance between nitrogen,
    phosphorus and Sulphur in *Pinus radiata* foliage. Symposium on Managing Nitrogen Economics

31. Rodríguez, V.; Rodríguez, O. Normas foliares DRIS para el diagnóstico nutricional del plátano

    preliminares DRIS desarrolladas para caña de azúcar a partir de un bajo número de muestras
    [Preliminary DRIS norms for sugar cane developed from a small number of samples]. *Pesquisa
    Agropecuaria Brasileira* 2009, 44, 1700-1706.

33. González, T.F.L. Respuesta de Teca (*Tectona grandis* L. f.) a una formulación de fertilizantes de
    liberación lenta e insecticida sistémico [Response of teak (*Tectona grandis* L.f.) to a formulation
    of slow-release fertilizer and systemic insecticide]. Bachelor thesis. División de Ciencias

34. Alvarado, A.; Raigosa, J. Nutrición y fertilización forestal en regiones tropicales [Forest nutrition and
    fertilization in tropical regions]. 1st ed. Asociación Costarricense de la Ciencia del Suelo (ed.). San