

Effect of fertilization and container size on the quality of “Sangre de Drago” (*Croton urucurana* Baill.) seedlings in a plug +1 production system.

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Tables 1-5 are available in the Supplementary Files section.

1 Effect of fertilization and container size on the quality of “Sangre de
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19

20 **ABSTRACT**

21 “Sangre de Drago” (*Croton urucurana* Baill.) is a native tree of South
America with high value
22 for urban afforestation or the restoration of riparian forests. This study
aimed to evaluate the
23 effect of container size and fertilization on the quality of seedlings produced
in a plug+1 system.
24 In the establishment phase, the impact of container size (110 mL or 250 mL)
and fertilization
25 (with or without) on seedling quality were evaluated before transplanting
into bigger containers.
26 In the rapid growth phase, the effect of container size (1 L, 1.5 L, and 3.5 L)
on survival and
27 seedling quality was evaluated. At the end of each phase, seedling quality
was evaluated
28 through morphological and physiological attributes. Data were analyzed
through linear models,
29 generalized linear models, and nonlinear regressions. In the establishment

phase, seedlings

30 grown in 250 mL containers with fertilization showed the best performance
in morphological

31 and physiological variables and the Dickson quality index. In the rapid
growth phase, seedlings

32 grown in 3.5 L containers showed the highest values in total height, stem
diameter, total dry

33 weight, Dickson quality index, and the lowest mortality at the end of the
experiment. The results

34 of this study suggest that the most efficient strategy for producing high-
quality dragon's blood tree

35 seedlings is to use 250 mL tubes during the establishment phase, followed
by transplanting them

36 into 3.5 L pots during the rapid growth phase.

37 .

Keywords: nursery, substrate, Dickson quality index, propagation, root growth, sangra d'água.

Author contributions

Conceptualization: all authors; Methodology: M. M., N. M., P. G., and N. A.; Investigation: M.M; Resources; N.M., M.B., and D.C.C.; Formal analysis: M. M. and D.C.C.; Writing - original draft preparation: M. M.; Writing - review and editing: D.C.C., N. M., P. G. and N. A.; Funding acquisition: M. B.; Supervision: D. C. and M.B.

Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

60 Introduction

61 Argentina has a great diversity of environments with a vast
vascular flora with high
62 landscaping potential that has not been well explored, leading to a
limited supply of
63 seedlings for landscape architecture projects (Soto et al. 2011).
Previous studies have
64 shown that there is a local demand for native plants for
landscaping architecture
65 purposes (Mancini et al. 2018; Villanova et al. 2018). Thus,
research and associated
66 technologies to achieve the cultivation of native species are
increasingly important to
67 improve the competitiveness of the nursery business related to
ornamental horticulture.
68 One native tree of the riparian forests of the Argentinean Chaco
region with high
69 economic potential is *Croton urucurana* Baill., commonly known as
"Sangre de Drago",
70 "Everá-caá-berá", "Ivirá-caá-berá", "Sangre de dragón", "Sangre
drago", "Sangregado"
71 and "Urucurá" (Anton and Zuloaga 2014). "Sangre de Drago" is a
phylogenetic resource
72 with high landscape value because it is a pioneer species in the
natural succession of
73 riparian forests, which has shown good performance for reforesting
degraded forests
74 (Gerber et al. 2022). It could also be used for urban afforestation in
different situations
75 (e.g.: to enhance stormwater infiltration on urban retention ponds
and as street trees in
76 modified urban soils) since urban environments have a high degree
of disturbance of
77 their pre-existing natural conditions and are environmentally hostile
to most species.
78 Further, this species have a high value for the pharmaceutical

industry as bark extract

- 79 are effective against the hemorrhagic activity of some snake's venom
(Esmeraldino et al.
- 80 2005) and for the treatment of cardiovascular diseases related to liver
injury (Gasparotto
- 81 Junior et al. 2022). However, to obtain a plant with high quality
and commercially
- 82 competitive for restoration purposes, medicinal use, or urban
forest afforestation,
- 83 quality standards must be achieved. The total height (H), total
dry weight, stem
- 84 diameter (d), root dry weight (R), shoot dry weight (S), assimilation
capacity (indirectly

85 measured by total leaf area), and non-structural carbohydrate (NSC)
reserve level are

86 the most important seedling quality attributes (Marshall 1985;
Thompson 1985; Hunt

87 1990; Ritchie et al. 2010). Using these attributes, some quality
indices such as the

88 Dickson index (DQI), the S/R ratio, and the sturdiness quotient (H/d)
can be calculated,

89 which are directly related to performance and survival in the
planting site (Dickson et

90 al. 1960; Thompson 1985; Bayala et al. 2009). Furthermore, all
these attributes and

91 quality indices are closely correlated with nursery cultivation,
particularly with plant

92 density, substrate fertility, container volume, and dimension, among
others (NeSmith

93 and Duval 1998; Martínez and Rubio 2005; Ritchie et al. 2010;
Gutiérrez et al. 2010;

94 Bernaola-Paucar et al. 2015; Senilliani et al. 2021; Venancio
Nabor et al. 2022).

95 Adjusting the nursery cultivation process of *C. urucurana* is a key
factor in producing

96 high-quality plants. Previous research has addressed sexual (Scalon
et al. 2012; Ghio et

97 al. 2021) and asexual propagation (Biganzoli et al. 2021) generating
replicable protocols

98 on a commercial scale. Other studies have evaluated the effect of
light intensity

99 (Alvarenga et al. 2003), nutritional deficiencies (Sorreano et al.
2008, 2011), and

100 production costs for seedlings of *C. urucurana* in different types
of substrates and

101 fertilizers in container-grown plants (Uesugi et al. 2019). The
survival rate of *C.*

102 *urucurana* seedlings has also been evaluated in the restoration of
natural forests under

103 different environmental conditions (de Oliveira et al. 2012; Lima et al.
2016; Fragoso et
104 al. 2016). However, there are gaps in the technological knowledge
of the seedling
105 production process for this species. The seedlings production
process in a nursery can
106 be divided into three phases: establishment, rapid growth, and
hardening (seedlings in
107 each of these phases have distinct environmental and crop needs).
The most important
108 gaps in containerized *C. urucurana* seedlings production are
related to the effect of

109 container size and fertilization level in the establishment phase
and the effect of
110 container size in the rapid growth phase.
111 Therefore, the objective of this work was to evaluate the effect of
container size and
112 fertilization on the quality of seedlings produced in a plug+1 system.

113 **Methods**

114 *Plant material and experimental design*

115 Seeds of *C. urucurana* collected from natural forests in Santa Fe
province, Argentina
116 were used. Reference material of mother seed plants is deposited
in herbarium SF
117 (herbarium voucher Pensiero et al. – 11785(SF)). The container
production system used
118 was plug+1, which means that the establishment phase of the
seedlings was produced in
119 plastic containers of 110 mL or 250 mL and then had an additional
growth season in
120 larger containers (rapid growth phase). The seedlings were assessed
before undergoing
121 the hardening phase, as determining the requirements of the
seedlings during this phase
122 was not a research objective.

123 *Experiment 1: Effect of container size and fertilization level on* 124 *seedling quality in the* *establishment phase.*

125 In April 2019, seeds were sown in 880 plastic containers of two
different volumes (110
126 mL and 250 mL), using commercial substrate (GROWMIX®
Multipro). The set of
127 containers of each volume was divided into two subsets: one received
fertilization with

- 128 50% Hoagland solution (100 ppm N) and the other did not (control
with substrate's
- 129 fertility: 250-450 $\mu\text{g.L}^{-1}$ of nitrate, 30-100 $\mu\text{g.L}^{-1}$ of phosphate and
200-300 $\mu\text{g.L}^{-1}$ of
- 130 potassium). Fertirrigation (treatments) or irrigation (control) was
done with a watering
- 131 can whenever the actual container weight was 20% lower than the
container capacity

weight. The end of the establishment phase was considered when the seedling could be

easily lifted from the container with its intact and well-formed root ball. The duration of

the establishment phase was 4 months.

At the end of the experiment, the following response variables were determined:

a. Total seedling height (H): measured with a 1 mm precision measuring tape.

Considering the distance from the substrate surface to the apex of the plant in

the main stem. Results were expressed in centimeters.

b. The number of leaves: registered by manual counting of the number of fully

expanded leaves.

c. Stem diameter (d): measured using a digital caliper with 0.1 mm precision at the

stem base. Results were expressed in millimeters.

d. Stem (SDW), root (RDW), and leaf (LDW) dry weight: a sample of 10 seedlings

of each treatment was taken and placed in the oven at 40 °C until it reached a

constant weight. Then, each corresponding portion was weighed using a

precision balance. Results were expressed in grams.

e. Shoot to root ratio (S/R): relates the shoot dry weight (SDW+LDW) to root dry

weight (RDW), as follows
$$\frac{S}{R} = \frac{(SDW + LDW)}{RDW} \quad (1)$$

f. Sturdiness quotient (H/d): a dimensionless quotient calculated as the ratio of

total height in centimeters (H) to stem diameter (d) in millimeters.

g. Dickson Quality Index (DQI), is a dimensionless index that

relates the total

152 seedling dry weight (TDW = SDW + RDW + LDW), the
sturdiness quotient,

153 and the biomass partitioning between the shoot and root
(S/R) defined by

154 equation 2: $DQI = \frac{TDW}{\left(\frac{S}{d} + \frac{R}{R}\right)} \quad (2)$

h. Total leaf area (LA): was determined using an LI-COR electronic leaf area meter, model LI-3050C, and expressed in square centimeters.

i. SPAD index: is a dimensionless index that indirectly measures the amount of chlorophyll present in the leaves. Its value is proportional to the chlorophyll content. It was measured with a Minolta SPAD-502 Plus model, averaging 2 measurements in the first fully expanded leaf.

j. Soluble non-structural carbohydrate (NSC): samples of each treatment dried for SDW, LWD, and RDW determination were used for soluble NSC determination according to Dubois et al (1956). Results were expressed as mg of glucose per 100 grams of fresh tissue.

A 2×2 factorial experiment (2 container volumes × 2 levels of fertilization) was used within a completely randomized design with 220 replicates per factorial combination, with a seedling as the experimental unit.

Experiment 2: Effect of container volume in the rapid growth phase on plant quality.

At the end of the establishment phase, all seedlings from the best treatment resulting from experiment 1 were chosen for experiment 2. At this time, a random sample of 10 seedlings was taken to assess the quality of the batch before starting the rapid growth phase, to obtain information about the mean starting point for all treatments. Seedlings at the beginning of experiment 2 had an average height of 20.8 cm ($\pm$ 5.0 cm), an

174 average DAC of 2.41 mm ($\pm$ 0.47 mm), an average TDW of 0.73 g
($\pm$ 0.30 g), an
175 average S/R ratio of 3.28 ($\pm$ 0.85), an average H/d ratio of 7.1 ($\pm$
1.3 cm), and an
176 average DQI value of 0.07 ($\pm$ 0.02). The remaining seedlings were
randomly assigned to
177 one of the following container sizes: 1L, 1.5L, or 3.5L, where they
underwent one
178 growth season. The substrate in these containers was composed
of a mixture of

179 commercial GROWMIX® Multipro substrate and composted organic
material in a ratio

180 of 1:3. The experimental design was completely randomized with
60 replicates per

181 treatment, with a plant in its corresponding pot as the experimental
unit.

182 During the experiment, the following response variables were
recorded:

183 a. Total plant height (H): measured every 14 days after transplant
and at the end of

184 the experiment, following the procedure described in
experiment 1 and

185 expressed in centimeters.

186 b. Stem diameter (d): measured every 14 days after transplant and
at the end of the

187 experiment, following the procedure described in experiment 1
and expressed in

188 millimeters.

189 c. Mortality: visually evaluated by counting the number of dead
plants relative to

190 the total, every 14 days and at the end of the experiment.

191 At the end of the experiment, the following response variables were
recorded:

192 a. Stem (SDW), root (RDW), and leaves (LDW) dry weight: using
the procedures

193 described in experiment 1.

194 b. Shoot to root ratio (S/R), according to equation 1.

195 c. Sturdiness quotient, as described for experiment 1.

196 d. Dickson quality index (DQI), according to equation 2.

197 e. Total leaf area (LA), following the procedure described in
experiment 1.

198 f. SPAD index, following the procedure described in experiment 1.

199 g. Soluble non-structural carbohydrate (NSC), using the
procedure described in

200 experiment 1.

201

202 *Data analysis*

203 The statistical analysis was performed using the R programming
language environment
204 (R Development Core Team 2017), using the RStudio interface
(RStudio 2016). The
205 effect of the container volume and the fertilization level (experiment
1), or the container
206 volume (experiment 2) on the morphological and physiological traits
of the seedlings
207 was analyzed using general linear models (LM) through the `lm()`
function of the `stats`
208 package. Normality was evaluated using `shapiro.test()` function and
homoscedasticity
209 was tested using both `leveneTest()` and `barlett.test()` functions of
package `stats`.
210 Normality and homoscedasticity were also tested graphically (Q-Q
plot, and studentized
211 residuals vs. predictors plot, respectively). When the variables
did not meet the
212 assumptions of normality and homoscedasticity, generalized linear
models (GLM) were
213 adjusted using gamma or log-normal distribution using the `glm()`
function of the `stats`
214 package. The number of leaves was analyzed using GLM with
Conway-Maxwell
215 Poisson distribution, to control for over or under-dispersion of the
data using the `glm()`
216 function of the `stats` package. The factorial treatment (experiment 1)
and the container
217 volume (experiment 2) were considered explanatory variables.
Tukey's multiple
218 comparisons test was performed using the `TukeyHSD()` function of
the `stats` package.
219 For the height and diameter growth data throughout experiment 2,
generalized additive
220 models (GAM) were adjusted with random effects by type of pot

and sample, and a

- 221 first-order autoregressive temporal correlation structure (AR1).
Because plant growth is
- 222 not linear over time, GAMs are useful for modeling them since they
have a parametric
- 223 part and a series of smoothing functions whose polynomial function
is not defined a
- 224 priori (Pedersen et al. 2019). The adjustment of GAM models was
performed using the
- 225 gam() function of the mgcv package(Wood 2019). Mortality data was
modeled using
- 226 the drm() function of the drc package (Ritz and Strebig 2022). Log-
logistic parameter

227 models or Michaelis-Menten models were adjusted. The best models
were chosen using
228 the `mselect()` function of the `drc` package. The level of statistical
significance was
229 established as $p < 0.05$. Figures were done using the `ggplot2` package
(Wickham 2016).

230 **Results**

231 *Effect of container size and fertilization level on seedling*
quality during the
232 *establishment phase.*

233 Tables 1, 2, and 3, as well as Fig. 1, provide details on the
morphological and
234 physiological characteristics exhibited by the seedlings at the end of
experiment 1. For
235 most of the response variables evaluated, an interaction was observed
between the effect
236 of container volume and fertilization level (Table 1). The interaction
container volume $\times$
237 fertilization was not observed in for SPAD index, and soluble NSC
content. As shown
238 in Table 2, an increase in fertilization resulted in a rise in total
height (H), number of
239 leaves (NL), total dry weight (TDW), leaf area (LA), and a greater
partitioning of
240 growth towards aerial organs (greater S/R ratio) regardless the
container volume.
241 Fertilization enhanced the sturdiness quotient in 110 mL containers
but not in 250 mL
242 containers. Conversely, fertilization improved the stem diameter
(d) and Dickson
243 quality index (DQI), but only in 250 mL containers. This effect was
particularly notable
244 in the case of DQI, which represented an increase of over 100% with
fertilization. For

245 most of the response variables tested, the variation in fertilization
level produced a

246 greater magnitude of enhancement in 250 mL containers than in 110
mL containers.

247 On the other hand, when the effect of container size was observed
within a given level

248 of fertilization, there was no clear trend in the variables (Table 2).
For example, an

249 increase in container volume did not result in an improvement in
total seedling height,

250 regardless of the fertilization level. Nevertheless, an increase in
container volume in

251 fertilized plants resulted in a notable increase in stem diameter,
number of leaves, TDW,
252 S/R ratio, leaf area, and DQI. On the contrary, in non-fertilized
seedlings, an increase in
253 container volume did not improve these traits.

254 The SPAD index values improved as a function of both fertilization and
pot size effects,
255 without interaction between both main effects (Table 1 and Table
3). SPAD index
256 values were higher in fertilized plants regardless of container volume
(Table 3). Higher
257 SPAD index values were also observed in seedlings grown in 250 mL
pots. However,
258 SPAD index values were better with fertilization than with increasing
container volume
259 (Table 3). Non-structural soluble carbohydrates (NSC) did not
show a clear trend
260 regarding fertilization level or container volume (Table 1). Stem
NSC content was
261 lower in 250 mL containers compared to 110 mL containers but
was not affected by
262 fertilization level (Table 3). In contrast, leaf NSC content did not
differ according to
263 container volume, but was lower in fertilized seedlings (Table 3).
Finally, total NSC
264 content followed the trend of stem NSC content, showing higher
values in 110 mL
265 containers (Table 3).

266 *Effect of container volume on the quality of seedlings during the rapid
growth phase.*

267 The container volume during the rapid growth phase significantly
influenced the total
268 height (H), stem diameter (d), total dry weight (TDW), leaf area (LA),
Dickson's quality

269 index (DQI), the stem NSC and mortality (Tables 4 and 5). For all
the mentioned

270 response variables, the explained variability was moderate ($R^2 =$
0.43-0.61), except for

271 the leaf area, whose model had low explanatory capacity ($R^2 = 0.28$).
Container volume

272 did not affect the S/R ratio, sturdiness quotient (H/d), and leaf NSC
(Table 4).

273 Seedlings grown in all containers showed a remarkable increase in
total height, stem

274 diameter, TDW, and DQI compared to initial values (Table 5). The
biggest increase was

275 observed in TDW (+680% to 2160%, depending on container volume)
and DQI (+686%
276 to 1900%, depending on container volume). The S/R ratio and H/d
quotient showed a
277 numerical increase (+0.6% and +17%, respectively) compared to
initial values in all
278 container sizes, but this difference has no biological relevance in
seedling survival.

279 As a general rule, a larger container volume produced an
improvement in most seedling
280 attributes; except for the S/R ratio, the H/d quotient, and the NSC
content (Table 5).

281 Seedlings produced in 1 L containers showed the lowest values in all
attributes except
282 for DQI, which not differed from seedlings produced in 1.5L
containers ($p > 0.05$). In
283 addition, seedlings grown in 1L containers had the highest stem NSC
(Table 5). Plants
284 grown in 3.5L containers showed significantly better values than
those grown in 1.5L
285 containers for stem diameter and DQI but not for the total height,
TDW, leaf area, and
286 stem NSC content.

287 Regarding Dickson's quality index, there was an 89% increase
when comparing
288 seedlings grown in 1L and 1.5L containers with seedlings grown in
3.5L containers.

289 This indicates that a considerable increase in plant quality was
achieved by increasing
290 the container volume from 1-1.5L to 3.5L, which also resulted in
lower plant mortality
291 during the trial (Fig. 3 and Table 5). Furthermore, growing seedlings
in 3.5 L containers
292 could allow for a longer plant nursery stay until sale. Stem diameter
was significantly

293 higher in seedlings grown in 3.5L containers than in those grown in
1.5L containers

294 (Table 5 and Fig. 2A). However such a small variation could have
low biological

295 significance in terms of seedling adaptability and survival after
transplantation.

296 Seedlings grown in all container volumes showed greater biomass
partitioning towards

297 the shoot than was considered appropriate (S/R dry weight ratio >
2.5). Slender plants

298 were produced in all container sizes. From a physiological point of
view, there were no

299 differences in leaf and total NSC between plants produced in the
different containers.

300 However, seedlings produced in 1L containers had the highest stem
NSC.

301 Total height and stem diameter growth showed significant
differences among different

302 container types, with a positive relationship observed between
growth and container

303 volume (Fig. 2). Total height growth showed a sigmoid trend for
seedlings grown in 1.5

304 and 3.5L containers but not for those grown in 1L containers (Fig.
2B). Slow growth

305 was observed until 28 days after transplantation in seedlings grown
in 1.5 L and 3.5 L

306 containers. Then, height growth showed an exponential trend
until 70 days after

307 transplantation. Starting at day 70 after transplantation, height
growth began to plateau.

308 In seedlings produced in 1L containers, height growth showed a
linear trend until 98

309 days after transplantation, after which a plateau occurred until the
end of the trial.

310 Seedlings produced in 1L containers had lower height than those
produced in larger

311 containers throughout the experiment. No differences in height
growth were observed

312 between the 1.5L and 3.5L treatments during the same period.

313 Regarding the evolution of the stem diameter, no sigmoidal trend
was observed (Fig.

314 2A). In seedlings produced in 1.5L and 3.5L containers, growth
followed a linear trend

315 until 56 days after transplantation, after which the DAC began to
grow at a slower rate.

316 Seedlings produced in 1L containers showed linear growth with a
shallower slope than

317 in seedlings produced in larger containers.

318 Mortality showed a trend of increasing over time for all treatments
(Fig. 3). However,

319 the progression of mortality varied between treatments; 50%
mortality was reached at

320 49 days, 55 days, and 126 days in seedlings grown in 3.5L, 1L, and
1.5L containers

321 respectively (Fig. 3). Final mortality was significantly different
between seedlings

322 grown in different container volumes (Table 5). Seedlings in 1L
containers had the

highest mortality (>90%), followed by those grown in 1.5L containers (53%), while seedlings produced in 3.5L containers had the lowest final mortality (37%) (Table 5 and Fig. 3). Therefore, initial mortality was faster in seedlings produced in 3.5L containers, but final mortality represented around 1/3 and 2/3 compared to those produced in 1L or 1.5L containers respectively (Fig. 3).

328 Discussion

Effect of fertilization on morphological variables and quality indices during the establishment phase.

Our results show that the fertilized treatments obtained the best values for the morphological traits (except for total height and H/d) (Table 2). This fact is consistent with previous research that found that fertilization and container size correlates positively with seedling size, nutritional status, quality, and survival in the field (Carrasco Manzano et al. 2004; Trubat Domenech et al. 2004; Martínez and Rubio 2005; Sorreano et al. 2008, 2011; Carpenedo Aimi et al. 2016). Regarding the quality indices evaluated, the H/d quotient reflects the robustness of a seedling and is useful for discarding disproportionately tall plants. A seedling with an H/d > 10 is susceptible to mechanical injury due to a lack of lignification. Adequate values of the sturdiness quotient are in the range of H/d < 6-8, which ensures resistance to mechanical damage and abiotic stress factors at the planting site (Thompson 1985; Hunt

1990). Our results

- 342 show that the most slender plants were those fertilized and among
them, those produced
- 343 in 110 mL containers, possibly in response to the higher plant
density per surface area
- 344 in 110 mL compared to 250 mL containers. However, all *C. urucurana*
seedlings grown
- 345 in the establishment phase showed $H/d < 8$ leading to the
absence of mechanical
- 346 damage once transplanted to larger containers in the rapid growth
stage.

347 The S/R ratio of a seedling shows the balance or imbalance between
transpiration and
348 water and nutrient absorption (Ritchie et al. 2010). Sáenz et al.
(Sáenz Reyes et al.
349 2010) established that good quality conifer seedlings should have an
S/R ratio between
350 1.5 and 2.5, while seedlings with an S/R ratio > 2.5 are considered
poor quality. On the
351 other hand, Thompson (1985) advises that the S/R ratio should be $\approx$
1 when the out-
352 planting site environment presents restrictive characteristics to root
growth. In our work,
353 a good S/R ratio was obtained in seedlings of all treatments, except
for those grown in
354 250 mL containers with fertilization where the S/R ratio was > 2.5 .
This fact did not
355 represent a problem in survival since those plants were not intended
for field planting,
356 but for larger containers in the rapid growth phase. In fact, after
lifting from 250 mL
357 containers and planting in 3.5 L containers, seedling survival was over
60 % (Table 5).

358 The SPAD index has a high correlation with chlorophyll content in
leaves, therefore, it
359 is expected that its value increases as a result of fertilization in
combination with the
360 larger container volume on seedlings in the establishment phase
(Table 3). This was
361 consistent with what was observed in *Lisianthus*, where nitrogen
fertilization increased
362 chlorophyll content and therefore SPAD values (Cunha et al. 2015).

363 Previous studies establish that a DQI value < 0.15 - 0.20 could harm
plant survival, while
364 a seedling with a DQI > 0.50 is considered of good quality (Dickson
et al. 1960; Sáenz

365 Reyes et al. 2010). The DQI values found in our work indicate
that no seedling
366 treatment produced seedlings suitable for field planting, given their
low DQI. However,
367 the *C. urucurana* seedlings were transplanted to larger containers
to go through the
368 rapid growth phase without limiting factors; under these conditions,
ICD values <0.15
369 did not affect the growth or initial survival of these plants when
grown in containers
370 with adequate volume (Table 5, Fig. 3).

371 *Effect of container volume on morphological variables and quality*
indices in the

372 *establishment and rapid growth phases.*

373 The container volume influences the potential size that a seedling
can reach because it

374 determines the amount and availability of water and nutrients for
growth, as well as

375 affecting root growth (Biran and Eliassaf 1980; NeSmith and Duval
1998; Ritchie et al.

376 2010). In the establishment phase, an increase in container volume
from 110 mL to 250

377 mL improved stem diameter, number of leaves, TDW, S/R ratio, leaf
area, and DQI in

378 fertilized *C. urucurana* seedlings (Table 2). In addition, an increase in
container volume

379 enhanced the total height, stem diameter, TDW, leaf area, and ICD
of *C. urucurana*

380 seedlings transplanted from 250 mL containers to their final
containers in the rapid

381 growth phase (Table 3). Previous studies in different species have
shown that container

382 size influences the morphological and physiological quality
attributes of seedlings

383 (Biran and Eliassaf 1980; NeSmith and Duval 1998; Carpenedo
Aimi et al. 2016;

384 Senilliani et al. 2021; Grossnickle and Ivetić 2022). Various
authors have also

385 established a direct correlation between container size and
morphological quality

386 parameters (Domínguez Lerena 1997; Dominguez-Lerena et al. 2006;
Bernaola-Paucar

387 et al. 2015). Of the attributes and indices that we tested, those
most related to plant

388 quality and survival in the out-planting site are TDW, S/R ratio, and
ICD (Dickson et al.

389 1960; Thompson 1985). The container volume is the main factor

that controls the

390 production of root system biomass, which in turn impacts the
ability of seedlings to

391 generate aerial growth due to its contribution of nutrients, water, and
growth regulators

392 (NeSmith and Duval 1998; Grossnickle and Ivetić 2022). Therefore,
a restriction on

393 root growth will negatively affect total biomass accumulation by
affecting aerial growth

394 (NeSmith and Duval 1998; Gutiérrez et al. 2010). The increase in TDW
as a result of an

395 increase in container volume has been documented in different
species (Dominguez-

396 Lerena et al. 2006) and is due to a larger root exploration volume
that allows for
397 increased nutrient and water uptake and gas exchange with the
substrate atmosphere, in
398 addition to the root being important for the provision of growth
regulators essential for
399 shoot growth. Additionally, when the container volume is not
adequate, root growth
400 occupies the porous space leading to changes in total porosity,
aeration porosity, and
401 water retention porosity, which causes growth restriction due to
changes in oxygen
402 concentration, carbon dioxide, and substrate water regime (Biran and
Eliassaf 1980).

403 As mentioned earlier, DQI allows for defining the balance between
mass partitioning
404 and the sturdiness quotient of a seedling (Dickson et al. 1960). High-
quality seedlings
405 should have an ICD > 0.5 (Dickson et al. 1960; Sáenz Reyes et al.
2010). In our study,
406 all treatments in the rapid growth phase produced seedlings with
DQI > 0.5, with the
407 best quality plants being produced in 3.5 L containers (DQI = 1.6).
Previous research in
408 different species also found a rise in DQI with an increase in
container size (Alzugaray
409 et al. 2004; Bernaola-Paucar et al. 2015; Carpenedo Aimi et al.
2016; Senilliani et al.
410 2021; Jesús Albino et al. 2021). The improve in DQI would be the
result of a significant
411 increase in TDW, given that the S/R ratio and H/d quotient are
usually not altered with
412 an increment in container size, as long as there is no restriction to
root growth. On the
413 other hand, the H/d quotient and S/R ratio of *C. urucurana* seedlings
grown in 1 L, 1.5

414 L, and 3.5 L containers were not significantly affected by container
volume. Previous
415 results in floral and horticultural crops indicate that container
volume does not have an
416 unequivocal effect on the S/R ratio (NeSmith and Duval 1998). A
rise in container
417 volume did not significantly affect the S/R ratio in *Pinus pinea*
(Domínguez Lerena
418 1997; Dominguez-Lerena et al. 2006), *P. pinaster*, *P. halepensis*
(Domínguez Lerena
419 1997), *Prosopis alba* (Senilliani et al. 2021), or *Quercus crassipes*
(Venancio Nabor et
420 al. 2022), while there was a vague effect in *Pinus hartwegii*
(Bernaola-Paucar et al.

2015). This suggests that although container size affects total biomass production, it does not affect biomass partitioning between aboveground and belowground seedling tissues unless there is severe root restriction (NeSmith and Duval 1998). *C. urucurana* seedlings in the rapid growth phase showed $S/R > 2.5$ in all container volumes, which could affect their growth and initial survival. However, in *Pseudotsuga menziesii* with a similar S/R ratio, growth and survival after two years of planting were higher in seedlings with greater root volume and TDW at planting (Alzugaray et al. 2004). According to Thompson (1985), the acceptable threshold for the S/R ratio tends to be higher as the plant grows larger. Furthermore, when the field site environment is not limiting, the root could have a growth stimulus that could compensate for an inadequate S/R ratio. According to Grossnickle and Ivetić (2022), the combination of root development and planting site environmental conditions will determine the survival after out-planting. Thompson (1985) warns that besides the S/R ratio, seedling survival in its final location will depend on its TDW and DQI value. Furthermore, unfavorable seedlings S/R ratios at the planting phase on *Acacia angustissima*, *A. mangium*, *Gliricidia sepium*, *Leucaena hybrid* (L×L), and *Leucaena leucocephala* did not affect survival or growth after 12 months with irrigation (Bayala et al. 2009). Therefore, even if there is an unfavorable S/R ratio, *C. urucurana* plants grown in 3.5 L containers in the

439 rapid growth phase have the best chances of survival in the field
before a proper

440 hardening.

441 Previous studies show that container volume has no direct effect on
sturdiness quotient

442 in *Pinus halepensis*, *P. pinea*, *P. pinaster* (Domínguez Lerena
1997), or *Quercus*

443 *crassipes* (Venancio Nabor et al. 2022). *C. urucurana* seedlings in
the rapid growth

444 phase would have an acceptable H/d quotient as explained by
Hunt (1990), which

445 would make them resistant to wind damage after transplantation to
the final site.

446 The growth in total height and stem diameter was significantly
higher in the larger
447 containers, possibly due to a combined effect of a larger leaf area
and greater root
448 biomass (data not shown), leading to increased water and nutrient
uptake, gas exchange,
449 and production of growth regulators (Ritchie et al. 2010).
Seedlings grown in 1 L
450 containers showed a restriction in root growth possibly due to
changes in total porosity,
451 aeration porosity, and water retention porosity, which negatively
impacts water and
452 nutrient uptake, gas exchange between the root and the substrate
atmosphere, and results
453 in lower production of important growth regulators (Biran and
Eliassaf 1980; NeSmith
454 and Duval 1998). Also, a restriction in root growth causes a decline
in root quality and
455 promotes root-bound conditions (Grossnickle and Ivetić 2022) that
could explain the
456 lower height and stem diameter growth rate and higher mortality
in *C. urucurana*
457 seedlings grown in 1L containers. The plateau produced on height
and stem diameter
458 growth in seedlings of all treatments could be due to all containers
becoming restrictive
459 for root system development towards the end of the experiment. This
restrictive effect
460 of the container on seedling growth was observed in different
herbaceous and woody
461 horticultural and ornamental crops (NeSmith and Duval 1998;
Gutiérrez et al. 2010;
462 Grossnickle and Ivetić 2022). Therefore, once the substrate type to be
used in the rapid
463 growth phase is defined, it is crucial to consider the container
volume, as it defines the

464 period during which the seedling will maintain a good quality until
the out-planting

465 date.

466 Our results have shown that the concentration of soluble NSC
was higher in the

467 establishment phase compared to the rapid growth phase (Tables 3
and 5). On the other

468 hand, in both phases, smaller containers had a higher
concentration of soluble NSC

469 (Tables 3 and 5). In addition, in the establishment phase, the
concentration of soluble

470 NSC was higher in unfertilized seedlings (Table 3). When there are
growth-limiting

471 factors, soluble NSC tend to accumulate in organs with
photosynthetic capacity as a
472 strategy to reduce photosynthetic rate through the down-regulation
of involved genes,
473 where soluble sugars function as signals (Marshall 1985; Pallardy
2008a). On the other
474 hand, when there are no limiting factors, a higher growth rate leads
to greater structural
475 carbon production (cellulose, hemicellulose, pectin) at the expense
of soluble and
476 insoluble NSC *concentration* (simple sugars, starch, etc.)(Marshall
1985; Pallardy
477 2008a, b). Our results agree with previous research where
decreasing growth-limiting
478 factors reduced the *concentration* of soluble NSC to use that
carbon for structural
479 growth (Peng et al. 2021; Chen et al. 2022). On the other hand,
while treatments with
480 growth restrictions showed a higher *concentration* of soluble NSC
(mg glucose per
481 100g of fresh weight), treatments without growth restrictions showed
almost three times
482 more biomass, leading to a higher *total content* of soluble NSC (total
mg of glucose per
483 seedling) (Tables 3 and 5). This is important because a higher *content*
of soluble NSC is
484 positively correlated with biotic or abiotic stress resistance and
field survival of
485 seedlings of different species (O'Brien et al. 2014; Grossnickle and
Ivetić 2022). Before
486 out-planting, *C. urucurana* seedlings must be hardened to enhance
survival chances by
487 acclimation to sunlight and water stress. Hardening also will lead to a
rise in soluble and
488 insoluble NSC concentration by reducing the photosynthetic rate
by generating

489 moderate water, nutrient, and light stress.

490 Viverization is the process by which ornamental, fruit, and/or forest
plants are cultivated

491 in nurseries using specific propagation and culturing techniques
and technologies for

492 each species or group of species. For sexually propagated seedlings,
these techniques

493 and technologies involve the selection of seed-bearing mother
plants, seed treatment,

494 substrate selection, container type and volume choice, fertilization
regime, irrigation

495 regime, and pest management at various stages of the process
(establishment, rapid

496 growth, and hardening phases) to achieve high-quality seedlings
adapted for the out-
497 planting site. Overall, the final quality of a container-grown plant is
determined by the
498 container type and volume, fertilization, substrate, and irrigation
regime used (Ritchie et
499 al. 2010). Our work studied an important and previously
unexplored aspect of the
500 viverization process for *C. urucurana*, namely the effect of
container size in the
501 establishment and rapid growth phases. We demonstrated that,
with other important
502 factors fixed (irrigation level, substrate type, pest management,
air temperature,
503 radiation, CO₂, and photoperiod), the quality of *C. urucurana*
seedlings depended on
504 container size and fertilizer level in the establishment phase.
Additionally, with the
505 fertilizer level as a fixed factor, seedling quality depended on
container volume during
506 the rapid growth phase of this species.

507 **Conclusions**

508 Container volume and fertilization level influenced the quality of
"Sangre de Drago"
509 seedlings in the establishment phase. Seedlings grown in 250 mL
containers with
510 fertilization resulted in better performance in morphological
variables. These seedlings
511 had lower *concentrations* of soluble NSC due to their higher
growth rate but had a
512 higher total soluble NSC *content* due to their greater total biomass.
This fact, along with
513 adequate Dickson quality index values, resulted in moderate to low
mortality during the
514 rapid growth phase in larger containers (1.5L and 3.5L).

515 In the rapid growth phase, container volume had a significant impact
on morphological
516 and physiological attributes, growth, and mortality of *C. urucurana*
seedlings. Seedlings
517 grown in 1 L containers showed over 90% mortality, making
them an unviable
518 commercial option. Seedlings produced in 3.5 L containers had the
highest survival rate,
519 total dry weight, Dickson quality index, and total soluble NSC
content compared to

520 other treatments, making them the most likely to survive in the final
planting site. The
521 use of 3.5 L containers also allows for greater flexibility in plant sale
timing as they can
522 last longer without quality losses compared to the smaller containers
tested.
523 To grow "Sangre de Drago" seedlings using a plug + 1 system, it is
recommended to use
524 250 mL containers with 100 ppm (N-P-K) fertilization in the
establishment phase and
525 3.5 L containers in the rapid growth phase. To expand knowledge
and efficiency in the
526 viverization of this species, it is necessary to evaluate different
substrate formulations
527 and fertilization levels in the growing stage using 3.5 L containers.

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536 **Data availability**

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

539 Competing Interests

540 The authors declare that they have no known competing financial
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Figures

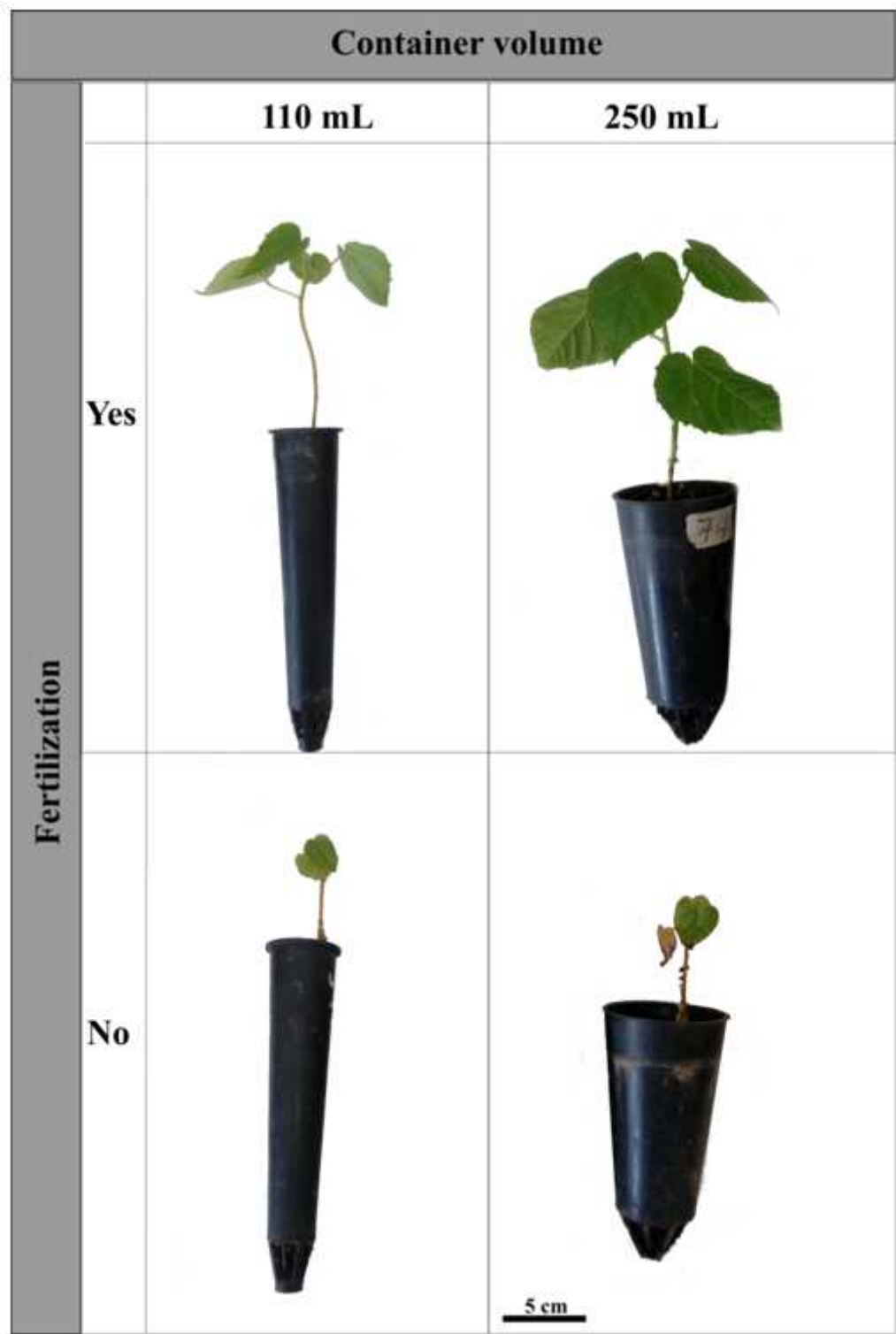


Figure 1

Effect of container volume and fertilization level on growth and quality of *C. urucurana* seedlings at the establishment phase

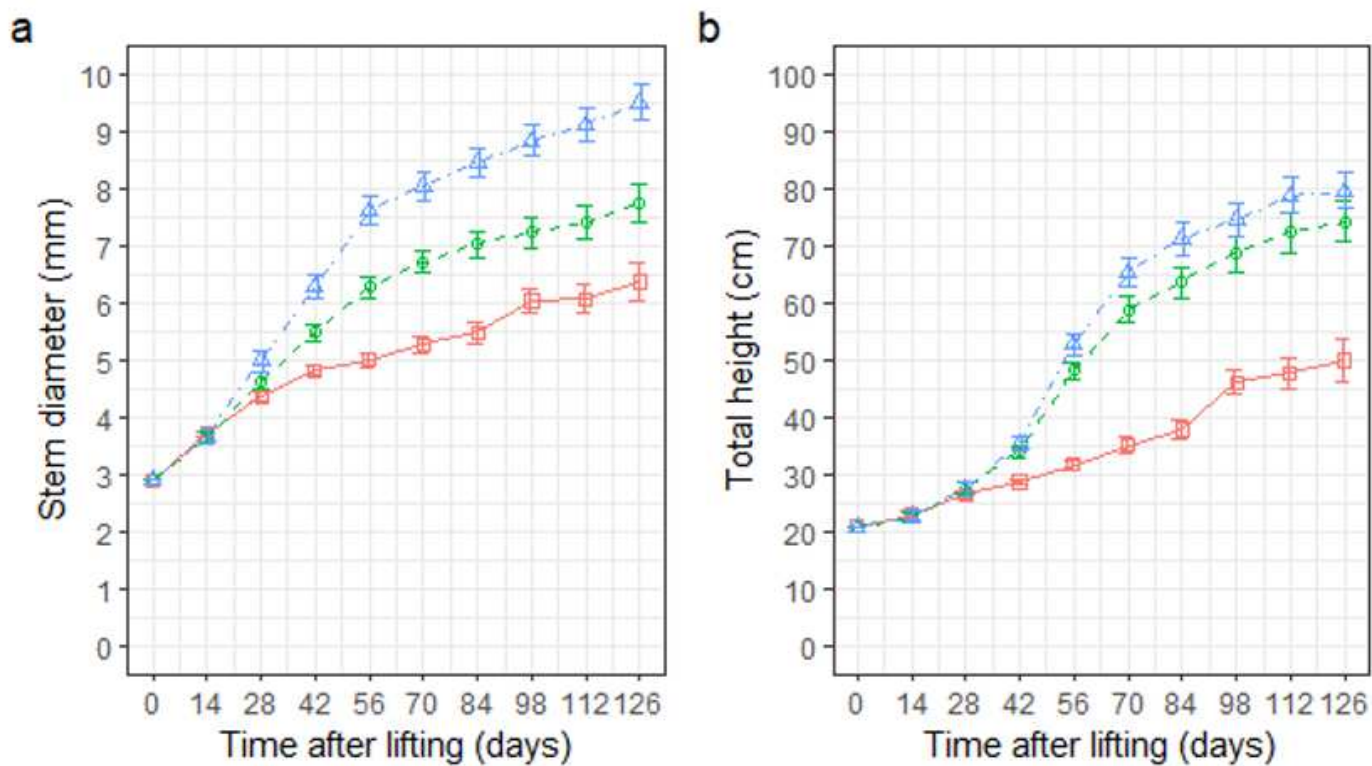


Figure 2

Effect of container size (1 L squares and red solid line; 1.5 L circles and green dashed line; 3.5 L triangles and blue dash-dot line) on stem diameter (a) and total height (b) evolution in *C. urucurana* seedlings in the rapid growth phase. Vertical bars indicate standard error of the mean

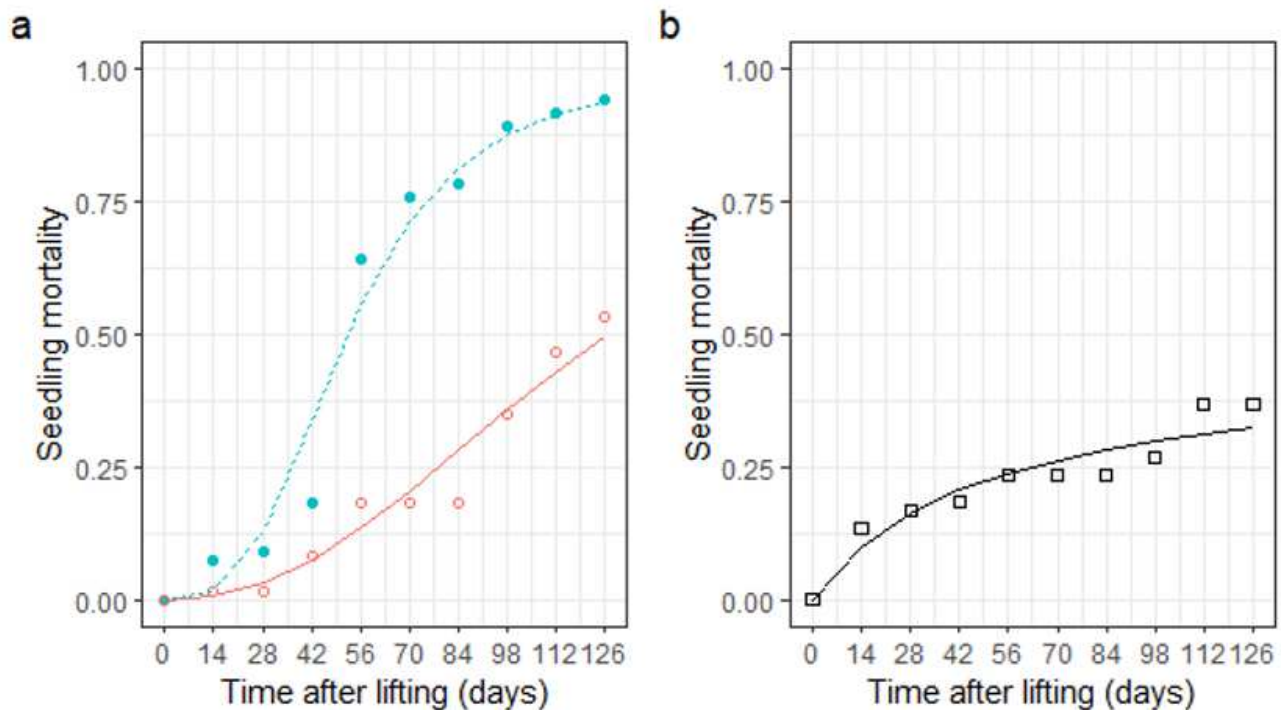


Figure 3

Effect of container size on mortality of *C. urucurana* seedlings during the rapid growth phase. a) 1 L containers (filled circles and blue dashed line) and 1.5 L containers (empty circles and red solid line). Curves correspond to the adjustment of 2-parameter log-logistic models. b) 3.5 L containers (empty squares and black solid line). The curve corresponds to the adjustment of the 2-parameter Michaelis-Menten model



Figure 4

Effect of container volume on the growth and quality of *C. urucurana* seedlings in the rapid growth phase

Supplementary Files

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- TABLES.docx