Radicle length and container size effects on root deformities in the Mediterranean oak *Quercus suber* L.

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Figure 1.
Root deformation on six-year-old cork oak seedling excavated from reforestation project in the Algerian north-eastern coast. The root deformation engendered in nursery persisted after plantation, increased with time and resulted in plant death.
Photos INRF - JIJEL.

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L’objectif de cette étude était d’évaluer les effets de la longueur des radicules au moment de l’ensemencement et de la taille du conteneur sur les déformations des racines chez le chêne méditerranéen Quercus suber L. Des glands de cinq méthodes d’ensemencement — glands intacts, glands germés pendant la conservation au froid, glands germés après stratification humide avec des radicelles (R) d’une longueur inférieure à 5 mm, des radicelles d’une longueur comprise entre 7 mm et 12 mm et des radicelles de plus de 15 mm — ont été semés individuellement en conteneurs WM de 400 cm³, 800 cm³ et 1200 cm³. À la fin de la période de croissance en pépinière, les racines des 1 080 semis échantillonés ont été examinées pour déceler les déformations des racines. Les résultats obtenus de l’expérience ont montré que la déformation des racines affectait près de la moitié (49 %) des plantules de chêne-liège cultivées en conteneurs et que l’état germinatif des glands au moment de l’ensemencement avait un effet significatif sur les déformations des racines, alors que la taille du conteneur n’avait aucun effet clair. Le plus grand nombre de racines déformées a été observé chez les plantules issues du semis de glands germés par rapport aux semis de glands intacts avec des pourcentages respectifs de 91 % et 9 % des semis inspectés. L’ensemencement de glands germés avec des radicelles de 15 < R < 20 mm et 7 < R < 12 mm de longueur a généré les pourcentages les plus élevés de déformations rédhibitoires. Indépendamment de sa taille, le type de conteneur WM sans fond s’est révélé très efficace contre la spirale radiculaire fréquemment observée dans les sacs en polyéthylène de forme cylindrique à fond fermé. Les résultats de cette étude peuvent contribuer à améliorer la qualité des semis de chêne-liège pour des programmes de reboisement plus efficaces.

Mots-clés : chêne-liège, matériel de pépinière, qualité des semis, déformations radiculaires, longueur des radicelles, volume des conteneurs, Algérie.

Resumen

El objetivo de este estudio era evaluar los efectos de la longitud de las radículas en el momento de la siembra y de las dimensiones del contenedor sobre las deformaciones de las raíces en el álamo mediterráneo Quercus suber L. Se incluyeron bellotas de cinco métodos de siembra: bellotas intac tas, bellotas germinadas durante el almacenaje, bellotas germinadas después de la estratificación húmeda con radículas (R) de una longitud inferior a 5 mm, con radículas con una longitud comprendida entre 7 mm y 12 mm y con radículas de más de 15 mm. Se sembraron individualmente en contenedores WM de 400 cm³, 800 cm³ y 1 200 cm³. Al final del período de crecimiento en vivero, las raíces de las 1 080 semillas de la muestra fueron examinadas para detectar sus deformaciones. Los resultados obtenidos de la experiencia mostraron que la deformación de las raíces afectó a casi la mitad (49 %) de las plántulas de álamo cultivadas en contenedores. Evidenciaron asimismo que el estado germinativo de las bellotas en el momento de la siembra tenía un efecto significativo en las deformaciones de las raíces, mientras que el volumen del contenedor no tenía ningún efecto claro. El mayor número de raíces deformadas se observó en las semillas de bellotas germinadas respecto a las semillas de bellotas intacas con porcentajes respectivos del 91 % y del 9 % de las semillas examinadas. La siembra de bellotas germinadas con raíces de 15 < R < 20 mm y 7 < R < 12 mm de longitud generó los porcentajes más elevados de deformaciones nocivas. Independientemente de sus dimensiones, el tipo de contenedor WM sin fondo resultó muy eficaz contra la espiral radicular observada frecuentemente en los sacos de polietileno de forma cilíndrica con fondo cerrado. Los resultados de este estudio pueden contribuir a mejorar la calidad de las semillas de álamo para que los programas de reforestación sean más eficaces.

Palabras clave: álamo, material de vivero, calidad de las semillas, deformaciones radiculares, longitud de las radículas, volumen de los contenedores, Argelia.
Introduction

Cork oak (*Quercus suber* L.) is one of the major and valuable components of the natural forests in the Mediterranean region. In Algeria, this evergreen woody species occupies 357,582 hectares (Abbas, 2013) mainly located in the North-East of the country. However, despite their ecological and socio-economical interests, cork oak forests are subjected to degradation under the combined action of human impacts (urbanization, wildfires, overgrazing, clearing and seed collection) and water stress due to recurring droughts under climate change. The combination of these factors, along with a lack of management practices adapted to the species resulted in a deficiency of natural regeneration. To address this worrying situation, many actions have been implemented, including several field plantings with container-grown seedlings in nurseries. Unfortunately, transplantation projects have not, in most cases, achieved their purposes. In fact, the poor survival of *Quercus* plantations in the field has most often been attributed to the low quality of the planted seedlings, the limited resources to grow (Tsakaldimi et al., 2005; Valdecanthos et al., 2006), the poor planting technique and the poor post-planting maintenance.

In addition to the genetic aspect, the quality of seedling is based on the physical quality, which is exhibited by the integration of a multitude of morphological attributes such as seedling height and weight, root collar diameter, root form and weight, the number of first order of laterals root, bud set, foliage color and various ratios such as shoot: root weight and sturdiness ratio, physiological attributes such as mineral nutrient and non-structural carbohydrate concentration, chlorophyll content, gas exchange, fine root electrolyte leakage and performance attributes such as root growth potential, drought resistance and frost hardiness. Accordingly, several works and reviews (e.g. Ritchie, 1984; Thompson, 1985; Mekal and Landis, 1990; Mattsson, 1997; Gregorio et al., 2010; Villar-Salvador et al., 2004; Grossnickle and MacDonald, 2018) have thoroughly addressed these quality attributes, their measurement and use in seedling assessment methods and their utility for predicting seedling outplanting performance for barefoot and containerized planting stock of numerous species.

Nursery cultivation regimes can strongly determine the functional characteristics of seedlings and their field performance (Landis et al., 1990; Villar-Salvador et al., 2004). A good root system able to perform their physiologic functions toward seedling is essential for seedling establishment and growth. The forming of a root system starts at the germination stage, and as a consequence nursery practices may greatly influence the root development of the seedlings used for reforestation (Hahn and Hutchison, 1978).

Because root growth is restricted to the volume of substrate a container can hold, it is not surprising that pot characteristics have a significant impact on root and plant growth (Mathers et al., 2007). Container of poor design such as solidwall containers made of hardplastic with smooth walls and small bottom drainage can obstruct roots, limiting their development or causing them to circle within the container or descend vertically to its base which resulted in root spiraling (Hultén and Jansson, 1978; Lindström and Rune, 1999). After plantation in field, circling roots formed at the bottom of the container becomes irreversible with the lignification of plant tissues (Amorini and Fabbio, 1992), commonly fail to grow out into the soil, resulting in reduced root growth and function (Ruter, 1993). As a result, it has been shown that deformed root systems can contribute to long-term tree growth problems such as instability in the landscape (Nichols and Alm, 1983), reduced shoot growth, tree decline and mortality (Ortega et al., 2006).

Oaks seedlings quickly develop a strong taproot, which usually grows to several inches in length within a few weeks after germination begins (Johnson et al., 2002). This early development of the taproot resulted in root spiraling on cork oak seedlings reared in polyethylene bag of a closed-bottomed cylindrical shape. The root deformation increase with increasing time of growth in the container (Grene, 1978) and lead to poor post-planting survival. This root deformation type have been minimized by the out of ground seedbed production system associating anti-spiraling WM type containers made of thin plastic with dihedral ridges (Riedacker, 1978). This cultivation system potentially allows air root-pruning which minimize root spiraling by modifying or killing root tips and favors a more fibrous root system (Fiore et al., 1998; March and Appleton, 2004; Gilman and Kempf, 2009). However, despite these innovations and the improvements that have been made on seedling quality, root deformations on cork oak container-grown seedlings, in particular collar nodes and sticks, remain observed in nurseries. Theses detrimental root deformations persist after plantation and lead to plant death few years later (figure 1).

Although previous studies have focused on the effects of growing media (Belghazi et al., 2013) and the orientation of the radicle at the moment of sowing (Chouial and Bena-mirouche, 2016) on root deformations of cork oak container-grown seedlings, the effect of radicle length at the time of sowing remain not documented. Moreover, despite the above advantages of the WM container type, there is little information about the ideal volume of this container type that allows better root and shoot attributes; the WM type container of 400 cm³, suggested as an alternative to the polyethylene bag, was used in empirc approach.

Thus, looking to the above elements, the purpose of this study was to assess the effects of the radicle length at the time of sowing and container size on root deformation on Cork oak seedlings. Although limited at the nursery development phase, the study provides also the opportunity to evaluate the effectiveness of the container type WM for producing well-conformed root system. The results would provide guidelines for enhancing cork oak planting stock quality by minimizing root deformations.
**Methods**

**Seed collection and handling**

The experiment was conducted in the open-air nursery of the regional station of forestry research in Jijel on the North-East-Algerian coast (36.79°N, 5.66°E, elevation 18 m). Seeds were hand-collected in mid-December 2015 from one mother *Quercus suber* tree growing in the surroundings of the nursery. After a vigorous cleaning by flotation method, apparently healthy acorns were stored in a cold room until the experiment. In April 2016, the stored acorns were removed from the cold room and separated into two lots: germinated acorns during storage and intact ones. A sub-lot from the intact acorns were put to germinate in moist sawdust under laboratory environment.

**Seed sowing and seedling cultivation**

Five sowing methods were tested (figure 2): sowing of intact acorns (IA) as a control, sowing of acorns germinated during storage (AGS), sowing of acorns germinated after moist stratification with radicle less than 5 mm length (R<5), radicle of length ranging between 7 and 12 mm (7 < R < 12) and radicle of length ranging between 15 and 20 mm (15 < R < 20).

The acorns were individually sown in containers types WM of Riedacker made of thin plastic with dihedral ridges of three different volumes of 400 cm³, 800 cm³ and 1,200 cm³ filled with the same growing mixture made of a compost of *Acacia cyanophylla* and forest brush (1v:1v). The containers of each size were arranged in perforated plastic boxes which were randomly placed on benches elevated at 30 cm from the surface of the ground to allow root air-pruning.

**Experimental design and cultural treatments**

The study consisted of a randomized complete block design with three replications. There were 15 treatments (5 sowing method x 3 containers size). Each treatment combination (sowing method) was represented by 75 acorns (25 x 3). Thus, the total number of acorns sown in the experiment was 1,125 acorns (25 acorns x 5 sowing methods x 3 container sizes x 3 replications).

Even though the compost reduces the germination of weeds, the growing medium can be infested by some weeds that compete the plants for water and nutrients. To improve weed control, continual hand suppressing of the emerged weeds was ensured to keep containers weed free. The irrigation was uniformly given throughout a semi-automatic irrigation system. No fertilization was applied.

**Sampling and roots examination**

At the end of the vegetation period (six months after sowing), all the 1,080 survived seedlings (24 x 3 per treatment) were destructively sampled for roots deformations examination. After carefully washing roots systems free of growing media, the roots were examined for root deformations and classified in one of the three categories: collar nodes, collar sticks and bad pruning of the taproot. If no deformity was observed, the root was classified as normal.

**Statistical analysis**

An ANOVA was performed to ascertain significant effects of radicle length and container volume on root deformations. A Newman-Keuls test was used to separate means obtained from different treatments (sowing method x container volume). Correlation coefficients were calculated between root deformation and the various treatments. Percentage values were transformed through the formula arcsine (where x is the percentage value divided by 100) before data were subjected to one-way analysis of variance (Amoroso et al., 2010). All statistics were performed with XLSTAT software package.
Results

Overall, the results show that almost half (49%) of the 1,080 seedlings inspected at the end of the nursery period were affected by root deformations (table I). The other seedlings representing a percentage of 51% were free of deformation and were thus classified as normal.

All the root deformations were observed on the taproot, of which the root collar node, the root collar stick and the bad pruning of the taproot (L-shaped taproot) with respective percentages of 20.91%, 21.09% and 0.62% of the inspected seedlings (table I).

Furthermore, no root spiraling was recorded, while 6.43% of the inspected seedlings showed multiple taproots which was proving to be an advantage for seedlings intended for arid environments. Therefore, we presented only results of detrimental root deformations expected to have harmful influences on survival and growth of transplanted trees (collar node, collar stick and L-shapes taproot).

Effects of sowing methods

Among all inspected seedlings, sowing with radicle of \(15 \, < \, R \, < \, 20\) mm length generated the highest percentage of deformed roots (14.83%), followed by sowing with radicle of \(7 \, < \, R \, < \, 12\) mm length, sowing of acorns germinated during storage and sowing with radicle of length less than 5 mm with respective percentages of 10.01%, 7.51% 7.33%, whereas the lowest percentage of deformed roots (3.84%) was observed on seedlings grown from sowing of intact acorns (table II). Accordingly, the most deformations were generated by sowing of germinated acorns both during storage or after moist stratification compared to sowing of intact acorns with respective percentages of 91% and 9% of the total of seedlings.

Moreover, sowing method showed a marked effect on the type of the root deformation observed (table II). Seedlings grown from acorns with radicle of \(15 \, < \, R \, < \, 20\) mm and \(7 \, < \, R \, < \, 12\) mm length showed high percentages of collar nodes, around 15% of seedlings grown from acorns with radicle \(R \, < \, 5\) mm length and those germinated during storage showed nodes, whereas less than 5% of seedlings grown from intact acorns showed this detrimental deformity. Likewise, the collar stick was observed in particular on seedlings grown from sowing of acorns with radicle of \(15 \, < \, R \, < \, 20\) mm and \(7 \, < \, R \, < \, 12\) mm length and those germinated during storage with respective percentages of 25.68%, 26.83% and 21.32%.

Effects of container size

As shown in table III, the highest percentage of deformed root was observed on seedlings grown in the container of 400 cm\(^3\) (24.03%), followed by seedlings grown in the containers of 800 cm\(^3\) (11.20%) and 1,200 cm\(^3\) (7.95%). Despite the expected increase in root deformation on the container of 400 cm\(^3\), the registered values were statistically non-different (\(P = 0.167\)).

In the same way, the container size had no clear effect on the type of the root deformation observed.
Results from this study confirm the occurrence of root deformities on cork oak seedlings grown in container as has long been reported for container-grown seedlings of a wide range of species, such as Jack pine (Segaran et al., 1978; Carlson and Naim, 1978), Black spruce (McClain, 1978), Lodgepole pine (Van Eerden, 1978), Stone pine (Ben Salem, 1978), Red oak and Sessile oak (Chagnon, 1995; Guibert and Le Pichon, 1998). The overall results showed that 49% of the 1,080 seedlings inspected at the end of the nursery period are affected by root deformities. These findings are in line with previous findings of Bouderrah et al. (2017) about root deformations on cork oak container-grown seedlings in some Moroccan nurseries.

Although, both treatment combinations cause root deformation, the germination status of acorns at the time of sowing was shown to exert the most significant effect. In accordance with a previous research (Chomial and Benamirouche, 2016), the greatest number of deformed roots was observed on seedlings grown from sowing of germinated acorns than on seedlings grown from direct sowing of intact acorns with respective percentages of 91% and 9% of the inspected seedlings. Thus, direct sowing of intact acorns could significantly reduce the occurrence of root deformations. However, the presence of root deformations on seedlings grown from direct sowing of intact acorns suggests that the root deformations also may be governed by other factors than those studied here, such as plant growth regulators (Coutts and Bowen, 1973; Sutton, 1980), genetic quality of acorns, physiological quality of acorns (both initial and post-storage qualities) and germination conditions (temperature and humidity).

Sowing of acorns germinated during storage provided 7.51% of seedlings with deformed roots predominantly detrimental deformations. These results confirmed the statement of Corbineau and Bernard (2001), that sowing of acorns that germinate during storage lays out the future root system with severe and often detrimental deformations. In fact, the tendency of acorns to germinate in the storage container constitutes a real problem in acorn storage (Bonner and Vozzo, 1987; Benamirouche et al., 2018). Thus, ensuring good storage conditions is needed to reduce the pre-sprouting of acorns and to avoid sowing of acorns germinated during storage for seedlings production.

The length of the radicle at the time of sowing had a marked effect on the percentage and type of the observed root deformities. In agreement with earlier results for the two oaks Quercus rubor and Quercus petraea (Chagnon, 1995; Guibert and Le Pichon, 1998), our results indicated that sowing with radicle of more than 15 mm in length engendered more root deformations than sowing with radicle less than 12 mm in length. Moreover, it appears that the longer the radicle the higher the percentage of the collar nodes and sticks that can occur on seedlings. These findings corroborate with Madeore (1996) who stated that the percentage of the collar sticks in Quercus petraea increases with the length of the radicle at the time of sowing. In addition, from a practical point of view, if the radicles become too long, it may be difficult to plant without causing root deformation (Luna et al., 2009). Hence, sowing of acorns with long radicle is more difficult to achieve and could induce several root deformations especially when germinated seeds were poorly picked out from the germination bed to the container.

The third detrimental root deformation observed in the current study was the L-shaped taproot (Lacaze, 1968; Brissette et al., 2012), which represented less than 1% of the inspected seedlings. Apparently, this detrimental deformation may occur when the taproot reaches the bottom of the container, it can eventually not correspond with the perforations of the plastic boxes, which are not primarily designed for such a use. This forces the taproot to grow horizontally forming a taproot L-shapes deformation. This deformation of the taproot persist after transplanting and lead to serious consequences, such as growth reduction reported for L-taprooting seedlings of spruce after two years in the field (Lacaze, 1968). Thus, this deformation of the taproot should be corrected prior to transplanting by the hand pruning of the deformed part of the root, as has been well demonstrated for planting stocks of several species affected by the L-taprooting deformation (Ball, 1976; Stone and Norberg, 1978).

By its design and restrictions, the container type can alter root orientation and morphology (Marshall and Gilm, 1998; Mathers et al., 2007; Kostopoulou et al., 2011), which seriously affected seedling post-transplanting. The

### Discussion

**Table III.** Effects of container size on type and percentage of detrimental root deformations observed on six-months-old cork oak container-grown seedlings.

| Container volume (cm³) | Collar node | Root deformation type (%) | L-shapes |
|------------------------|-------------|---------------------------|----------|
| 400                    | 24.77 a     | 21.76 a                   | 0.50 a   |
| 800                    | 16.85 a     | 20.48 a                   | 0.56 a   |
| 1,200                  | 18.33 a     | 20.28 a                   | 0.92 a   |
| P value                | 0.471       | 0.92 a                    | 0.800    |

In each column, values with no letters in common differ significantly at p = 0.05 according to Newman-Keuls ranking test.
results regarding the container size effect showed that root deformations were more pronounced with smaller container of 400 cm³ than with larger containers of 800 cm³ and 1,200 cm³, thought differences were no statistically significant (P = 0.167). These findings are in agreement with NeSmith and Duval (1998) who stated that altered root morphology may be more pronounced with smaller containers. Moreover, no root spiraling was recorded for the three container size tested in the current study. Thus, irrespective of its size, the bottomless WM container type was shown highly effective against this root deformation commonly observed in polyethylene bag of a closed-bottomed cylindrical shape. In fact, as has been well demonstrated by Riedacker (1978), the internal dihedral ridges of the bottomless container type WM induce roots to grow downward and air-pruned when they reached the atmosphere avoiding them to circle.

Furthermore, after the visual examination of the roots of the survived seedlings in the current study, we observed that the air-pruning of the tap root enhance root proliferation and induces the formation of root tips (De Champs, 1978; Amorini and Fabbio, 1992; Fiorino and Cork, 1996). NeSmith and Duval (1998) who stated that altered root morphology may be more pronounced with smaller containers. Moreover, no root spiraling was recorded for the three container size tested in the current study. Thus, irrespective of its size, the bottomless WM container type was shown highly effective against this root deformation commonly observed in polyethylene bag of a closed-bottomed cylindrical shape. In fact, as has been well demonstrated by Riedacker (1978), the internal dihedral ridges of the bottomless container type WM induce roots to grow downward and air-pruned when they reached the atmosphere avoiding them to circle.

Furthermore, successful seedling establishment is partially dependent on the capacity of seedlings to initiate new roots quickly and establish a vigorous root system before initiation of rapid new shoot growth (Sutton, 1980; Grossnickle, 2005). When outplanted to field or potted to a larger container, cork oak seedlings initiated as bottomless-contain-er stock generated new root from the root tips near the cut end of the primary root, the generated root grew in-line with the primary root as we observed on a root growth potential nursery trial (Benamirouch et al., 2018). On cork oak young trees excavated after six years from field transplant as WM container type stock, the replacement taproot penetrate with positive geotropism and have vertically reached a depth of more than 140 cm in a soil with no visible physical barriers (data not shown). Besides ensuring good anchorage and stability, the strong development of the taproot intends to acquire water from dipper soils with relatively more available moisture in summer (Devine et al., 2009).

Conclusion

The overall results of this study confirmed the influence of nursery practice on root quality of containerized seedlings. More especially, sowing method showed a marked effect on root deformation on cork oak seedlings. The root deformations on cork oak containerized seedlings can be significantly minimized by sowing of intact acorns than germinated acorns for seedling production. Besides this relevant advantage, sowing of intact acorns is easier and faster to carry out and thus cost-effective and so more interesting for nurseries. If, however, we must sow germinated acorns, sowing should be down with acorns with radicle less than 5 mm in length to reduce the occurrence of detrimental root deformations. The study also highlighted the need to a safe storage of acorns to reduce the acorns pre-sprouting since the occurrence of detrimental root deformations on seedlings grown from sowing of acorns germinated during storage.

Although the container size had no clear effect on the type and percentage of root deformations, it is evident that breeding of cork oak seedlings in WM container type may be a good nursery practice that could enhance seedling quality by significantly preventing root spiraling frequently observed on seedlings raised in polyethylene bag. In addition, this container type is reusable and offers an excellent opportunity for mechanization. Thus, although more expensive than the usual polyethylene bag, its advantages are worth the cost for better root conformation.

The results of this study indicate that cork oak seedlings with perfect roots can be produced in nurseries through suitable sowing method and appropriate container and growing medium. These good practices will certainly lead to enhance seedlings quality for more successful restoration plantings especially under the harsh site conditions of the Mediterranean areas, where the post-planting recovery and survival are extremely linked to root quality.

Finally this study has been focused on the nursery phase; we emphasize the need to an in-depth and continued study of the long term effects of root deformation on the post planting performance of planted cork oak seedlings with deformed roots.
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