Abstract

Root growth potential (RGP) is used to evaluate seedling vitality from nurseries prior to outplanting. Because results from previous studies indicate mixed results, there is still interest in exploring if a correlation between RGP and outplanting performance exists. This study tested RGP for 44 western larch (Larix occidentalis Nutt.) and 24 Interior Douglas-fir (Pseudotsuga menziesii [Mirb.] Franco var. glauca [Beissn.] Franco) seedlots using mist chambers followed by outplanting at three sites in the Inland Northwest. Survival exceeded 95 percent for both species at all three sites and was not related to RGP. RGP was not correlated with aboveground growth for western larch but was positively correlated for Douglas-fir at one site. Weather during early summer was suitable for new root growth (warm temperatures and average precipitation) and most likely caused the high survival and growth during the first year. This paper was presented at the Joint Annual Meeting of the Western Forest and Conservation Nursery Association and the Intermountain Container Seedling Growers Association (Coeur d’Alene, ID, October 25–26, 2018).

Introduction

Root growth potential (RGP; root production under optimal controlled conditions) is one of many seedling quality tests used to assess vitality of seedlings grown in nurseries prior to outplanting (Haase 2008). RGP was first proposed by Stone (1955) to assess seedling physiology in response to claims that seedling physiological grades were equally or more important than morphological grades (Wakeley 1954). Stone’s experiment was simple: he grew conifer seedlings in a greenhouse, observed their root development, and related root development to seedling survival. The idea that a simple test of seedling root development under controlled conditions may relate to field performance spurred rapid development of RGP research and methodologies from the 1970s through the 1990s and their applications continue today.

Literature reviews from the peak of RGP research show inconsistent correlations between RGP and outplanting performance that vary by species, RGP testing procedures, and outplanting site conditions (Ritchie and Dunlap 1980; Ritchie 1985; Ritchie and Tanaka 1990). Variability led to a debate about the relevance of RGP to predict outplanting performance given the other factors that can influence seedling outplanting performance such as site quality and climate (Simpson and Ritchie 1996). The debate continues today in the Inland Northwest and other regions as landowners contract with private nurseries to grow seedlings, with overall goals of improving seedling quality and outplanting success.

The ability of seedlings to produce new roots is strongly controlled by their physiology. Seedling physiological potential is developed in the nursery by manipulating nutrient inputs, watering regimes, light quality and quantity, temperature and relative humidity, and seedling dormancy. RGP, like dormancy, shows seasonal cycles that are regulated by internal factors. RGP typically peaks when shoots are not actively growing but dormancy intensity is weak, possibly due to available assimilates and hormonal signals to promote root elongation (Ritchie and Tanaka 1990). Villar-Salvador et al. (1999) found that aleppo pine (Pinus halepensis Mill.) seedlings hardened in the autumn under severely dry conditions produced 27 percent less new roots in RGP tests compared to seedlings hardened under no water stress. Even though they found RGP was lower in water-stressed seedlings, no significant differences were found in survival or
growth 2 years after planting. Similar results were found for Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) seedlings (Tinus 1996).

Given the simplicity of measuring new root growth under controlled conditions, various methods have been developed to test RGP. Testing systems can be divided into three broad classes: (1) seedling potted in soil medium; (2) seedling placed into hydroponic water baths; and (3) seedlings suspended in aeroponic mist chambers where water is misted onto the roots to avoid desiccation. Even though the testing systems expose seedling roots to different environmental conditions, results between the three methods are often correlated (Rietveld 1989). The variety of testing systems, but also the diversity of testing regimes among investigators, makes a comparison of results among studies difficult. To make inferences on seedlot performance, it is thus best to use a consistent testing system and regime to ensure repeatability of results.

Mist chamber RGP is a desirable method since multiple seedlots can be tested within a compact space while being exposed to similar environmental conditions. The mist chamber method was first used by Lee and Hackett (1976) to examine root regeneration of Chinese pistache (*Pistacia chinensis* Bunge). The method was later adapted for conifer seedlings by Harvey and Day (1983) using a system that continuously misted roots with fine droplets of water recirculated within the chamber. The U.S. Department of Agriculture, Forest Service Lucky Peak nursery was one of the first to develop an operational mist chamber system (Hileman 1986). It tested seedlings during packing by misting roots for 10 days then counting new white root tips and measuring the length of the longest new roots (Dolata 1986). The system was further refined by Rietveld and Tinus (1987) to become portable and provide uniform conditions for the roots. The mist chamber method continues to be used to assess RGP (Tinus et al. 2000).

The outplanting environment substantially influences the relationship between RGP and seedling performance (Ritchie et al. 2010). Results typically show low RGP and poor site quality result in poor seedling performance, while high RGP and good site conditions result in good seedling performance. These generalities are pieced together from multiple studies where RGP testing procedures and species differed. Jenkinson et al. (1993) provides one of the most comprehensive examination of the topic, where seedlings were grown at the same nursery, RGP was tested using the same method, and seedlots with different RGP were planted at more than 30 sites across the Pacific Northwest. They classified sites based on critical RGP, where harsher site conditions exhibited higher thresholds of RGP for adequate seedling survival. Seedlots that did not produce RGP values above the critical RGP for the site did not have good first-year survival. Burdett et al. (1983) and Grossnickle (2012) found a similar positive correlation between RGP and seedling survival. In contrast, Ritchie (Simpson and Ritchie 1996) argued that RGP is not a good indicator of field performance. He used a dataset derived from Binder et al. (1988) to demonstrate poor correlation between RGP and first-year seedling survival. Binder et al. (1988) suggested the high variability in first-year mortality of seedlings of three seedlots with moderate RGP was due to microsite conditions such as location from a shading object and proper site preparation.

Silviculture and planting within proper microsite conditions has advanced substantially in the Pacific and Inland Northwest regions, prompting reexamination of the relevancy of RGP for predicting field performance within a contemporary reforestation context. Extensive research suggests RGP is not the “holy grail” for predicting early seedling outplanting performance, but testing can still be beneficial to evaluate if seedlings are physiologically damaged, and thereby assist in the prediction of seedling performance. RGP data can vary by individual seedling responses within seedlots, but especially among seedlots. Only rarely have numerous seedlots been tested simultaneously and then outplanted in common-garden experiments that minimize within-site variability. Therefore, the objective of this study was to examine the first-year survival and growth of western larch (*Larix occidentalis* Nutt.) and Interior Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco var. *glauca* [Beissn.] Franco) seedlings in relation to RGP mist chamber results at three sites in the Inland Northwest.

**Methods**

**Seedlings**

Seedlings for RGP testing and outplanting were grown at various nurseries located in western and
central Oregon, western Washington, Idaho, and British Columbia. All seedlings were 1-year-old containerized seedlings grown in 91/130 Styroblock® containers (Beaver Plastics, Alberta, Canada) with cavity volume of 130 ml (7.9 in³). Only seedlots derived from seed sources from the east side of the Cascade Mountains were tested. Seedlings were grown using operational growing regimes at different private nurseries. Minimum morphological specifications were 2.7 mm (0.1 in) stem diameter and 15 to 30 cm (5.9 to 11.8 in) tall.

Each nursery shipped 90 seedlings per seedlot for this study. Nurseries were instructed to randomly select the seedlings from across the crop to avoid sampling bias. Seedlings were immediately placed in freezer storage at -2.0 °C (28.4 °F) at the University of Idaho Center for Forest Nursery and Seedling Research (CFNSR) until testing. A total of 24 Douglas-fir seedlots from 10 nurseries and 44 western larch seedlots from 9 nurseries were used for this study. Of the 90 seedlings from each seedlot, 15 seedlings were randomly selected for RGP testing and 75 were reserved for outplanting.

**Root Growth Potential**

RGP was tested in mist chambers at the CFNSR Seedling Quality Lab starting in January 2018 using chest freezers with the lids removed (figure 1). The freezer’s internal dimensions were 137.2 by 50.8 by 71.1 cm (54 by 20 by 28 in), and external dimensions were 156.2 by 70.0 by 82.5 cm (61.5 by 27.5 by 32.5 in). Each chamber was filled with 76 to 113 L (20 to 30 gal) of water, which was recycled throughout the testing. A hose with an attached strainer was submerged in the water to pump water to three superfine misting nozzles (Fogg-it Nozzle Co., Belmont, CA) that sprayed 1.9 L (0.5 gal) of water per minute using a 115-volt diaphragm pump operating at 11.4 L (3 gal) per minute at a pressure between 276 and 345 kPa (40 and 50 lbs/in² [PSI]). The three nozzles were equally spaced 44.5 cm (17.5 in) apart and mounted to a polyvinyl chloride (PVC) frame that was centered in the chamber, approximately 25 cm (9.8 in) from all chamber walls (figure 2). The PVC frame was designed to be 31 cm (12.2 in) from the chamber floor and approximately 28 cm (11.0 in) from the bottom of the seedling root plug. The pump was plugged into a timer that misted for 5 seconds followed by 4 minutes and 55 seconds of no misting. The system ran 24 hours per day throughout the test.

Supplemental light was provided to seedlings using Phillips light-emitting diode (LED) linear light modules for 12 hours during the day. Each of the 16 lighting modules in the lab has 87 bulbs emitting 85:10:5 (red:blue:green) light (DR/W LED 120-110V, Phillips, Texas, USA). The lights were suspended 140 cm (55.1 in) above the tops of the chambers and were evenly spaced 12.7 cm (5.0 in) apart (figure 1). Blackout curtains were hung around the sides of the chambers from the ceiling to the top of the chambers to control light intensity and quality (figure 3).
Air and water temperature were maintained at 21 °C (69.8 °F), and an airstone was inserted into the water at the bottom of the chamber to increase the amount of oxygen in the water. Between each round of testing, the chambers and pump system were sterilized using a 1:8 bleach:water solution that circulated within the system for 24 hours. Prior to suspending seedlings in the mist chambers, Styrofoam insulation boards were cut to the dimensions of the chambers and placed over the top, and the mist system was run for approximately 1 hour to raise internal relative humidity to 100 percent and water temperature to 21 °C.

Seedlings were removed from freezer storage and thawed in a refrigerator set to 4 °C (39.2 °F) for 2 days prior to RGP testing. Seedling roots were then washed in room-temperature water to remove soil medium, then measured for root-collar diameter (RCD; mm) and height from the root collar to the tip of the terminal bud (cm). Seedlings were suspended in the chambers in plastic slats with square rubber mats to hold the seedlings upright (figure 1). Slat dimensions were 57.1 by 7.6 cm (22.5 by 3.0 in) to align with the internal chamber width. Five circular (10.2-cm [2-in] diameter) holes were cut out of each slat. Rubber squares were 7.6 by 6.7 cm (2.6 by 3.0 in) with a slit cut halfway through the mat and a small hole cut out of the center for the seedling.

Douglas-fir seedlots were tested for 16 days and western larch seedlots were tested for 20 days, based on preliminary research to identify the minimum number of days required to achieve consistent seedling performance in the mist chambers. At the end of testing, seedlings were removed from the chambers and the number of new white roots 1 cm (0.39 in) long or longer were counted (figure 4).

**Field Experiment**

Three study sites were selected on private land in the Inland Northwest (figure 5) that had similar climate but different soil characteristics (table 1). All sites were harvested and treated with chemical site preparation the year before planting using standard operational mixtures to control shrubs, forbs, and grasses. The

Figure 3. Blackout curtains are suspended from the ceiling around all the chambers. (Photo by Andrew Nelson, 2018)

Figure 4. Example of western larch new root growth at the end of root growth potential testing in the mist chambers. (Photo by Andrew Nelson, 2018)

Figure 5. Location of the three RGP outplanting sites in the Inland Northwest. One site was in the Blue Mountains of northeastern Oregon, while the other two sites were located in northcentral Idaho.
amount of slash left after harvest was minimal at all three sites.

Two days before planting, seedlings were removed from the freezer and thawed in a shaded warehouse with an air temperature of approximately 10.0 °C (50.0 °F). The 68 seedlots were planted in a completely randomized block design where each site served as a block (n=3) (figure 6). At each site, 15 seedlings from each seedlot were planted in a row with a spacing of 0.91 m (3 ft) between seedlings within a row and 1.22 m (4 ft) spacing between rows. Seedlots were randomly assigned to rows and all rows were oriented up-down the slope. Seedlings were shovel planted during a 2-week period starting 25 April 2018. Initial height and RCD were measured within 3 weeks after planting. Seedlings were remeasured at the end of September 2018. Mortality was also recorded. Seedlings that were missing and those that died due to animal damage were excluded from the analysis.

**Data Analyses**

Generalized additive models (GAMs) were used to examine the relationships between RGP and survival and RGP and growth. GAMs are semi-parametric extensions of generalized linear models (GLMs) (Hastie and Tibshirani 1990) and have been used extensively in ecology (Guisan et al. 2002, Yee and Mitchell 1991). GLMs examine the relationship between the mean of the response variable and the linear combination of explanatory variables using a link function, while GAMs use the link function to examine the relationship between the mean of the response variable and a smoothed function of explanatory variables. This makes GAMs very effective for analyzing nonlinear relationships.

Individual GAM models were developed using the data for western larch and Douglas-fir seedlings for both survival and growth. Survival models used a binomial link, since survival was a binary variable (alive or dead). Growth was expressed as the 1-year increment of volume index (cm$^3$) calculated as RCD$^2$ × height. Models tested the relationship for each of the three sites using a thin plate regression spline (Wood 2003) of RGP using the “mgcv” package (Wood 2019) in R version 3.4.3 (R Core Team 2017).
Results

Root Growth Potential

RGP varied considerably among seedlots (figure 7). Average RGP of western larch and Douglas-fir was 25 and 43 new white roots, respectively. Even though RGP varied among seedlots within a nursery, nurseries 8 and 9 grew western larch seedlots with relatively low RGP, while nurseries 6, 8, and 9 grew Douglas-fir seedlots with low RGP (figure 7).
RGP Effects on Seedling Field Survival

RGP was a poor predictor of first-year survival of western larch and Douglas-fir seedlings. For example, nursery 4, which produced seedlots with higher RGP values (figure 7) had the lowest average survival, while nursery 9, which had the lowest average RGP, had higher survival (table 2). The western larch survival GAM model did not find a relationship between RGP and survival at the Northeast Oregon (NEO) and St. Joe (STJ) sites (estimated degrees of freedom [edf] = 1.000, p ≥ 0.376), and only a slightly nonlinear relationship at the Clearwater (CLW) site (edf=1.048, p=0.056) (table 3). The deviance explained by the model was only 3.90 percent. The same was found for Douglas-fir, where the smoothed term for RGP had an edf of 1.000 for all three sites and only explained 0.23 percent of the deviance (table 3).

RGP Effects on Field Volume Index Growth

RGP had a greater effect on volume index growth than on seedling survival, but the effect was still small. RGP was not a significant smoothed term for western larch at any of the three sites (p≥0.146) and the deviance explained by the model was only 4.06 percent (table 4). Volume index plateaued at a RGP value of approximately 25 new roots at the CLW and STJ sites, while the relationship was flat at the NEO site (figure 8). Douglas-fir volume index was positively related to RGP at the CLW site (edf=1.296, p=0.021) with the model accounting for 14.5 percent of the deviance (table 4). Douglas-fir showed a continual increase in volume index with increasing RGP values at all three sites (figure 8).

Discussion

The relationship between RGP and outplanting survival and growth during the first year is not always consistent and often lacks correlation (Simpson and Ritchie 1996). The same was found in the current study for several western larch and Interior Douglas-fir seedlots planted at three sites across the Inland Northwest. This contrasts with other syntheses that found strong correlations between RGP and field performance. Ritchie and Dunlap (1980) reported that 85 percent of 26 papers reviewed showed a positive relationship, while Ritchie and Tanaka (1990) found 75 percent of 12 studies reported a positive correlation. Ritchie and Tanaka (1990) recognized, however, that a relationship does not always occur and postulated three reasons: (1) inadequate testing procedures, (2) poor seedling handling after leaving the nursery, and (3) site and weather conditions.

RGP testing procedures vary considerably among investigations, including differences in procedure with the same testing method. This makes it difficult to draw broad conclusions about the utility of RGP for assessing seedling vitality and outplanting performance. The aeroponic mist chamber RGP testing system used in this study is based on previous iterations of similar systems (Day 1982, Hileman 1986) and was designed to rapidly test multiple seedlots within a limited space. Most published studies that examined the relationship between RGP and seedling performance used potted RGP tests, especially studies comparing multiple...
Results from different testing methods are correlated, but mist chamber systems typically produce less new roots than potted tests under similar environmental conditions (Rietveld 1989). The differences in the number of new roots produced could be due to a lack of dissolved oxygen in the water sprayed onto the roots, as roots are usually coated with fine droplets throughout testing even though they are surrounded by oxygen in the aeroponic environment. To overcome this potential limitation, nozzles with larger droplet sizes can be used to increase oxygen to the roots or an aeration stone could be added to the water at the bottom of the chamber, as was done in this study, so that fine-droplet nozzles could still be used to maintain moistened roots.

RGP testing conditions intentionally diverge from field conditions, where seedlings are exposed to warm conditions that favor root proliferation. This led Ritchie to argue that the logic behind the RGP-outplanting performance relationship is flawed (Simpson and Ritchie 1996) since proliferative root production under warm, controlled conditions does not reflect root growth in the field, when soil temperatures are low. Soil temperature was not measured at the three outplanting sites in the current study, but the minimum and maximum air temperature in May when the seedlings were planted were 6.4 °C and 19.7 °C (43.6 °F and 67.5 °F), respectively, which is approximately 2.0 °C (1.8 °F) warmer than the 30-year normal (Hege-

### Table 2. Average and one standard deviation of seedling size and survival at the beginning and end of the first growing season for western larch and interior Douglas-fir at three sites in the Inland Northwest.

<table>
<thead>
<tr>
<th>Site</th>
<th>Initial</th>
<th>End of season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height (cm)</td>
<td>Diameter (mm)</td>
</tr>
<tr>
<td>Clearwater (CLW)</td>
<td>33.6 (7.2)</td>
<td>3.8 (0.4)</td>
</tr>
<tr>
<td>Northeast Oregon (NEO)</td>
<td>32.4 (5.9)</td>
<td>3.8 (0.6)</td>
</tr>
<tr>
<td>St. Joe (STJ)</td>
<td>33.8 (6.7)</td>
<td>3.9 (0.6)</td>
</tr>
<tr>
<td>Clearwater (CLW)</td>
<td>32.1 (7.3)</td>
<td>4.3 (0.7)</td>
</tr>
<tr>
<td>Northeast Oregon (NEO)</td>
<td>30.8 (7.0)</td>
<td>4.3 (0.7)</td>
</tr>
<tr>
<td>St. Joe (STJ)</td>
<td>31.7 (7.2)</td>
<td>4.3 (0.7)</td>
</tr>
</tbody>
</table>

1 inch = cm/2.54; 1 inch = mm/25.4; 1 in$^3$ = cm$^3$/16.387

### Table 3. Generalized additive model results testing the correlation between RGP and first-year survival for western larch and Interior Douglas-fir.

#### Western Larch

<table>
<thead>
<tr>
<th>Parametric variable</th>
<th>Estimate</th>
<th>St. Error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.966</td>
<td>0.001</td>
<td>156.2</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smooth variables</th>
<th>Est. DF</th>
<th>Ref. DF</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s(RGP-Count): CLW</td>
<td>1.048</td>
<td>1.093</td>
<td>3.77</td>
<td>0.056</td>
</tr>
<tr>
<td>s(RGP-Count): NEO</td>
<td>1</td>
<td>1</td>
<td>0.79</td>
<td>0.376</td>
</tr>
<tr>
<td>s(RGP-Count): STJ</td>
<td>1</td>
<td>1</td>
<td>0.004</td>
<td>0.992</td>
</tr>
</tbody>
</table>

Deviance explained: 3.90%

#### Interior Douglas-fir

<table>
<thead>
<tr>
<th>Parametric variable</th>
<th>Estimate</th>
<th>St. Error</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>0.977</td>
<td>0.001</td>
<td>108</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Smooth variables</th>
<th>Est. DF</th>
<th>Ref. DF</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>s(RGP-Count): CLW</td>
<td>1</td>
<td>1</td>
<td>0.108</td>
<td>0.743</td>
</tr>
<tr>
<td>s(RGP-Count): NEO</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.999</td>
</tr>
<tr>
<td>s(RGP-Count): STJ</td>
<td>1</td>
<td>1</td>
<td>0.036</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Deviance explained: 0.23%

CLW = Clearwater; NEO = Northeast Oregon; STJ = St. Joe; DF = degrees of freedom; RGP = root growth potential.
Precipitation at the sites also persisted through the end of June, with average precipitation of 76 mm (3 in) during that month, which is the 30-year normal (Hegewisch and Abatzoglou 2019). The warmer-than-normal temperatures, typical early season precipitation, and deep surficial volcanic ash deposits that help maintain soil moisture during the summer may have resulted in conditions conducive to root growth and good seedling survival.

The hypothesized link between RGP and outplanting performance assumes that seedlings need to produce new roots following planting to absorb water from the soil. Although new root production is important for seedling survival (Grossnickle 2005), suberized roots can absorb water (Kramer 1946). Seedling morphology (e.g., height, stem diameter, and root mass) at the time of planting are typically positively related to aboveground seedling growth, while the relationship between RGP and shoot growth is more mixed (Grossnickle and MacDonald 2018). Grossnickle and MacDonald (2018) report that, of the 10 studies reviewed between 1991 and 2016, an equal split between positive and neutral responses was found. Our results align with the mixed results from other studies, where a significant relationship was not found for western larch at any of the three sites and for Douglas-fir only at the CLW site. In one of the few studies to examine western larch, L’Hirondelle et al. (2007) found a positive asymptotic relationship ($R^2 = 0.66$) between RGP and shoot dry mass of first-year coastal and interior western conifer species at moderately productive sites. When examined by species, however, the results were more variable: western larch seedlots that produced zero new roots in the RGP tests produced only about 10 percent of the maximum shoot dry mass, while seedlots that produced between 80 and 120 new roots had 80 percent of maximum shoot mass. This suggests a threshold value of RGP at which more new roots do not result in greater aboveground growth.

RGP varied considerably among seedlots and nurseries, but seedlings performed well overall across all three sites. It is unlikely that the lack of relationship between RGP and survival was caused by inadequate testing procedures or poor handling practices. The most likely reasons for the good performance were the favorable site and weather conditions during the period of observation. Mild site conditions can help seedlings overcome vitality issues because of fewer resource limitations (Burdett 1987; Ritchie et al. 2010; Ritchie and Tanaka 1990). Research with this mist chamber system will continue to refine assessment of seedling vitality, examine potential changes in the relationship between RGP and field performance in the second year after planting, broaden the scope of the outplanting sites to encompass a greater range of site quality in the Inland Northwest, and potentially observe the relationship during drier and warmer field seasons. Additional research on harsher site conditions is especially important as climate predictions suggest the region will experience increased mean temperatures and slightly lower precipitation in summer through 2100 (Joyce et al. 2018). Characteristics that define seedling quality may be revised to match site conditions as climate change progresses, including seedling physiology and specifically drought resistance. This may necessitate modifying nursery cultural practic-
es to adjust seedling physiology to withstand harsher site conditions. Since mist chamber RGP testing can produce results in a short time, the system could be used in future investigations to evaluate potential seedlot performance on harsh sites that may be common across the region in the future.

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**Acknowledgments**

The author thanks Hancock Forest Management and PotlatchDeltic Corporation for supplying the seedlots and field sites for this study, Anthony Davis for initially designing the root growth potential chambers, and Lori Mackey for refining the mist chamber system and performing the root growth potential testing.

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