Outplant Evaluation of Container Red Alder Grown with Bonzi® Plant Growth Regulator

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Abstract

Red alder (*Alnus rubra* Bong.) seedlings are challenging to grow due to their tendency to reach excessive heights in nursery culture. We evaluated several Bonzi® (paclobutrazol) plant growth regulator frequency by rate applications on large plug seedlings (15 in3 [250 ml]) then compared the best treatment (three applications of 25 ppm Bonzi®) with our standard Plug+1/2 stocktype at two outplant sites. We also evaluated all nursery treatments, including non-treated plugs, at a garden plot. Large plugs, even without treatment, broke bud several days earlier than P+1/2s and this effect increased with increasing Bonzi® intensity. The P+1/2s had greater height and stem diameter growth at one location and greater height growth at the other. Survival did not differ between stocktypes. The nursery grower can use Bonzi® to produce a balanced plug that is easier to frost protect in the nursery and less susceptible to mechanical damage, with overall higher average nursery packout (yield). This study shows, however, that reducing treatment intensity may lead to better outplant success. 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

Red alder (*Alnus rubra* Bong.) production at the Washington Department of Natural Resources' Webster Nursery in Tumwater is currently grown as a Plug+1/2 (P+1/2) stocktype, where a small plug is started in the greenhouse then transplanted for the remainder of the growing season into a bareroot field. It is sometimes referred to as a "4 by 4," as the seedling spends 3 to 4 months in the greenhouse and an additional 4 months in the bareroot field prior to outplanting. The primary advantage of the P+1/2 is that transplanting to a lower density produces a seedling with a large stem diameter, an important characteristic for resisting freeze damage of thin alder bark, both in the nursery and up to the first 3 years in the forest (Dobkowski 1996). Large seedlings are also less likely to be overtopped by competing vegetation (Dobkowski et al. 2006). The P+1/2 stocktype also has its downsides, including some mortality at the time of transplant. Seedlings that withstand or avoid transplant stress within the nursery may grow taller than desired later in the season. Seedlings in excess of 3.5 ft (1 m) are more likely to suffer mechanical damage during nursery frost protection (figure 1). Alder stems and roots are relatively brittle compared to conifers and are prone to breakage in lifting and packing operations, as well as damage from handling in the woods.

We have trialed application of the plant growth regulator paclobutrazol (Bonzi®, Syngenta, Wilmington, DE) in the plug stage of the P+1/2 stocktype over the last several years (Khadduri 2015) to control excessive height growth and the drawbacks that go with it. Fine-tuning application rates and timing has led to improved packout due to better root-to-shoot balance of the plugs at the greenhouse-to-bareroot transition and less transplant stress in the bareroot field. Even with aggressive root culturing and reduced watering to limit seedling growth, however, the P+1/2 stocktype still has a tendency to have excessive growth late in the growing season, when temperatures decline, nighttime humidity rises, and moisture (precipitation) cannot be controlled.

Given these challenges, we decided in 2017 to evaluate large-plug production as an alternative to our standard P+1/2 stocktype. Whereas P+1/2s start with a

Figure 1. Bareroot alder seedlings can be prone to frost damage in the nursery. Excessively tall seedlings are particularly subject to damage, not only from ice accumulation during frost protection, but also during lifting, packing, and outplanting. (Photos by Nabil Khadduri, 2003)

small plug that finishes in a bareroot field, large plugs remain in greenhouse production the entire season, although they may be moved outdoors for hardening during summer months. Since they remain in containers, large plugs can be moved back under cover for both moisture control and frost protection in the fall (figure 2). The plugs are grown at a higher density than the P+1/2, 26.5 stems per ft^2 (284 per m^2) versus 6 stems per ft^2 (64 per m^2), respectively. Accordingly, plugs are not expected to reach the stem diameter of P+1/2s. The goal of growing a large plug is to attain a seedling with as much stem diameter as possible, but with good balance between shoot-to-root systems. A preferred shoot-to-root target for container seedlings should be 2:1 or less (Haase 2011). Even with better moisture and nutrition manipulation, growers may still struggle to control height in container production, leading to spindly seedlings with a poor sturdiness quotient (height-to-stem-diameter ratio) (Ahrens 2006). Some growers may top mow or pinch off seedlings to control height, but we have observed that this often leads to a loss of apical dominance and subsequent low forking of the tree structure. While this may be acceptable for alder used in restoration efforts, alder used for saw log production should be free of defects in the bottom portion of the tree (Plank and Willits 1994).

The objective of our study was to evaluate the effects of two rates of Bonzi® at various application frequencies on subsequent morphology of large plugs. Since we had never grown large plugs with Bonzi®, the goal was to identify the best treatment in the nursery, then compare it against the standard P+1/2 stocktype in outplant environments.

Materials and Methods

Seedling production

In 2017, we evaluated two red alder stocktypes in a nursery trial using the same seed source (Washington Seed Transfer Zone 05 [Upper Chehalis], 0 to 1,000-ft elevation, woods-run collection, 2009). We started our standard P+1/2 in a 2 in^3 (40 ml) 240-cell Styroblock™ Container (Beaver Plastics, Acheson, Alberta, Canada) in the greenhouse with subsequent transplant to a bareroot field for the remainder of the growing season. We sowed seed February 28 and inoculated with 0.035 oz (1 g) blended fresh alder nodules per 1,000 seedlings at germination. As per our new standard, we applied two spray-to-wet applications of 25 ppm Bonzi® solution at approximately 0.5 fl oz (15 ml)

Figure 2. Large plugs set bud sooner (foreground) and can be easily protected under cover. P+1/2s set bud later due to late-summer/early-fall precipitation and must be frost protected with irrigation. (Photo by Nabil Khadduri, 2018)

Table 1. Bonzi® application frequency, rate, and treatment date..

Number of Bonzi® applications	Rate	Treatment dates
0	N/A	N/A
2	25 or 50 ppm	May 7, June 4
3	25 or 50 ppm	May 7, June 4, July 20
4	25 or 50 ppm	May 7, June 4, July 20, Aug 17

ppm = parts per million

per cell in the container stage at week 8, and again at week 12, just prior to transplant. Plugs were lifted and transplanted May 24–25, grown in the bareroot field for about 6 months, lifted December 7, and stored at 30 °F (-1 °C).

For comparison with the standard P+1/2, we grew large-plug seedlings in 15 in^3 (250 ml) 60-cell Styroblock™ Containers with a range of treatments. We sowed seed March 13 and inoculated with 0.035 oz (1 g) blended fresh alder nodules per 1,000 seedlings at germination. We applied Bonzi® at two rates (25 ppm or 50 ppm) for 0 (non-treated control), 2, 3, or 4 times through the season (table 1). Spray volume for spray-to-wet application to large cells was approximately 0.5 fl oz (15 ml) solution per cell initially, and increased to 1.0 fl oz (45 ml) for the third and fourth applications to account for larger leaf area. Large plugs were lifted December 22 and placed in storage at 30 °F (-1 °C).

We noted widespread presence of nitrogen-fixing *Frankia* bacteria nodules on both stocktypes by early summer. Artificial inoculation of alder seedlings with *Frankia* bacteria has been linked to improved seedling growth (Martin et al. 1991) and improved field performance, particularly in nutrient poor soils (McNeill et al. 1989). While excessive nutrient load in the nursery can limit alder nodulation, we did not know what effect adding a plant growth regulator to a peat-only growing medium might have. We did not see nodule inhibition in this trial.

We recorded final seedling height and stem diameter for both Plug+1/2 and S-15 stocktypes the week before harvest. We conducted a factorial analysis (frequency by rate) on treatment responses using the R statistical package (R Core Team 2019). Means were subjected to Tukey's Honestly Significant Difference (HSD) test and considered significantly different at the $p<0.05$ level.

Nursery Results for Selection of Large Plugs for Outplant Evaluation

Bonzi® applications at the 25 ppm rate provided an effective seedling response (figure 3). Two applications slowed seedling height growth compared with the control, and three applications further retarded growth compared with two applications. Four applications, however, had no additional growth reduction compared with three applications. Bonzi® applications at the 50 ppm rate (data not shown) proved to be excessive. Unlike the lower rate, the 50 ppm Bonzi® treatments significantly reduced both height and stem diameter in comparison with the 25 ppm rate or non-treated seedlings at as few as 2 applications. Three applications of Bonzi® at 25 ppm significantly controlled height with minimal impact to stem diameter in comparison with non-treated seedlings (table 2).

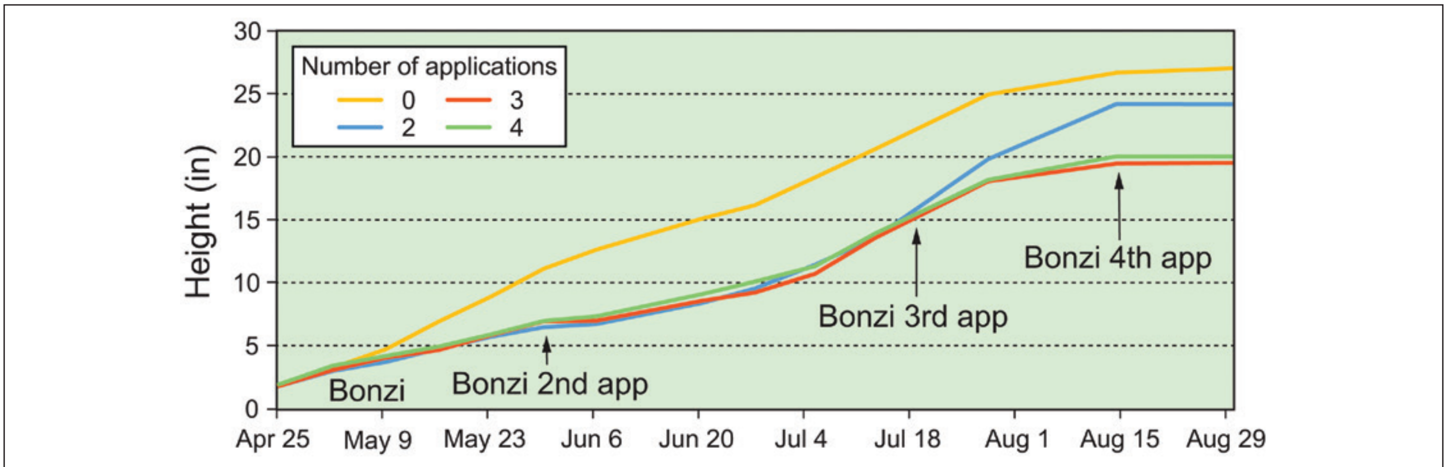

Figure 3. We applied Bonzi® at 25 and 50 ppm solutions 0, 2, 3, or 4 times through the growing season in response to height growth. For clarity, only 25 ppm rate is shown. Note that the 4th application applied late in the season did not have an effect on height growth as seedlings were in the process of shutting down on their own.

Table 2. Morphology averages for 25 ppm Bonzi®, applied 0, 2 or 3 times compared with the standard P+1/2 alder stocktype. Sturdiness ratio is height divided by stem diameter. Means with same letter are not significantly different at the p<0.05 level.

# Bonzi® apps	Height (cm)	Stem diameter (mm)	Sturdiness ratio (height/stem diameter)
0	68.6b	5.8b	11.8a
2	61.5c	5.7b	10.8a
3	49.5d	5.6b	8.8b
P+1/2 alder	102.9a	11.1a	9.3b

ppm = parts per million

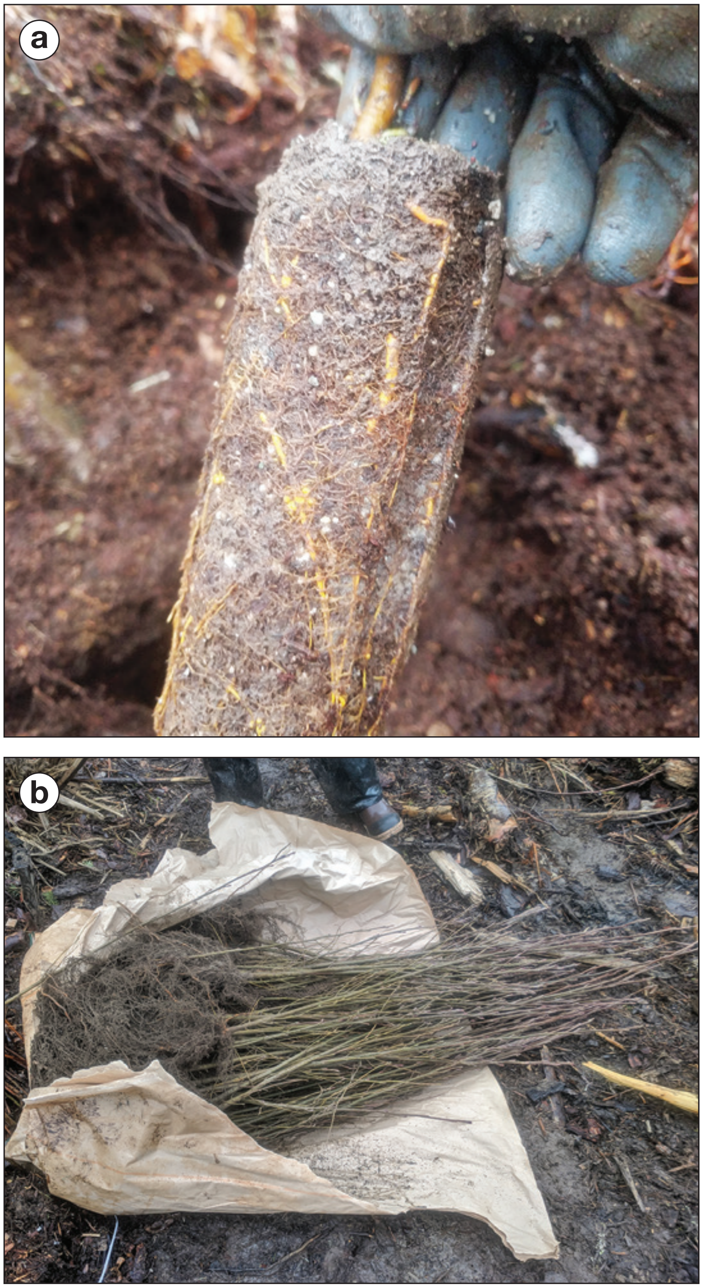

Figure 4. Shoot-to-root ratio averaged 1:1 for (a) large plugs (3 Bonzi applications at 25 ppm rate) compared with (b) 3:1 for P+1/2s. (Photos by Nabil Khadduri)

These data resulted in a superior sturdiness quotient (ratio of height to stem diameter in cm/mm) averaging close to 9 which is similar to the standard P+1/2 alder stocktype. Ahrens (2006) assumes a maximum sturdiness quotient of 10 for culling standards. In an effort to minimize the sturdiness ratio of the treated plugs, we selected the 3 application @ 25 ppm Bonzi® treatment for comparison with the standard P+1/2 alder stocktype for outplant evaluation.

Outplant and Garden Plot Evaluations

In 2018, we compared field performance between the P+1/2 and large plug stocktypes. We used a water displacement method to determine shoot and root volumes (all soil media or soil washed from roots). Shoot-to-root ratios (n = 30) averaged close to 1:1 for S-15 (3 apps at 25ppm) versus a more top-heavy 3:1 for the P+1/2 stocktype (figure 4). Seedlings were planted at two forest sites and a nursery garden plot (figure 5). The University of Washington Pack Forest site, near Eatonville, is at 1,100 ft (335 m) elevation, with an east aspect and a Scamman silty clay loam. The Louie site is outside of Castle Rock, WA, at an elevation of 750 ft (230 m), with a northeast aspect and an Olympic cobbly silt loam. The nursery garden plot is near Tumwater, WA, at 200 ft (60 m), with a flat aspect and Yelm sandy loam soils. Since the nursery site is a

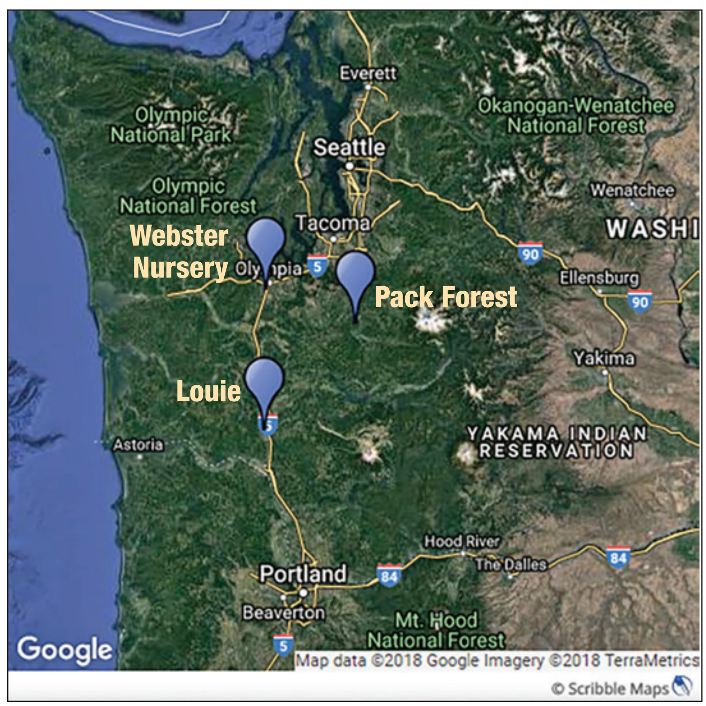

Figure 5. Two forest sites and nursery garden plot location of outplanted seedlings.

Figure 6. P+1/2 seedling at Pack Forest site at end-of-season measurement in October 2018. (Photo by Nabil Khadduri, 2018)

cultivated agricultural field, we placed fixed irrigation to avoid excessive drought mortality, but due to natural soil moisture conditions, it was not used.

We planted four replications (blocks) of 25 seedlings per stocktype at Pack Forest and Louie for a total of 100 seedlings of each stocktype per site. We planted 30 seedlings, without blocking, of every container treatment at the nursery site along with the P+1/2 stocktype (table 1).

We started thawing seedlings on March 10 by moving stock to a cooler kept at 36 °F (2 °C) until planting. We planted the nursery garden plot March 20, Pack Forest on March 22, and Louie on April 17.

At the garden plot, we evaluated budbreak three times weekly on all seedlings for 5 weeks, from the end of March through April. We measured chlorophyll fluorescence and chlorophyll content at all three sites the last week of August with a SPAD 502 Plus Chlorophyll Meter (Spectrum Technologies, Aurora, IL). Thirty seedlings were measured per treatment. We placed three leaf clips (replicates) per plant and allowed foliage to adjust to baseline light levels for 20 minutes for stabilization before measurement. We measured predawn moisture stress (PMS Instrument Company, Albany, OR) on 12 seedlings per treatment at the nursery garden plot and Pack Forest the third week of August. We measured seedlings at all sites for height and stem diameter and tallied survival in early October (figure 6). We conducted ANOVA analyses using the R statistical package (R Core Team 2019) and subjected treatment means to Tukey's HSD test and considered means significantly different at the $p<0.05$ level.

Outplant Results and Discussion

Overall, container seedlings broke bud earlier than the bareroot stock, and tended to be earlier with increasing Bonzi® applications. While there were no differences in survival, the P+1/2 seedlings started and remained taller by the end of the first growing season, with significantly greater stem diameter at all three sites.

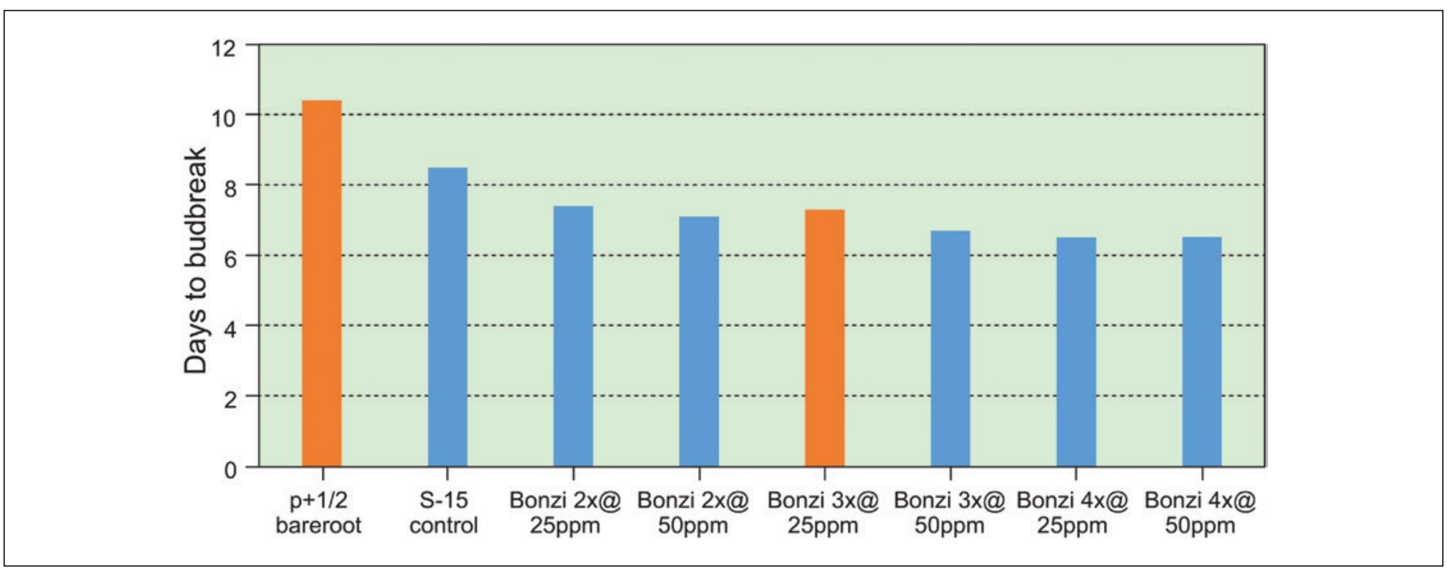

Figure 7. Day of budbreak in April by treatment at nursery garden plot following planting on March 20. Outplant-evaluated treatments are highlighted for clarity.

Budbreak

In the nursery garden plot, the P+1/2 stocktype broke bud later on average than all container treatments (figure 7). Within the plug treatments, increased Bonzi intensity (rate combined with application frequency) tended to have earlier budbreak (figure 7).

Budbreak was not measured at the forest sites. We did, however, note at Pack Forest that approximately 60 percent of large-plug stock had broken bud 3.5 weeks after planting whereas less than 20 percent of P+1/2 stock had broken bud at that time.

Dobkowski (2006) noted earlier budbreak in red alder container stock in comparison with bareroot stocktypes. This effect may be attributed to earlier budset in the nursery, or different hardening or de-hardening conditions while finishing in a greenhouse environment. We did not find mention in the literature of gibberellin-inhibiting growth regulators further hastening budbreak in Pacific Northwest tree species. Paclobutrazol has been shown to hasten flowering time in other plants, for example lupine (*Lupinus varius* L.) (Karaguzel et al. 2004). The important biological ramification is that alder seedlings breaking bud even a few days earlier risk increased exposure to spring freezes. Peeler and DeBell (1987) list spring freeze damage as one of the primary obstacles to successful alder seedling establishment.

Physiology

We saw no significant differences between stocktypes for chlorophyll fluorescence or plant moisture stress (table 3).

Chlorophyll content readings were significantly higher at Pack Forest for the large-plug stocktype compared with P+1/2. Paclobutrazol, the active ingredient in Bonzi®, has been shown to concentrate chlorophyll in other species, and this is attributed to a larger number of chloroplasts in a relatively smaller leaf area compared with non-treated plants (Khalil et al. 1995). Our data indicate that for red alder this effect may last late into the first growing season, but it is not clear what impact this may have had on growth or survival.

Survival

We saw no significant survival differences between stocktypes at any of the sites. Survival was 97 to 100 percent at Pack Forest and the nursery garden plot. At the Louie site, however, both stocktypes had less than 20-percent survival, which is most likely due to later planting and drought stress caused by an early dry season and heavy vegetation competition. Precipitation at the Louie site from the time of planting to the end of August was 4.1 in (10.4 cm) compared with 9.2 in (23.4 cm) and 8.6 in (21.8 cm) at the nursery garden plot and Pack Forest sites, respectively. A challenge for planting alder is to weigh the risk of late freezes into March against drought onset that can occur as early as May in some years. Tanaka and Dobkowski (unpublished data) found that seedlings planted in mid-March had approximately two and four times more roots in July, respectively, than seedlings planted in mid-April or early May.

Morphology

At all sites, P+1/2 seedling heights were initially taller at planting and remained significantly taller at the end of the first growing season (figure 8). At the Pack Forest site and nursery garden plot, P+1/2 seedlings had significantly greater height growth during the growing season. Similarly, P+1/2 stem

Table 3. Physiology measurements conducted in August of first growing season. Chlorophyll content (SPAD) readings were significantly higher for large plugs than the P+1/2 stocktype at Pack Forest.

State	Chlorophyll fluorescence (Fv/Fm)		Chlorophyll content (SPAD)		Plant moisture stress (Mpa)	
	P+1/2	Plug	P+1/2	Plug	P+1/2	Plug
Oregon	0.771 a	0.785 a	30.2 a	38.0 b	-0.36 a	-0.41 a
Washington	0.740 a	0.767 a	36.9 a	42.3 a	n/a	n/a
Washington	0.814 a	0.816 a	39.2 a	43.8 a	-0.43 a	-0.46 a

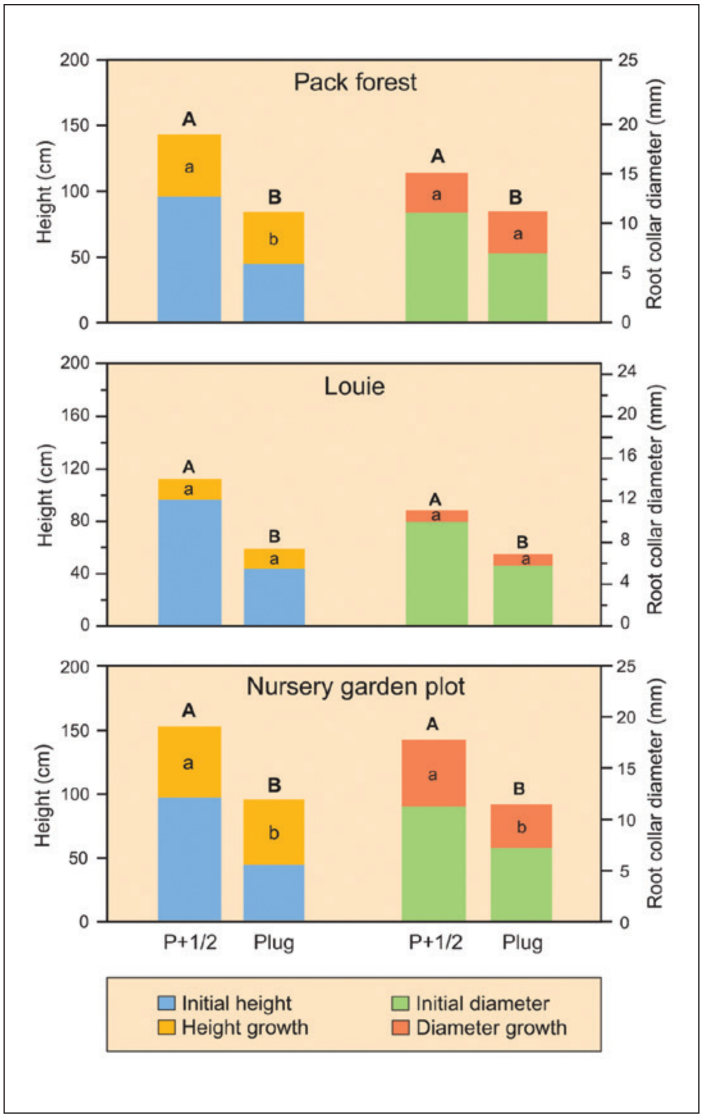

Figure 8. Final height and height growth were significantly greater for P+1/2 seedlings at Pack Forest. At Pack Forest, P+1/2 seedlings averaged greater initial and final root collar diameter, but we saw no significant difference in root collar diameter growth. At Louie, final height and root collar diameter remained significantly taller for P+1/2 seedlings, but we saw no difference in height and root collar diameter growth compared to plugs. At the nursery garden plot, P+1/2s had greater final height and root collar diameter, as well as greater root collar diameter growth compared to plugs. We saw no difference in height growth.

diameters started and remained significantly larger than large plug stem diameters on all three sites. Stem diameter growth did not differ among stocktypes at the Pack Forest or Louie sites but P+1/2 had greater stem diameter growth than large plugs at the nursery garden plot (figure 8).

All large-plug treatments were included at the nursery garden plot and showed a significant trend between Bonzi® plant growth regulator intensity (rate by frequency of application) and decreased stem diameter growth (figure 9). Anecdotal evidence in early Bonzi® studies on reforestation species suggested the plant growth regulator might stunt seedlings for several growing seasons. At least with the lower intensities applied in this trial, we did not observe dramatically negative effects in the first growing season. The correlation between paclobutrazol intensity and decreased stem diameter growth at the nursery garden plot, however, suggests limiting nursery applications to avoid negative impacts on outplant performance.

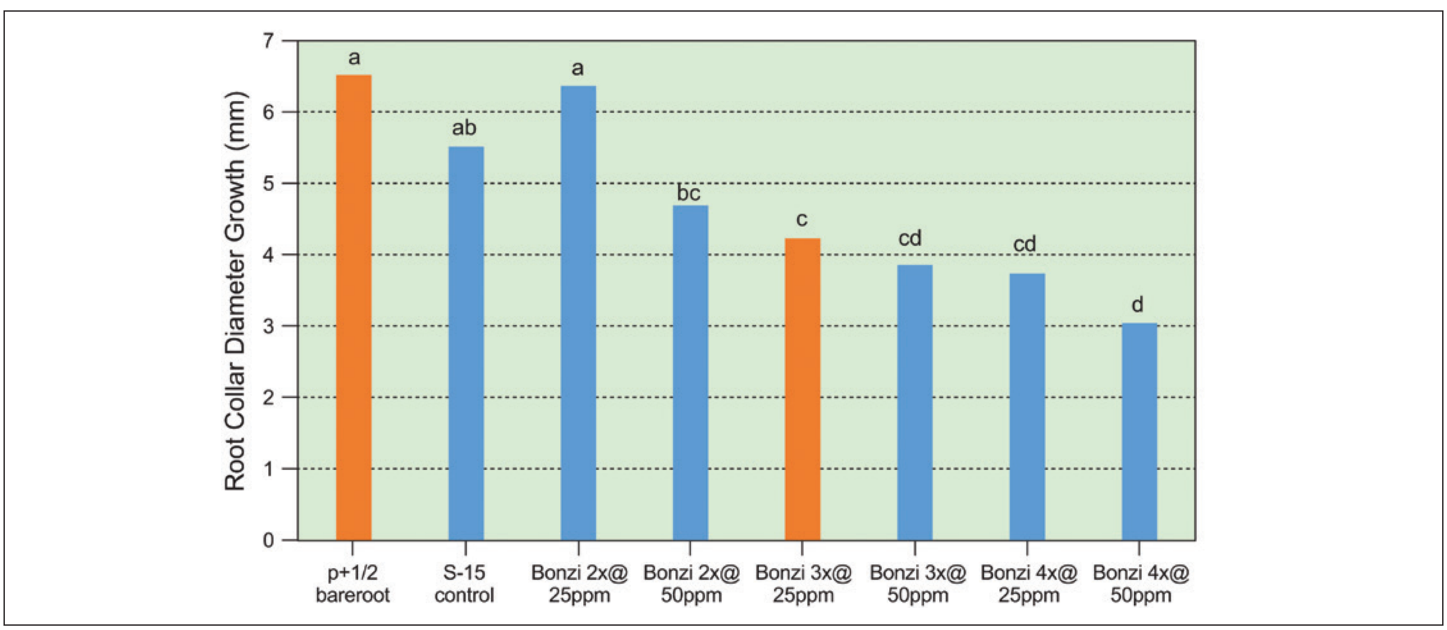

Figure 8. In the nursery garden plot evaluation of all treatments, increasing Bonzi® treatment intensity (rate by application frequency) significantly decreased root collar diameter growth. Outplant-evaluated treatments are highlighted for clarity.

Conclusion

We advise growers to minimize rate and frequency of Bonzi® applications to large plugs to effectively limit height growth while avoiding unnecessary side effects such as early budbreak or reduced stem diameter growth after outplant. Large plugs already tend to break bud earlier than bareroot stocktypes, and, as found in this study, a plant growth regulator can exacerbate that effect.

Despite these potential drawbacks, using a plant growth regulator for large-plug production can result in a seedling with a balanced root-to-shoot ratio and reasonable sturdiness. This balance may reduce stem breakage during handling and provide a more resilient plug root system for shallow soil or in mild drought. Although noted but not measured in this study, another benefit may be the increased density of buds along the stem in Bonzi®-treated large plugs. Large plugs without treatment had fewer buds, spaced farther apart along the stem. An increased density of buds on the lower portion of the stem has been attributed to a reduction of sunscald (Harrington et al. 1994), though we did not observe sunscald on any seedlings at our sites. Perhaps the greatest benefit of the large plug is an increase in expected nursery packout.

We emphasize the importance of appropriate outplant timing to avoid both late freezes and early drought. The current recommendation of mid-March to mid-April planting holds, but early drought years may pose as much or more of a risk than late frosts. It may be better to err on planting earlier in this window rather than later. As always, drought and/or severely frost-prone sites should be avoided altogether when planting red alder, especially with P+1/2s for the former and large plugs for the latter.

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Acknowledgments

The authors thank Kila Benge, Greenhouse Manager at WADNR Webster Nursery; Jeff Kelly, Forest Manager, Center for Sustainable Forestry at Pack Forest; and Gregory J. Ettl, Associate Professor and Director, Center for Sustainable Forestry at Pack Forest, School of Environmental and Forest Sciences, University of Washington.

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