WHITE OAK EPICOTYL EMERGENCE AND 1-0 SEEDLING GROWTH FROM SURGICALLY ALTERED GERMINATING ACORNS

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Abstract—Open-pollinated white oak (*Quercus alba* L.) acorns were collected and stored at 4 °C in November 2004. Three days before sowing in early December, we treated germinating acorns in five ways: no surgery (C); one half of the radicle cut off (HR); whole radicle cut off (WR); one cotyledonary petiole severed (OP); and both cotyledonary petioles severed, which resulted in no embryo axis (NE). Seedlings were lifted in February 2006. The NE treatment had two percent epicotyl emergence and was not included in our statistical analysis. The OP seedlings were shorter and had greater root-to-shoot ratio than seedlings in other treatments. For most of the families, the OP treatment had less percent epicotyl emergence; OP seedlings also had smaller root collar diameter and less biomass than C. More HR and WR seedlings had a forking root system than C. Our study showed that white oak acorns should be sown before radicle protrusion to avoid damage, which may reduce growth or result in loss of the epicotyl.

INTRODUCTION

White oak (*Quercus alba* L., WO) is among the most valuable and abundant oak species in the Eastern United States. It often coexists with northern red oak (*Q. rubra* L.) in natural stands. The number of both species is declining, and their prominence on high-quality mesic sites has diminished throughout their ranges due to lack of adequate natural regeneration. Artificial regeneration of oaks has not been successful on those sites due to severe competition from faster growing or more shade tolerant species. The absence of quality oak planting stock is another reason given for the regeneration failure (Kellison 1993, Lorimer and others 1994).

Since the early 1990s, scientists and staff officers from the USDA Forest Service Southern Research Station, Southern Region, and the Georgia Forestry Commission have developed and implemented an integrated artificial oak regeneration program. The program's purpose is to establish oak seedling plantings on several national forests in the Southeastern United States, in order to meet land management goals and objectives (Kormanik and others 1994, 2000, 2002, 2006). The goal of an integrated regeneration program is to establish seed-production areas, to obtain desirable stocking in reforestation areas, and to augment species composition in such areas. Attributes of successful oak stand establishments are good survival, fast growth, and early acorn production. Growing and selecting the bareroot oak seedlings that possess the most desirable root system and stem characteristics, as well as protecting planted stands from competition for several years after establishment, will help ensure success (Clark and others 2000, Kormanik and others 1997, 2002, 2004, 2006). A full cycle of this artificial regeneration program begins with acorn collection, handling, and storage. The next phases in the program include growing, grading, and outplanting seedlings, as well as selecting, preparing, and maintaining sites. Such a program results in fully stocked oak stands that can be used for timber, wildlife, or aesthetic purposes, as well as sites from which to collect acorns for future stands (Kormanik and others 2006).

Among many successful applications of this regeneration program, however, some failures also came to light. In some cases the lack of effective and timely vegetation-control regimes in planted stands may have caused stand establishment failures (Kormanik and others 2004, 2006). Sometimes, due to errors in acorn collection and storage prior to sowing, the entire seedling crop was discarded at the nursery. This was especially true with WO (Kormanik and others 2000, Sung and others 2002, 2006). White oak acorn germination is highly sensitive to acorn desiccation (Connor and Sowa 2003, Sung and others 2006), and WO acorns with less than 30 percent moisture content had less than 12 percent germination (Sung and others 2006). Nevertheless, WO acorn moisture contents at sowing did not affect subsequent seedling growth in the study by Sung and others (2006). Factors other than low acorn moisture contents at sowing must be contributing to poor WO seedling growth in the nursery (Kormanik and others 1997, Sung and others 2002).

Unlike the red oak species group, acorns from the white oak species group do not need stratification to germinate. It is commonly observed that WO acorns germinate during storage at 4 °C. After the radicle extends, the germinating WO acorn extends its cotyledonary petioles out of the acorn fruit pericarp between which the epicotyl (future shoot) eventually extends. The protruding parts of the germinating acorns are vulnerable to physical damage during acorn storage, handling and sowing. Our study objective is to evaluate the effects that varying degrees of physical damage to the radicle and the cotyledonary petioles of WO acorns prior to sowing have on epicotyl emergence and subsequent seedling growth.

MATERIALS AND METHODS

Preliminary Study

White oak acorns were collected from a mother tree in Athens, GA within 24 hours after dropping. The acorns were soaked in water for 30 minutes. All floating acorns and

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Citation for proceedings: Stanturf, John A., ed. 2010. Proceedings of the 14th biennial southern silvicultural research conference. Gen. Tech. Rep. SRS-121. Asheville, NC: U.S. Department of Agriculture, Forest Service, Southern Research Station. 614 p.

acorns with holes, cracks, or any signs of blemish were discarded. The rest were placed in moistened paper towel-lined Pyrex glass pans, covered with plastic wrap, and set on laboratory benches. Temperatures in the laboratory were kept between 25 and 28 °C. When most acorns had the radicle (less than 2 cm) and two cotyledonary petioles (less than 1.5 cm) protruding, we imposed surgical treatments on the germinating acorns. The five treatments were: no surgical treatment (C); radicle cutoff at half the distance between the radicle tip and the base of the hypocotyl, which had a slightly darker color than the radicle (HR); whole radicle cutoff (WR); one cotyledonary petiole severed at 1 cm from where it connects with the embryo axis (OP), and both cotyledonary petioles severed at 1 cm from the embryo axis, resulting in acorns with no embryo axis (NE). Each treatment had 30 acorns. Acorns from each group were then placed into glass pans lined with fresh moist towels. One week later, we assessed morphological development of the embryo axis. We took no measurements of radicle or epicotyl growth in this preliminary study.

Field Study

In early November 2004, WO acorns were collected within 24 hours after dropping from seven mother trees in Athens, GA. The same procedures for floating and selecting acorns were followed as in the preliminary study. A sub-sample of 50 acorns was randomly selected from each family for moisture content determination (Sung and others 2006). Acorns from all seven families had a moisture content of at least 43 percent, indicating the absence of desiccation. The rest of the acorns were stored at 4 °C until 3 days prior to sowing. Most acorns had radicle and cotyledonary petiole protrusion on the day of surgical treatments. However, acorns from families 2 and 5 did not have much radicle or cotyledonary petiole protrusion to permit surgical treatment. These acorns were brought out of the cold storage and set at room temperature one week before surgical treatment to promote radicle and cotyledonary petiole extension. We then implemented the same surgical treatments as we had in the preliminary study. All treatments were completed within 10 h. Within a family, acorns of similar sizes were evenly distributed over the treatment groups to avoid size-associated variations among treatments (Kormanik and others 1998). After the surgical treatments, all acorns were carefully placed in plastic bags and stored at 4 °C until sowing. Acorns were sown the first week of December, 2004, at the Georgia Forestry Commission's Flint River Nursery (near Montezuma, GA) and grown using the standard oak nursery protocol for WO (Kormanik and others 1994, 2000). Epicotyl emergence was scored at the end of April 2005, and 1-0 seedlings were lifted in February 2006. Because the extent of leaf abscission varied greatly among seedlings within the same family and among families, we removed all remaining leaves before determining stem and branch dry weight. Seedling flush number, stem and branch oven dry weight, root system oven dry weight, and forking root system were recorded. Seedlings with taproots that forked within 7.5 cm beneath root collar were counted as having a forking root system. As in a study by Sung and others (2002), the flush elongated from the resting bud of the epicotyl was counted as the first flush.

Experimental Design and Statistical Analysis

The field study was conducted as a split-plot design with replication by means of four blocks. For logistic reasons, acorns from each family were planted together in each block with treatment groups randomly assigned within the family whole plot. Within each block, all seven families were randomly assigned. A total of 720 acorns (36 acorns per treatment x 5 treatments x 4 blocks) from each of the seven families were sown. Distance between treatment subplots, family whole plots, and blocks was 1 m. The whole-plot factor was family and the split-plot factor was treatment. The family and treatment factors and their interaction were considered fixed effects. The NE treatment had very low percent of acorns with epicotyl emergence, thus this treatment was not included in the statistical analyses involving seedling growth or morphological parameters. The split-plot design was a mixed model which was analyzed using PROC MIXED (SAS 2004) where the 0.05 alpha level was used for all tests on the main effects and interaction. The multiple comparisons for the four treatments within a family were performed using the Bonferroni method, where the six comparisons within a family were each tested at the 0.05/6=0.0083 level. The six multiple comparisons were performed across families when there were no treatment x family interactions. The six multiple comparisons were performed within each family when there were treatment x family interactions. No other multiple comparisons were of interest.

RESULTS AND DISCUSSION

During surgical treatment for both preliminary and field studies, the two protruding cotyledonary petioles from some WO acorns were no longer compressed against each other. The epicotyl of these acorns was visible but not extending at the time. White oak acorns often start germination (radicle protrusion) within days of dropping if the environmental condition is moist enough. The epicotyl usually does not extend until the temperature warms up in the spring. Germinating acorns with their epicotyl not protected by the two protruding cotyledonary petioles may be vulnerable to physical destruction and desiccation if they are not covered by leaf litter or soil throughout the winter.

Preliminary Study

Seven days after surgery, some acorns in the C group grew radicles as long as 7 cm and epicotyls as long as 2 cm. All acorns in the HR group developed one or multiple adventitious roots from the cut surface of the severed radicle. Those acorns with only a single new adventitious root were similar to the C in appearance. Epicotyl development from all HR and WR acorns was similar to that of the C. Some WR acorns grew multiple adventitious roots from the cut surface or from the lower portion (near the remaining embryo axis) of the cotyledonary petioles. Other WR acorns grew a single new root from the cut surface. The OP acorns showed no differences in epicotyl or radicle development from those of the C acorns seven days after surgery. Some NE acorns developed adventitious roots near the severed ends of the cotyledonary petioles. No epicotyl development was observed in the NE acorns but the stored reserves in cotyledons of these acorns were able to sustain adventitious root growth.

Field Study

No treatment and family interaction was observed for seedling height, flush number, or root-to-shoot ratio. There were treatment and family interactions for epicotyl emergence percent, root collar diameter, shoot and root dry weight growth, and root forking. Therefore, treatment effects were analyzed within each family for these parameters.

Epicotyl emergence—Theoretically, the NE acorns whose embryo axes were cut off should have had zero percent epicotyl emergences. However, this treatment had two percent epicotyl emergence, and all except families 1 and 4 had at least one epicotyl emerging. It is known that some oak acorns have multiple embryos. Acorns with more than one radicle protruding on the day of surgery were not used in the study. It is possible that some multiple-embryo acorns had only one embryo axis protruding at the time of surgery. These NE acorns which still had intact embryos contained within the pericarp at surgery were able to develop into seedlings of normal appearance. Indeed, values of the few NE seedlings were in the similar range as those from seedlings of other treatments (table 1). Another less likely possibility is that the severed cotyledonary petioles not only developed adventitious roots but may generate adventitious buds, which could develop into shoots as in a *Q*. *coccifera* L. and *Q*. *ilex* L. study by Pascual and others (2002).

Some of the NE acorns were excavated in June 2005. A few of these excavated acorns did not develop any recognizable adventitious roots. But most of the excavated NE acorns developed one or more adventitious roots from the lower part of one or both cotyledonary petioles. These adventitious roots ranged from 5 to 18 cm in length. Farmer (1977) described in his study that some WO acorns grew roots but not much of the epicotyl (1 to 7 cm in length). He reported as high as 50 percent of freshly collected WO acorns had this type of epicotyl dormancy (Farmer 1977). These dormant epicotyls did not have any leaf development after an eight week greenhouse culture. Our study did not support epicotyl dormancy as reported by Farmer (1977). The number of epicotyl emergence scored at the end of April was very close to the number of 1-0 seedlings lifted in February 2006. We found that the almost non-emergence of epicotyl by NE acorns was directly caused by severance of both cotyledonary petioles, and thus the physical destruction of epicotyls.

Table 1—Growth of 1-0 white oak seedlings from surgically altered germinating acorns. Surgical treatments were: C-control, HR-half radicle cutoff, WR-whole radicle cutoff, OP-one cotyledonary petiole severed, and NE-both cotyledonary petioles severed resulting in no embryo axis

Treatment	HT	Flush	Root shoot ratio
	cm	#	
C	28.9a[b]	2.79ab	4.77b
HR	30.4a	2.85a	4.60b
WR	29.7a	2.78ab	4.68b
OP	22.5b	2.67b	5.29a
NE[a]	22.8	2.70	4.52

[a]Due to low epicotyl emergence percent for this group, its growth parameters were not included in the statistical analysis.
[b]Least square means with the same letter for a given variable were not significantly different at the 0.05 level using the Bonferroni method.

All the acorns we used were collected and stored properly as evidenced by a close to 100 percent of radicle protrusion, which of course was the basis for this study. However, epicotyl emergence of C acorns varied from 28 percent in family 3 to 75 percent in family 4 (fig. 1). The reasons for this less than satisfactory percent of epicotyl emergence were not clear. As mentioned previously, the separation of two cotyledonary petioles before sowing, which makes the non-protected epicotyl more vulnerable, may be one reason for the low percent of epicotyl emergence. Excavation of the non-epicotyl emerging acorns of treatments other than NE would provide some insights to this phenomenon. But excavation was not conducted in this study. Nevertheless, results in figure 1 support earlier observations made by Kormanik and others (1997) and Sung and others (2002, 2006) that many factors in addition to acorn moisture content can impact WO epicotyl emergence and subsequent seedling growth.

Treatment effects on epicotyl emergence were not consistent across families (fig. 1). Radicle treatments (HR and WR) did decrease epicotyl emergence for four families, and removal of one cotyledon (OP) reduced epicotyl emergence for three of the seven families. These results indicated that it is best to machine sow WO acorns before radicle and cotyledonary petiole protrusion to avoid radicle or cotyledonary petiole severance that results in growth reduction or epicotyl destruction. Hoss (2006) reported that most of the swamp white oak (*Q*. *bicolor* Willd.) acorns did not germinate during 1 year storage at 1 °C. Seedlings from these long-term and low-temperature stored acorns grew as well as those from acorns sown immediately after collection (Hoss 2006). Acorns of sessile oak (*Q*. *petraea* (Matt.) Liebl.), another white oak species, did not germinate during the 6 month storage at -1 °C (Zitnik and others 1999). It would be interesting to test whether WO acorns can be stored at 1 °C or -1 °C to prevent radicle and cotyledonary petiole protrusion and still retain viability.

Growth of 1-0 seedlings—The OP seedlings, fed by one cotyledon before they became photo-autotrophic, were shorter than C, HR, and WR seedlings (table 1). Seedling heights for the other three treatments did not differ from each other (table 1). Flush number of the OP seedlings was less than that of the HR seedlings (table 1). Between 62 and 64 percent of seedlings in C, HR, and WR treatments had three or more flushes and only 56 percent of the OP seedlings had three or more flushes. Total flush number for all seedlings in this study (table 1) were generally one less than that reported by Sung and others (2002).

The OP seedlings in all but families 1 and 3 developed smaller root collar diameter (fig. 2), had less stem and branch

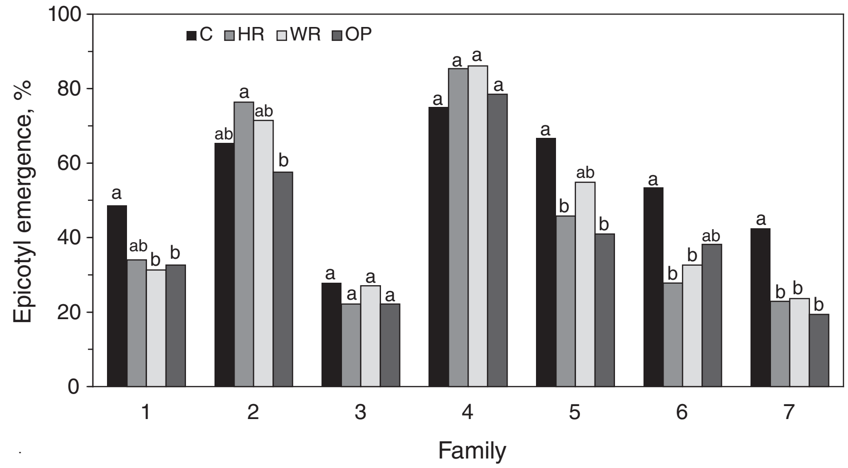

Figure 1—Within-family comparisons of epicotyl emergence as affected by surgical treatments of germinating white oak acorns. Surgical treatments were: C- control, HR-half radicle cutoff, WR-whole radicle cutoff, and OP-one cotyledonary petiole severed. Within each family, bars with the same letter are not significantly different using the Bonferroni method, where the six comparisons within a family were each tested at the 0.05/6=0.0083 level.

dry weight (fig. 3), and root dry weight (fig. 4) than C, HR, and WR seedlings. The effect of cotyledon reserve amount on seedling growth was still evident even after seedlings have become photo-autotrophic. This observation agreed with the report that within a half-sib northern red oak family, large-sized acorns (with more cotyledon reserve) always produced larger 1-0 seedlings than smaller-sized acorns (Kormanik and others 1998). Similar result of acorn size positively affecting seedling growth was reported with *Q. rugosa* L. (Bonfil 1998). In the Bonfil (1998) study, removal of both cotyledons one month after germination had a negative impact on that species' survival and growth after one growing season.

During the first year of seedling growth and development, WO allocated more than three-fold the amount of dry weight biomass to root systems than to stems and branches. The root-to-shoot ratio would be lower if leaf dry weight were included as a part of the shoot dry weight. Sung and others (1998) reported root-to-shoot ratios of 1.86 and 3.27 with and without leaf weight included in shoot dry weight, respectively. The root-to-shoot ratio for OP seedlings was significantly greater than seedlings of the other three treatments (table 1). These data suggest that when the carbohydrate supply is limiting, WO roots exhibited even greater sink strength for carbohydrate over the stems.

In general, seedlings of HR and WR treatments did not differ from C in most of the growth parameters assessed (table 1, figs. 2, 3, and 4) except for having more seedlings with forking root systems (fig. 5). This result was the opposite of findings published by Barden and Bowersox (1991). They found that clipping off one half length of the radicle slightly increased northern red oak seedling height growth during the first year. Their study did not assess root forking. Despite a slight increase in growth that results, this practice is not operationally practical.

In our study, fewer than eight percent of C seedlings from all but family 2 had forking roots. In family 2, 27 percent of C seedlings had forking roots. Yet, seedling biomass for C

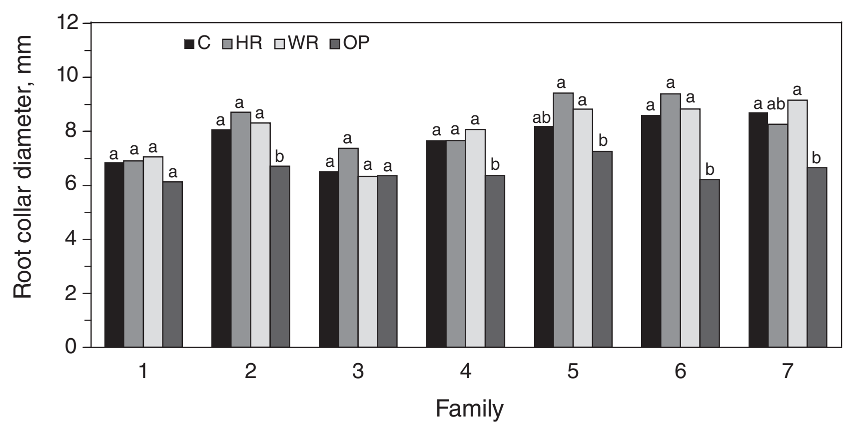

Figure 2—Within-family comparisons of root collar diameter of 1-0 seedlings from surgically altered germinating white oak acorns. Treatments and statistical analyses were the same as in Figure 1.

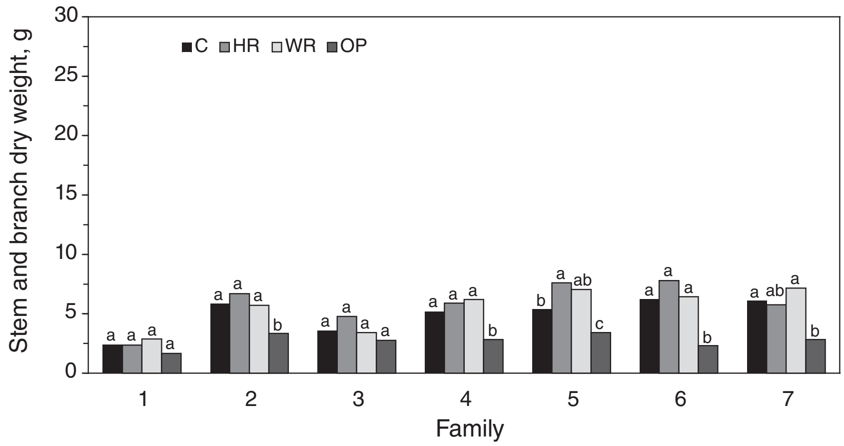

Figure 3—Within-family comparisons of stem and branch dry weight of 1-0 seedlings from surgically altered germinating white oak acorns. Treatments and statistical analyses were the same as in Figure 1.

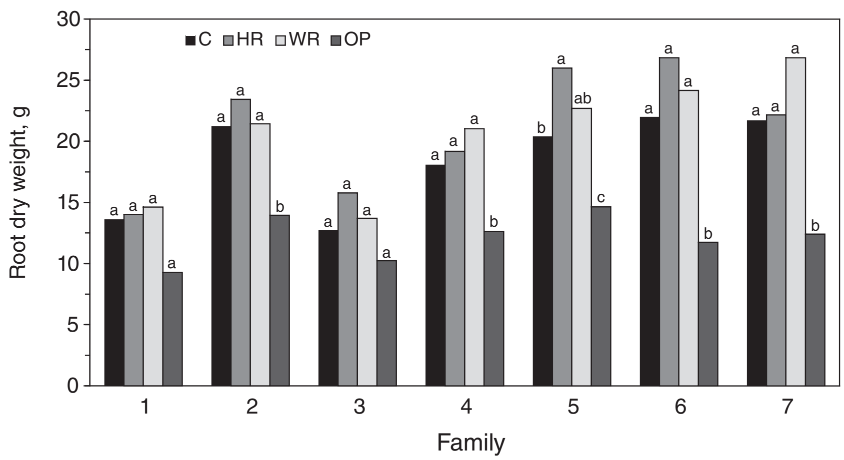

Figure 4—Within-family comparisons of root dry weight of 1-0 seedlings from surgically altered germinating white oak acorns. Treatments and statistical analyses were the same as in Figure 1.

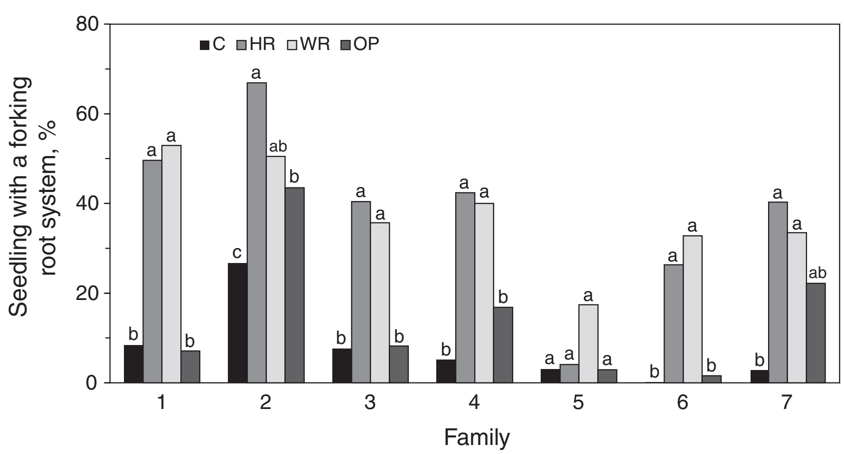

Figure 5—Within-family comparisons of root system forking of 1-0 seedlings from surgically altered germinating white oak acorns. Treatments and statistical analyses were the same as in Figure 1.

seedlings of family 2 was among the greater ones in all C seedlings from all families (figs. 2, 3). These results indicate that a forking root system is partially controlled by genetics, but that the physiological impact on seedlings is not clear. Tamasi and others (2005) reported that forking root systems negatively affect the physical stability of trees in areas where high wind can uproot or topple trees. Seedlings with forking root systems also are more difficult to plant with standard planting bars and augers, and may leave seedlings more susceptible to desiccation due to air pockets that can be left around the root system after planting.

CONCLUSIONS

There was much variation in WO seedling growth within each family and within each treatment. But the basic biology of the first year WO seedlings, such as the greater dry weight allocation to roots than to shoots remains the same across treatments and families. Oak acorn cotyledon reserves were critical for 1-0 seedling growth. Some WO acorns developed adventitious roots even when their embryo axes were removed. Any factors, such as desiccation or physical damage, which affect an epicotyl, will result in no shoot emergence. For operational purposes, it is best to machine sow WO acorns before radicle and cotyledonary petiole protrusion to avoid cotyledonary petiole severance that results in growth reduction or no epicotyl emergence. This means that the nursery personnel need to get the nursery beds ready in time for sowing non-protruding WO acorns.

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