

# Seed Propagation Protocol for Pure and Hybrid Butternut (*Juglans cinerea* L.)

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## Abstract

Butternut (*Juglans cinerea* L.) is a native, eastern North American hardwood tree with economic and ecological value. It is severely threatened by butternut canker disease, which is rapidly killing the species range-wide. Hybrids of butternut and butternut canker-resistant Japanese walnut (*Juglans ailantifolia* Carr.) have been proposed as an alternative to planting pure butternut. Information on pure and hybrid butternut seed harvest, preparation, stratification, germination, planting, and initial seedling care is lacking. Methods and results are described from a project growing these species at Purdue University, forming a seed propagation protocol for the species. Germination was first observed 14 days after stratification. After 17 days, 64 percent of seeds germinated using the current method. Alternate methods to those used in this project are provided when possible, so growers can tailor protocols at different scales.

## Introduction

Butternut (*Juglans cinerea* L.) is a medium-sized, exceptionally cold-hardy (USDA zone 3) hardwood tree native to Eastern North America (Dirr 2009, Rink 1990). The economically valuable wood of this species is easily worked and rot-resistant, making it ideal for furniture, paneling, veneer, and carving (Goodell 1984, Michler et al. 2005, Ostry et al. 1994). Butternut also holds ecological value as a mast species, providing energy-rich food for wildlife (and humans) with its large, oily kernels (Ostry et al. 1994). However, butternut canker disease, caused by the fungus *Ophiognomonia clavignenti-juglandacearum* ([Nair, Kostichka, & Kuntz] Broders & Boland), has caused rapid declines in butternut populations since its discovery in 1967 (Broders and Boland 2011). The species is now classified as “endangered” by the International

Union for Conservation of Nature (Stritch and Barstow 2019) and is listed under Canada’s Species At Risk Act (SARA) (Environment Canada 2010). In the United States, butternut has a conservation status of either “critically imperiled,” “imperiled,” or “vulnerable” in 21 States (NatureServe 2019). While butternut was never as widely produced as the closely related black walnut (*Juglans nigra* L.), the severity and prevalence of butternut canker disease has recently made butternut less viable for nurseries to produce and sell.

Butternut is readily able to hybridize with Japanese walnut (*Juglans ailantifolia* Carr.) and the resulting hybrids have naturalized in some parts of butternut’s range (Hoban et al. 2009). Researchers have only recently begun comparing the biology and performance of pure and hybrid butternuts. Crystal and Jacobs (2014) found that the hybrids were intermediate to butternut and Japanese walnut in terms of drought and flood stress tolerance. Morphologically, the hybrids have shown great variability and can hold the phenotypical features of either of the progenitor species (Crystal et al. 2014). The hybrids have also shown initial tolerance to butternut canker disease (Boraks and Broders 2014, Orchard et al. 1982), and are now being proposed by some as a possible alternative for butternut restoration (Boraks and Broders 2014, Michler et al. 2005).

Detailed and illustrated guidelines on the care of pure and hybrid butternut seeds and seedlings would aid in both restoration and research efforts, while also making it easier for growers to propagate and increase butternut in the landscape. This article contains seed-propagation protocols for pure and hybrid butternut, including information on seed harvest, preparation, stratification, germination, planting, and initial seedling care. Pure and hybrid butternut seedlings were recently grown at Purdue University (West Lafayette, IN) for

a project comparing their cold tolerances and phenology. Specific details from the seed propagation portion of the project are recorded here, but alternative methods are also included for use by growers at different scales with varying resources.

## Step 1: Seed Harvest, Preparation, and Stratification

### Harvesting

Harvest butternut and hybrid butternut fruits after ripening in autumn, preferably before they fall to the ground (Bonner 2008, Woeste et al. 2009, Young and Young 1992). For our project, fruits were harvested from September to October 2017 from the U.S. Department of Agriculture National Germplasm Repository (NCGR) and from six orchards of the Hardwood Tree

Improvement and Regeneration Center (HTIRC) at Purdue University (table 1). Fruits were stored in plastic ventilated bags to allow airflow (figure 1).

Fruits can be planted directly into the ground (direct seeding) immediately after harvest or after removal of the green husks. As it requires fewer steps, direct seeding can be more efficient, allowing you to skip stratification and pre-germination, and may be most useful for large-scale plantings. Stratification and pre-germination, however, allow for more control over the entire process, protection from predation, and the ability to screen out nonviable seeds and unhealthy seedlings prior to planting. If direct planting the seeds, make sure the fruits are covered with a 1- to 2-in (2.5- to 5-cm) layer of soil and consider using screens to protect the planted seeds from rodent predation (Bonner 2008). See step 3 in this article for information on site selection and seedling care if direct planting.

**Table 1.** Butternut and hybrid butternut seed and germination information for Purdue University project. Seeds were harvested in fall 2017 and germinated in spring 2018.

Accession	Name	Orchard	Species	Origin	Quantity	Avg. Wt/nut (g)	No. Germ.	% Germ.
PI 666982	CJUG 1. 002 PL: Ayres	NCGR (Corvallis, OR)	butternut	MI	42	12.5	40	95.2
PI 666983	CJUG 4. 002 Chamberlin	NCGR (Corvallis, OR)	butternut	NY	33	13.0	33	100.0
PI 666987	CJUG 9. 001 PL: Herrick	NCGR (Corvallis, OR)	butternut	IA	29	22.7	24	82.8
PI 666992	CJUG 14. 001 PL: Booth	NCGR (Corvallis, OR)	butternut	NY	53	9.1	44	83.0
# 719	Part: 9906 OS-23 Slocums Woods	HTIRC (Walla Walla, WA)	butternut	WI	39	13.9	37	94.9
# 856	Hadley #1' Dave Hadley	HTIRC (West Lafayette, IN)	butternut	MI	40	12.8	35	87.5
03-713	Prog. OS-14 - #2097	HTIRC (West Lafayette, IN)	butternut	WI	37	11.4	31	83.8
PI 666997	CJUG 42. 001 Collier #2	NCGR (Corvallis, OR)	butternut	WV	34	16.8	32	94.1
# 968	Haberle # 1	HTIRC (West Lafayette, IN)	butternut	KY	40	15.5	36	90.0
# 979	Rickey #2 - Chilicothe	HTIRC (West Lafayette, IN)	butternut	OH	40	14.3	30	75.0
# 1073	Maxwell #5	HTIRC (West Lafayette, IN)	butternut	OH	40	12.3	37	92.5
# 1090	Hoosier #2	HTIRC (Huntingburg, IN)	butternut	IN	40	18.3	39	97.5
# 1083	Part: 9903 Indiana -Hoosier # 3/HNF	HTIRC (Walla Walla, WA)	butternut	IN	40	20.0	40	100.0
# 701	11th Road Hyb. Marshall Co	HTIRC (Plymouth, IN)	hybrid	IN	86	11.4	71	82.6
# 1093	Kellogg Comp. Hyb	HTIRC (West Lafayette, IN)	hybrid	MI	43	13.7	29	67.4
OS-222	'LaCrosse' Hybrid	HTIRC (West Lafayette, IN)	hybrid	WI	40	14.9	29	72.5
HYB 212	'Vrana' Fulton Co.	HTIRC (Plymouth, IN)	hybrid	IN	84	15.1	78	92.9
# 2033	Prog. No. 1-OS-191 / HTI #750	HTIRC (Wanatah, IN)	hybrid	IA	20	16.9	7	35.0
# 1000	Norristown # 2	HTIRC (West Lafayette, IN)	hybrid	IN	42	12.1	31	73.8
# 696	'Bountiful' grafts	HTIRC (Vera, MO)	hybrid	MO	79	12.3	77	97.5
<b>Total</b>					<b>901</b>		<b>780</b>	<b>86.6</b>



**Figure 1.** Freshly harvested butternut and hybrid butternut seeds placed in ventilated plastic bags prior to preparation for stratification. (Photo by A.N. Brennan 2017)

## Husk Removal

While not necessary, removing the husks before stratifying and storing the seeds is helpful for preventing mold growth (Bonner 2008, Woeste et al. 2009). Remove the husks when they are firm, yet slightly soft; after this point, they can become too soft and quite difficult to remove (Bonner 2008, Young and Young 1992). We removed husks in our project within approximately 1 month of harvest.

Remove the major portion of the husk using any form of abrasion that can safely remove the husks without cracking the shell (Hartmann et al. 2002, Woeste et al. 2009). Possible methods conducted on a hard surface (driveway, garage floor, etc.) include: pounding with a metal rake (figure 2a), running over with a light- to mid-weight vehicle (figure 2b), and stomping and twisting while wearing hard-soled shoes (figure 2c).



**Figure 2.** Husks of butternut and hybrid butternut seeds can be removed by (a) pounding with a metal rake, (b) running over with a light- to mid-weight vehicle, (c) stomping and twisting while wearing hard- soled shoes, and (d) repeated abrasion over a raised metal grill-like structure that also allowed the husks to fall through to the floor. A garden hose was used in a, b, and c to contain the seeds and prevent them from rolling away. (Photos by A.N. Brennan 2017)



**Figure 3.** Power-washing can be used to remove the final bits of husk from butternut and hybrid butternut seeds. (Photo by A.N. Brennan 2017)

The husk can also be manually peeled off. Another method is to remove by repeated abrasion over a raised, metal grill-like structure that allows the husks to fall through, but the seeds to remain above (figure 2d). Throughout the husking process, a garden hose or similar object can be used to set a perimeter and provide a barrier to prevent seeds from rolling away (figure 2). Be advised that skin and clothes that come in contact with the husk and seed during this process are likely to become stained. Once the majority of the husk is removed, a power-washer or garden hose can be used to remove remaining bits of husk, but is not necessary (Woeste et al. 2009) (figure 3).

### Rogueing and Sanitization

Within a few weeks of husk removal, prepare the seeds for stratification. To rogue out nonviable seeds, submerge the seeds in water and discard those that float (Woeste et al. 2009). For our project, we sanitized seeds in November 2017 with a 1:10 bleach:water solution to help prevent fungal and bacterial growth (Fraedrich and Cram 2012, Reil et al. 1998). Dip and swoosh batches of seeds using a large colander in a bucket of the bleach solution for approximately 15 seconds (figure 4a) followed by a 15-second rinse under plain water (figure 4b).

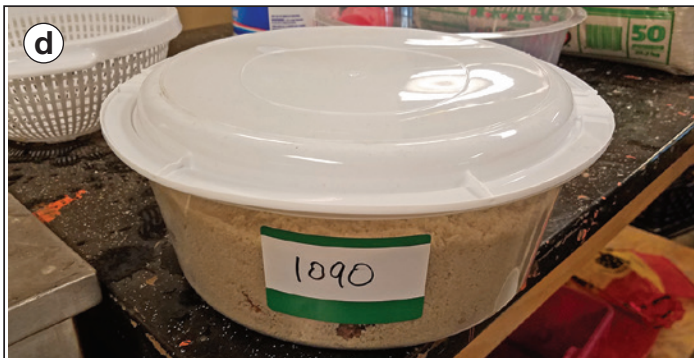
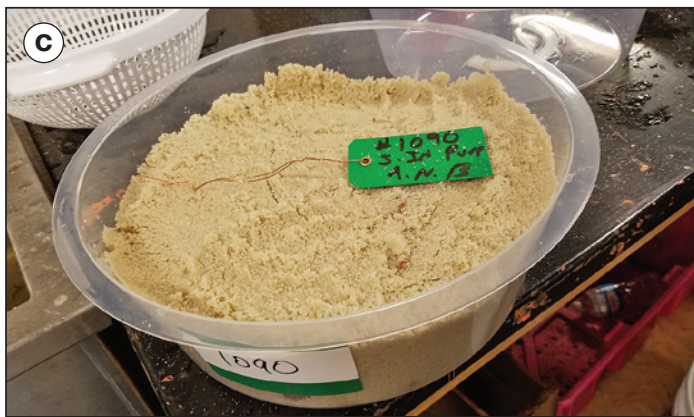
### Stratification Preparation and Storage

For our project, we placed cleaned seeds in moist, but not wet, sand (just enough so no water could be



**Figure 4.** Sanitizing butternut seeds prior to stratification can be accomplished by (a) placing them in a colander and immersing for 15 seconds in a 1:10 bleach:water solution followed by (b) rinsing under plain water. (Photos by A.N. Brennan 2017)

squeezed out by hand from a fistful of sand) (figure 5a and b). Other stratification media, such as peat, sphagnum moss, or vermiculite can also be used (Reil et al. 1998, Woeste et al. 2009). Ensure that each seed is completely surrounded by the medium (figure 5c) and that a small amount of airflow can pass through the container—enough so that the seeds can respire, but not enough to dry out the medium (Woeste et al. 2009). We



**Figure 5.** To prepare for stratification, sanitized butternut seeds can be (a) placed in a single layer on a shallow layer of moist sand using inverted cake containers. (b) Seeds should be covered with another shallow layer of sand, ensuring that each seed is surrounded by the moist sand. (c) This process is repeated for three layers of seeds. (d) The finished container should be covered with a loose-fitting lid to allow for a small amount of air circulation. Small holes can also be drilled near the top to further aid in circulation. (Photos by A.N. Brennan 2017)

accomplished this by drilling small holes (3/32-in [2.4-mm] drill bit) into inverted cake-storage containers with loose-fitting lids (figure 5d). If preparing multiple seed batches, make sure to appropriately label containers.

Store the seed containers in a cool area, such as a cooler or well-insulated garage or shed, just above freezing (34 to 41 °F [1 to 5 °C]) for stratification (Bonner 2008, Woeste et al. 2009). Juglans seeds are very attractive to wildlife, so ensure they are stored such that wildlife cannot access them (Bonner 2008, Woeste et al. 2009). For our project, seeds were stored in a walk-in cooler at 37 to 41 °F (2.8 to 5.0 °C) (figure 6).

### Stratification Duration and Monitoring

Stratify the seeds for 90 to 120 days (Bonner 2008, Young and Young 1992). We stratified the seeds for our project for 120 days and removed them from cool conditions in mid-March 2018. Check seeds weekly throughout the stratification period for mold growth and to ensure the sand is not drying out. If mold growth does occur, discard the moldy sand, re-sanitize the



**Figure 6.** Butternut and hybrid butternut seeds packed in moist sand in inverted and non-airtight containers and stored in a walk-in cooler for stratification. (Photo by A.N. Brennan 2017)



**Figure 7.** Brown staining (circled) in the moist sand surrounding butternut seeds after 45 days in stratification is suspected to be leached tannins from the seed and leftover husk pieces. (Photo by A.N. Brennan 2017)

affected seeds as described previously, and replace them in a new batch of moist sand. Other techniques, such as the application of fungicides or hydrogen peroxide can also be used, although fungicides may negatively affect germination and should be used with caution (Cram and Fraedrich 2012). If the sand is too dry, add just enough water to keep the sand moist, but not wet. In our project, we noticed dark-brown staining in the sand surrounding some of the seeds (figure 7). We took a small sample of seeds from different batches, including from those where the surrounding sand was stained, and cracked them open with a hammer to check the endosperm health. All endosperms from the samples looked healthy: bright cream to nearly white and a bit “gummy” (figure 8). Given this, we suspected the brown staining to be leached tannins from the seed itself, particularly from any bits of remaining husk.

## Step 2: Seed Germination

Upon completion of the stratification period in early spring, seeds can be planted directly into the ground or moved to warmer conditions for pre-germination before planting. Germinating the seeds in ideal conditions before planting into pots or in the field will encourage more expedient and uniform germination and allow for selection of the most viable and healthy seedlings.

### Germination Container and Medium Selection

Use moderately shallow, broad containers or trays, at least 7-in (17.8-cm) deep to ensure adequate depth for



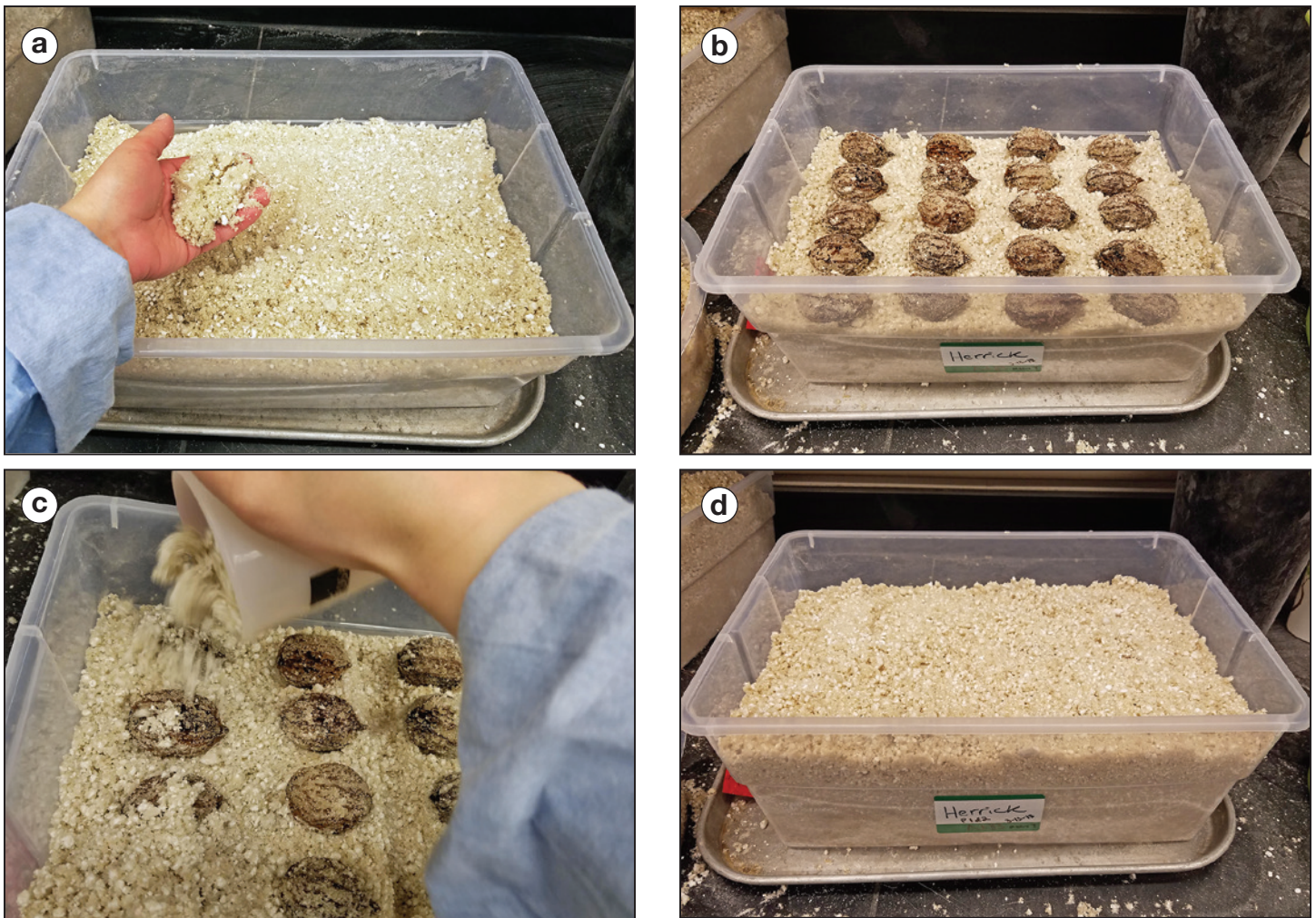
**Figure 8.** To ensure seed health in the middle of stratification, a small sample of butternut seeds were cracked open to reveal healthy, cream- to nearly white-colored endosperm. (Photo by A.N. Brennan 2017)

fast-growing roots. We used plastic storage containers (16.75-in length by 11.88-in width by 7.00-in height [42.5-cm by 30.2-cm by 17.8-cm]) and drilled nine small holes in the bottom of each container to allow for drainage of excess water (figure 9).

Fill the trays a little more than halfway with moist, but not wet, sand, peat, perlite, vermiculite, or soil,



**Figure 9.** Plastic storage containers are useful for germinating butternut and hybrid butternut seeds prior to planting. In this example, small holes were drilled in the bottom to allow for drainage. (Photo by A.N. Brennan 2018)



**Figure 10.** (a) A germination tray prepared for butternut seeds with a moist germination medium of 50:50 sand:perlite. (b) The seeds are placed on top of the medium lengthwise, on their sides. (c and d) Seeds are then covered with a shallow layer of medium. (Photos by A.N. Brennan 2018)

exclusively or in a combination (Bonner 2008). We used a 50:50 sand:perlite mixture (figure 10a).

### Preparing Seeds for Germination

Place the seeds in the substrate-filled trays. Lay each seed on its side, lengthwise (figure 10b). Butternuts have hypogeal (underground) germination, so it is important to then cover the seeds with a shallow layer (approximately 1 in [2.5 cm]) of substrate (figures 10c and 10d) (Rink 1990). Make sure there is enough room for the radicle (first seedling root) to emerge and grow downwards until transplanting or outplanting (otherwise, when the radicle reaches the bottom of the container, it will grow horizontally and “tangle” with other roots, making it difficult to extract for planting).

Label the container to identify the seed batch and cover it to help retain moisture but still allow a

small amount of airflow. We used the loosely fitting lids that came with the storage containers (figure 11), though other covers, such as loosely applied plastic wrap or tightly fitting lids with small holes drilled into them, could also be used.

### Germination Conditions

Place the seed trays into warm conditions (68 °F [20 °C] up to 86 °F [30 °C]) (Bonner 2008, Young and Young 1992). Light is optional for germination of *Juglans* species (Bonner 2008, Young and Young 1992). A greenhouse or growth chamber is ideal for providing warm, consistent temperatures, but if neither of these is available, germination heating mats can be used. These mats take up a small amount of space and are relatively inexpensive and easy to obtain from online vendors. *Juglans* seeds can also be germinated at room temperature, although it



**Figure 11.** Covered germination trays of butternut seeds in a growth chamber. (Photo by A.N. Brennan 2018)

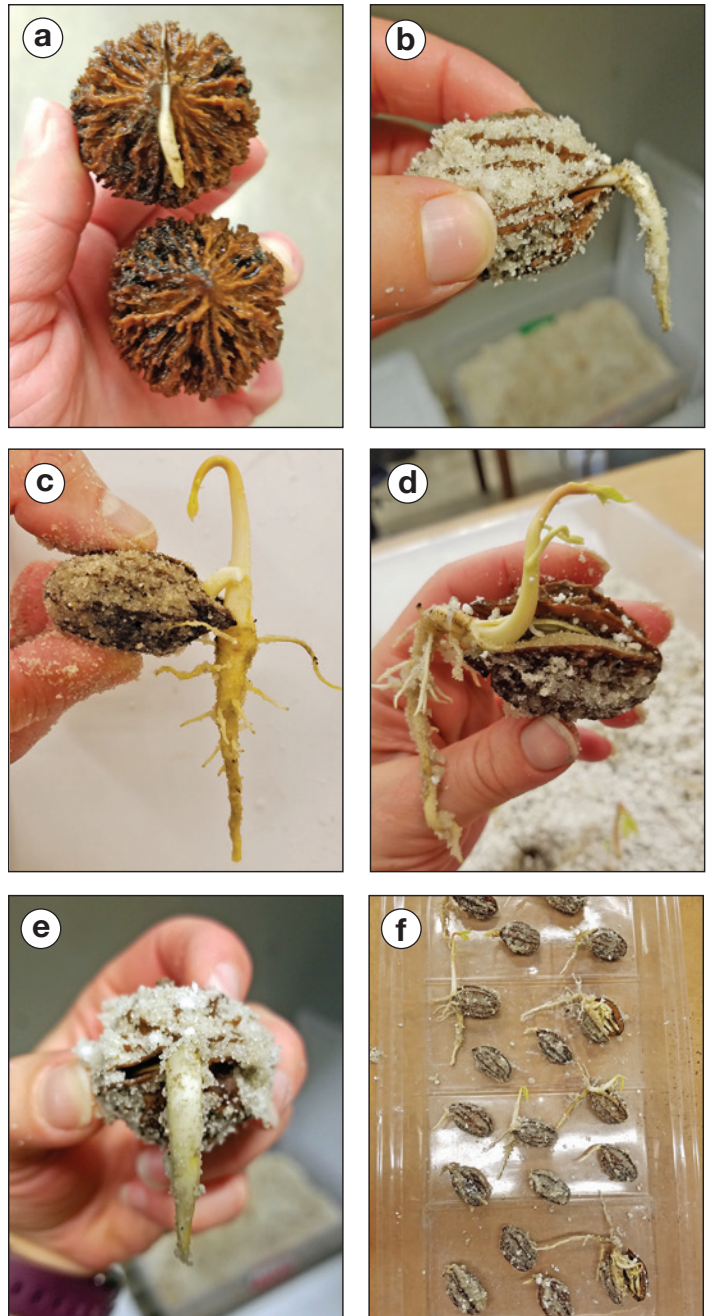
will take longer and may not be as uniform. In our project, we placed the seed trays into growth chambers (figure 11) with 8 hours of 86 °F (30 °C) day temperature alternated with 16 hours of 68 °F (20 °C) nighttime temperature (Bonner 2008). No light was used.

Check the germination containers every 4 days to ensure a consistently moist, but not wet, medium; add water as needed. At the same time, monitor for germination and fungal growth (the bleach sanitation method described previously will help prevent this). If serious fungal growth occurs, consider discarding the affected seeds or try treating them with a hydrogen peroxide solution (Fraedrich and Cram 2012). A general fungicide is also an option but could negatively impact germination (Fraedrich and Cram 2012).

## Germination

Seeds begin to germinate by cracking open at the main seam along the length of the shell. Soon afterwards, the radicle emerges from the crack (figures 12a-c) followed by the hypocotyl hook (curved stem that breaks through the surface of the growing medium) (figure 12d). The hook will straighten so that the epicotyl (terminal shoot) is on top (figure 12e). Seeds from the same family tend to germinate at a similar time, though there can be some variation in developmental speed (figure 12f).

In our project, germination was first observed after 14 days (late March). Generally, 50 to 80 days are



**Figure 12.** Germination of pure and hybrid butternut seeds begins with (a) a crack along the seam of the shell (b and c) from which the radicle will emerge. After the radicle emerges, (d) the hypocotyl hook will push out of the seed and the growing medium. (e) Eventually, the hypocotyl will straighten so that the epicotyl is pointing upwards. While related seeds will tend to germinate at a similar time, there is still some variation, (f) which can be seen by the different developmental stages of seeds of the same family. (Photos by A.N. Brennan 2018)

required for the majority of seeds to germinate and a germination rate of about 65 percent is expected (Bonner 2008, Young and Young 1992). Our method, however, resulted in a majority of seeds (64 percent) germinating by 17 days and 86.6 percent germinated within 45 days (table 1).



## Step 3: Planting the Seedlings

### Planting in the Field

Once the radicle is visible, germinated seeds can be carefully removed and planted directly in the ground or into pots. If planting directly in the ground, well-drained, rich loamy soils are ideal for butternut, but the species may also tolerate rocky, dry soils (Cogliastro et al. 1997, Rink 1990). Butternuts are shade-intolerant and must be planted in full sun (Rink 1990). Care must also be taken to protect the young seedlings from herbivore damage (particularly deer) by using fencing or tree shelters (Woeste et al. 2009). Once butternut seedlings are planted in the field, they generally require very little maintenance as long as the previously listed conditions are met. If the seedlings are planted on a particularly dry site or during a dry year, it is advisable to check if additional watering is required every few weeks during the first year of establishment.

### Planting in Pots

If planting butternut germinants into pots, start with 1-gal (3.8 L) or larger tree pots. We use TP414 “Tall One” pots (Stuewe & Sons, Inc., Corvallis, OR). Depending on individual growth rates, the seedlings may need to be transplanted into larger pots later in the growing season. Fill the pots with a coarse, well-draining medium that is predominantly bark and/or coir mixed with peat, perlite, and/or vermiculite, and a wetting agent. We used Metro-Mix 560 (Sun Gro Horticulture Distribution, Inc., Agawam, MA) for our project.

Plant the pre-germinated seedlings, radicle pointed down, about 1- to 2-in (2.5- to 5-cm) deep (Bonner 2008), so that the medium just lightly covers the seed shell (figure 13). A layer of vermiculite or perlite can also be added to the top of the pots to help retain moisture and prevent weed growth. Immediately after planting the germinated seeds, water well with unfertilized water (until water drains out the bottom).

Place the pots into a rack or other support structure (such as milk crates or inverted cow panels on supports) that will keep the long, narrow tree pots



**Figure 13.** Germinated butternut seeds, not yet covered, placed in pots (bottom right corner) and seeds that have already been covered with a shallow layer of potting mix (top left corner with red markers). (Photo by A.N. Brennan, 2018)

in an upright position. For our project, pots were placed in a greenhouse on a metal grid supported by a wooden frame and legs (figure 14). Butternut and butternut hybrid seedlings grow very quickly (figure 15), so will need to be spaced apart as they grow to accommodate the vigorous growth.

## Step 4: Culturing Seedlings in Pots During the First Growing Season

### Irrigation

Allow the medium to dry out somewhat, but not completely, between watering sessions. For our project, plants were watered when the medium turned from dark brown/nearly black (freshly watered) to light brown and felt dry below the top 1 to 2 in (2.5 to 5 cm). Regularly monitor the top few inches of the medium and check the moisture level from the bottom of the pots. Monitoring moisture levels is especially important until a deeper root system develops beyond the first few inches of growing medium. It is also important not to overwater, which can encourage damping-off. This fungal disease, especially prevalent in seedlings, causes the base of the stem to rot and the seedling to collapse (James 2012).



**Figure 14.** (a) Metal grids supported by a wooden frame and legs were used to support (b) containers of butternut and hybrid butternut seedlings. The seedlings were spaced more widely as they grew. (Photos by A. N. Brennan, 2018)



**Figure 15.** Butternut seedlings, (a) 1 week post-germination and (b) 6 weeks post-germination. (Photos by A. N. Brennan, 2018)

## Fertilization

Once seedlings have grown their first two or three true leaves, begin fertilizing them once a week. For field-grown hardwood seedlings, fertilization can be beneficial, but is not required for survival (Jacobs et al. 2005). If growing the seedlings in pots, however, fertilization is important due to the closed nature of the growth system. Pay special attention to the amount of nitrogen added. At least once monthly, irrigate beyond field capacity with clear water to rinse the substrate, thereby preventing salinization buildup.

Since there is currently no literature describing fertilizer regimes for butternut or hybrid butternut, we used the recommended nitrogen rate (luxury consumption point) for the closely related black walnut (Nicodemus et al. 2008) which is 1,200 mg N/seedling by the end of the growing season. The fertilizer concentration in our greenhouse fertigation water was 150 mg N/L. Thus, to apply 1,200 mg N/seedling by the end of the growing season, we needed to apply a total of 8 L (or 8,000 ml) fertigation per plant. By dividing the total fertigation needed by the 22 weeks in the growing period (May to September), we determined that the application rate should be 365 mL of fertigation water per seedling each week.

## Conclusion

Using our seed propagation methods, we found that overall, pure and hybrid seeds were both able to germinate quickly and uniformly. Eighty-six percent of the seeds germinated in 45 days; however, 64 percent had germinated by day 17, illustrating that this method can be used to germinate a majority of the seeds in just over 2 weeks. Our methods were also successful in producing strong, healthy seedlings, with all surviving through their first growing season (the duration of the project). The methods implemented in our project were designed specifically for our own research efforts, but additional methods were also provided to allow the protocol to be versatile for a variety of purposes and scales. This protocol is a valuable tool for butternut land managers and researchers wishing to use genetically diverse, seed-propagated material, while also supporting efforts to evaluate the suitability of hybrid butternuts as an alternative to the pure species.

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