



Asclepias speciosa, or showy milkweed, is the most common milkweed species in the Great Plains and western US. Photo by Tom Koerner, US F&WS

Successfully storing milkweed taproots for habitat restoration

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ABSTRACT

During a 3-y seed increase project, taproot size (volume and biomass) of showy milkweed (*Asclepias speciosa* Torr. [Asclepiadaceae]) increased annually, although top diameter of the taproot remained constant after the first growing season. At the end of the third year, taproots were harvested, held under different cold storage treatments, and subsequently outplanted. We observed that taproots can readily be stored in cardboard boxes placed in either cooler (0.5 to 2 °C [33–36 °F]) or freezer (–5 to –2 °C [23–28 °F]) conditions, and that success increases with the addition of protection from the cold in the form of plastic bag liners and (or) peat moss; survival was as high as 90%. These findings are promising because milkweeds are essential to the life cycle of the monarch butterfly (*Danaus plexippus* L. [Lepidoptera: Nymphalidae]), which has experienced large population declines during the past 20 y due primarily to habitat loss and fragmentation. Restoring milkweed to the environment is an important first step to help bolster populations. Seed increase is often used to produce seeds for specific restoration projects, but the taproots are often discarded once sufficient seeds have been attained. Harvesting the taproots is an effective way to efficiently use all parts of the increase bed. By utilizing cold storage, milkweed taproots can be harvested, stored, and outplanted at the proper time to produce viable and prolific plants for further restoration work and monarch habitat rehabilitation.

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KEY WORDS

monarch butterfly, *Asclepias*, Asclepiadaceae, vegetative propagation, cold storage, Lepidoptera, pollinators

NOMENCLATURE

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Monarch butterflies (*Danaus plexippus* L. [Lepidoptera: Nymphalidae]) are one of the most well-known and recognizable visitors to gardens and green spaces across North America (Figure 1). Known for their bold orange and black coloring and impressive migrations (USFS 2015; Semmens and others 2016; World Wildlife Fund 2018), monarchs have unfortunately experienced large population declines during the past 2 decades (Semmens and others 2016; Williams 2016). Climate change, logging in Mexico, pesticide use, and ultimately habitat loss and fragmentation are the major reasons why the monarch populations have declined so sharply (Toone and Hanscom 2003; Vidal and Rendón-Salinas 2014; Curry and Walker 2016; Thogmartin and others 2017). Species in the milkweed family, *Asclepias* spp. L. (Asclepiadaceae), are arguably the most important plants in the monarch butterfly life cycle because of their role as host for the obligate larvae that hatch and feed upon them (Figure 2) (Pleasants and Oberhauser 2013; USFS 2015; Monarch Joint Venture 2018). *Asclepias syriaca* L. (common milkweed) and *A. speciosa* Torr. (showy milkweed) are 2 abundant species



Figure 1. A male monarch butterfly nectaring on *Asclepias speciosa*. Photo by Thomas D Landis

across the US and are notable monarch hosts because of their large range (Malcolm and others 1989; Luna and Dumroese 2013).

Protecting milkweeds and restoring lost habitat is crucial to bolster monarch populations. The Xerces Society for Invertebrate Conservation considers habitat restoration the most important means to increase pollinator populations; specifically for monarchs, this means restoring native milkweed species (Borders and Lee-Mäder 2014; Pelton and others 2016, 2018) into landscapes where they have been eliminated. Habitat restoration for monarchs through the outplanting of milkweed and other native nectar plants is beneficial to many other pollinators and wildlife (Xerces Society for Invertebrate Conservation 2016) that are also experiencing large decreases in population numbers (Potts and others 2010; Landis and Dumroese 2015). Fortunately, certain crucial milkweed species, such as *A. syriaca* and *A. speciosa*, are easily grown from seeds or cuttings, and many native plant nurseries have started growing them for restoration purposes (Luna and Dumroese 2013; Landis and Dumroese 2015).

Propagating milkweeds from seeds has been readily practiced, and they have been shown to respond well to any standard seed propagation methods (Landis and Dumroese 2015). Seed propagation offers the advantage of higher genetic diversity and lower cost than does vegetative propagation but sometimes comes with complications that other propagation methods lack. Seed propagation can be challenging and time consuming (Luna 2009). Seed dormancy, an adaptation to ensure germination will occur at optimal environmental conditions, is one of these obstacles because it can vastly vary between species. Dormancy can be overcome by using a variety of stratification techniques, but that can add time and complications into the growing process that would not be faced when using vegetative propagation methods (Luna 2009). Milkweed, specifically, varies in stratification requirement times among ecotypes, species, and in harvesting locations within the same species (Luna and Dumroese 2013). Moreover, collecting milkweed seeds from the wild does not always result in the amounts needed for restoration work. A typical work-around to this issue is to use seed increase beds to boost the amount available for specific projects. These beds create uniform environments that aim to optimize the quantity and quality of seeds produced. Once sufficient seed requirements have been met from the increase plots, the plants left behind in the beds—referred to as mother plants—are no longer required.

Mother plants that have outlived their usefulness for seed production may, however, still have value. It may be possible to leverage milkweed's nature to readily sprout from underground tissues (Polowick and Raju 1982; Bhowmik 1994; Pellissier and others 2016). Taking advantage of this vegetative reproduction may be a viable way to propagate it for further restoration work. Indeed, vegetative reproduction has proved successful



Figure 2. A mid-instar monarch caterpillar on *Asclepias fascicularis*. Photo by Thomas D Landis

for wild milkweed, and Landis and others (2014) found that milkweed plants propagated from belowground structures produced larger plants that grew much faster in the first few years than those started from seeds. In their study, Polowick and Raju (1982) observed visible root buds, the means of their vegetative reproduction and spreading, on their seedlings 25 d after germination. Other studies, such as that by Jeffery and Robison (1971), concluded that *A. syriaca* can produce a plant

from a root segment as short as 2.5 cm (1.0 in). Landis and Dumroese (2015) reported that milkweed plants grown from first year taproots (see sidebar), when properly propagated, can produce flowers and seeds. Therefore, harvesting the taproots of the mother plants (in similar fashion to bareroot seedlings) and outplanting them may be another viable means to restore milkweed to the landscape. This harvesting method would likely be cost effective, as it does not waste the productive and easily propagated taproots that might be lost at the end of a seed plot's life. In their study, Pellissier and others (2016) found that 16 of the 49 species of milkweed (mostly North American; including *A. syriaca* and *A. speciosa*) showed clonality, which they defined as the presence of long rhizomes, as a means to grow successfully in disturbed and unstable environments. These clonal milkweed varieties could be other species to focus on for success with vegetative propagation techniques.

Taking advantage of taproots collected from decommissioned seed-increase beds and using them alongside milkweed grown from seeds allows the investment in the seed-increase beds to be used to its fullest potential. Milkweeds are perennials (Bhowmik and Bandeen 1976) whose taproots survive the winter underground, which sets them up well for overwintering in nursery cold storage. Storing harvested plants is a valuable tool that native plant nurseries utilize to help optimize their production potential. Often nurseries or seed production areas have climates that differ from restoration sites, so the optimum time for harvesting nursery plants to maintain their quality

TERMINOLOGY FOR THE BELOWGROUND PARTS OF MILKWEEDS

The terminology used for the belowground portions of milkweed plants varies among authors. Undoubtedly this reflects the background of the author, with weed scientists, plant physiologists, botanists, entomologists, plant anatomists, citizen scientists, and propagators applying terms from their perspectives. The debate generally involves whether milkweeds have rhizomes; this is compounded by various, nuanced definitions of rhizome and is further complicated because milkweed species generally fall into 2 forms, those that regularly produce classic, horizontal, underground structures and those that do not (Pellissier and others 2016). Because determining the anatomy of the belowground structures was not an objective of our study, we have decided to call the structures we measured “taproots,” because these belowground parts resemble what most propagationists would readily identify as a taproot.



Asclepias speciosa is an important host species for both monarch larvae and adult butterflies. Photo by Tom Koerner, US F&WS

may not coincide with the outplanting window—storage is an operational technique to overcome this discrepancy (Landis and others 2010). Harvesting and storing milkweed taproots could prove helpful for restoration projects and conservation efforts.

Our objective was to evaluate the potential of harvesting *A. speciosa* taproots from seed increase beds slated for decommission and storing them over the winter in anticipation of using them for restoration the following spring. We chose this species because of its wide range across the Great Plains and the western US and its known association as a monarch butterfly host. To better understand taproot development and to fill a void in the literature, we monitored its development in our seed increase plots for 3 y (the planned duration of these plots by nursery personnel) and then harvested them in a method similar to bareroot seedlings. Because storing plants for future outplanting can be a valuable operational tool to ensure plant quality is maintained, we evaluated storage conditions to identify the best practices that protected the taproots and resulted in stem growth following outplanting.

METHODS AND MATERIALS

Milkweed Establishment and Taproot Growth

In October 2013, *A. speciosa* seeds (Bureau of Land Management ASSP-OR110-36-Jackson-12; collected in 2012 in Jackson

County, Oregon, at 1100 m [3600 ft]) were sown into field A at the USDA Forest Service J Herbert Stone Nursery in Central Point, Oregon (lat 42.353830, long -122.942894). The nursery site is 388 m (1270 ft) above sea level and has a Mediterranean-like climate (hot, dry summers and wet, mild winters). Soils are fine to coarse loamy, mixed, Pachic Haploxerolls mainly in the Central Point soil series, with some representation of the Kubli, Barron, and Medford series (Soil Survey Staff 2018). Two uniform beds, each 99 m long and 1.2 m wide (325 ft × 4 ft), were fumigated, disked, and formed in September to October 2013. On 10 October 2013, seeds were sown with an Ojyord seed drill at a rate of 50 to 60 seeds per linear bed foot (0.22 g/m²) resulting in first-year bed density averaging 415 plants/m².

In September 2014, we designated 7.6 m (25 ft) buffers on each end of each bed, and then divided the remaining 84 m (275 ft) into 6 replicates (12 total) that were 14 m long and 1.2 m wide (46 ft × 4 ft). Within each replicate, we made 3 subplots, 120 cm × 30 cm (4 ft × 1 ft). Each fall (2014, 2015, 2016) we harvested all of the belowground structures (taproots and fine roots) from the root collar down in one subplot per bed per replicate. Samples were shipped to the USDA Forest Service Rocky Mountain Research Station in Moscow, Idaho, for processing.

Upon arrival in Moscow, samples were gently washed and sorted by size (Figure 3). Five plants were randomly chosen from each of the 12 subplots for measuring. Fine roots (< 5 mm



Figure 3. Harvested root systems ready for analysis. Photo by Katherine G McBurney

[0.19 in]) were removed from the taproot before measuring. Volume of taproots was determined by water displacement (Burdett 1979) as modified by Harrington and others (1994). Samples were then dried at 60 °C (140 °F) until they reached constant mass (24–72 h). Biomass of the taproots and fine roots and root-collar diameter (RCD) were recorded.

Final Taproot Harvest and Storage

In mid-November 2016, both beds were undercut to a depth of 17 to 20 cm (7–8 in) using a New Zealand root pruner, and taproots were hand-harvested by a crew and placed into temporary cold storage at just above 0 °C (32 °F). The next day, taproots were randomly assigned to 1 of 8 treatment combinations: 2 temperatures (cooler: 0.5 to 2 °C [33–36 °F]; freezer: –5 to –2 °C [23–28 °F]) × 2 liners (with or without) × 2 peat (with or without). Peat was Sun Gro Sunshine Canadian Sphagnum Peat-moss (Sun Gro Horticulture, Canada). Taproots were placed in typical cardboard shipping boxes. Plastic liners were ordinary plastic garbage bags.

Taproot Outplanting

In April 2017, 1.2 m (4 ft) wide beds at the USDA Forest Service J Herbert Stone Nursery were tilled to a depth of 20 cm

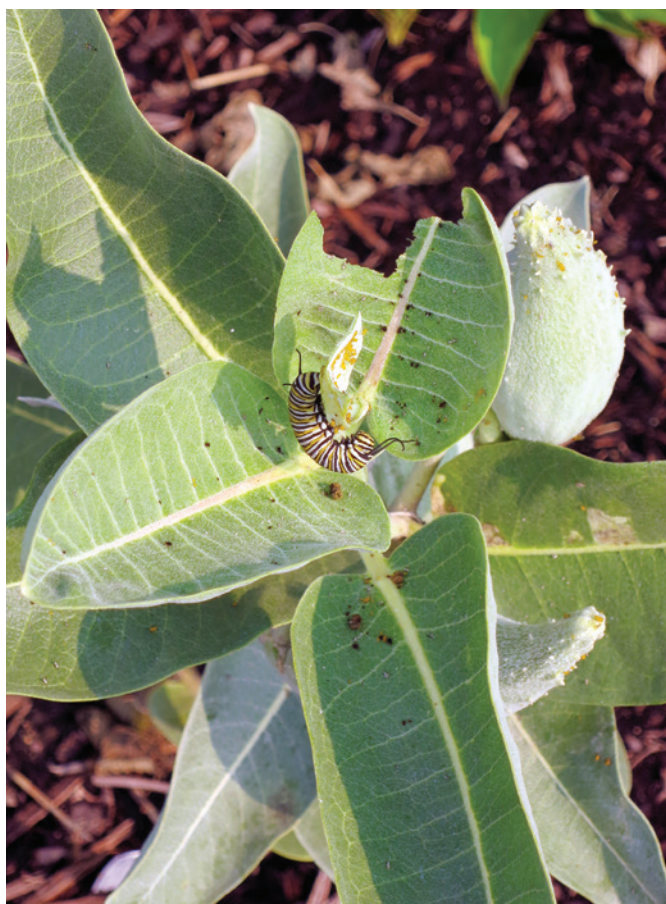
(8 in) using a Howard rototiller. The soil was not fumigated. After the taproots thawed for about an hour, 5 were randomly selected, hand-planted with their root crowns at the soil surface, and marked to facilitate future assessment. Each storage treatment was replicated 4 times. We expected high weed pressure because the soil was not fumigated, therefore beds were mulched to a depth of 5 cm (2 in) with sawdust and wood chips after planting (Figure 4). No additional water, fertilizer, or weeding was conducted. Survival and stem count were measured in June and again in November of 2017.

Statistical Analyses

Data were analyzed using SAS (version 9.4, SAS Institute, Cary, North Carolina). Analysis of variance using PROC GLM was performed on the root data from 2014 to 2016 to determine differences among years; pairwise comparisons were made using Tukey's HSD. Raw morphological data did not meet the assumptions for homoscedasticity or normality; therefore, a log transformation was used for the ANOVA on root volume, lateral root biomass, final biomass, and RCD data sets. Because fine root data did not meet the assumptions, even after transformation, we used a Kruskal-Wallis non-parametric analysis. We present raw data means in tables and figures. The main



Figure 4. Taproots were outplanted by hand in spring 2017 after storage treatments (left). After planting, the beds were mulched to control weeds (right). Photo by Thomas D Landis (left); photo by Steve Feigner (right)



Monarch larvae are obligate herbivores on the leaves of milkweed species. Photo by Thomas D Landis

effects of temperature, liner, and peat as well as their interactions were included in the model. Survival data were analyzed using the PROC LOGISTIC function in SAS. The stem count data were analyzed using the PROC GENMOD function in SAS. Analyses were considered significant at $\alpha = 0.05$.

RESULTS

During their time in the seed increase beds, taproots grew significantly larger (Table 1). Taproot volume increased by 30% from 2014 to 2015 and another 21% the following year, for an overall increase of 57% from 2014 to 2016. Biomass also increased during the 3 y, specifically by 21% in 2014 to 2015 and 35% the following year with an increase of 63% during the study. RCD, recorded in 2015 and 2016, remained constant between years.

We did not observe a temperature $\times$ liner $\times$ peat interaction (Table 2). Although we did not detect a 3-way interaction, we did note significant interactions for temperature $\times$ liner and temperature $\times$ peat (Table 2). For both interactions, adding the liner or peat during storage improved subsequent outplanting survival, but more so for freezer-stored taproots (Figure 5). Adding a liner or peat increased survival of taproots stored in the cooler by 25 or 40 percentage points, whereas survival of taproots stored in the freezer was increased 80 or 70 percentage points.

Of the main effects, temperature was not significant (Table 2). Adding a liner or peat did, however, significantly affect outplanting survival from 56 to 89% and 58 to 88%, respectively.

TABLE 1

Asclepias speciosa mean ($\pm$ SE) root system morphological data and root-collar diameter (RCD) for 3 y of bareroot bed nursery culture.

Year	Taproot volume (> 5 mm) (ml)	Taproot biomass (> 5 mm) (g)	Fine root biomass (< 5 mm) (g)	Total biomass (g)	RCD (mm)
2014	13.6 (1.1) c	4.9 (0.4) c	0.9 (0.08) ab	5.7 (0.5) b	—
2015	16.5 (1.0) b	6.4 (0.4) b	0.7 (0.05) a	7.1 (0.4) a	11.9 (0.4)
2016	23.6 (1.6) a	9.1 (0.7) a	1.1 (0.09) b	9.4 (0.7) a	11.4 (0.4)
P value	< 0.0001	< 0.0001	0.0022	< 0.0001	0.1591

Notes: Different letters within columns indicate significant difference at $\alpha=0.05$.

TABLE 2

Sources of variation and P values for ANOVA model main effects and their interactions ($\alpha = 0.05$) for outplanting survival data of *Asclepias speciosa* taproots exposed to 1 of 8 cold storage combinations.

Source of variation	P values
T	0.1317
L	< 0.0001
P	0.0016
T $\times$ L	0.1585
T $\times$ P	0.0019
L $\times$ P	0.0019
T $\times$ L $\times$ P	0.4793

Notes: T, temperature; L, liner; P, peat.

After growing 3 mo in the field, we counted stems originating from each outplanted taproot to see if treatments affected this variable; if so, this metric would have potential to evaluate milkweed plant quality. We detected no significant differences among treatments ($P \geq 0.4944$, data not shown). Taproots grew 1 (67% of plants), 2 (27%), 3 (9%), or 4+ (2%) stems.

DISCUSSION

The increase in taproot biomass and volume across the study is supported by milkweed’s tendency to spread aggressively through adventitious root buds. Honeyvine (*Cynanchum laeve* (Michx.) Pers.) (Robinson 1968), oval-leaf milkweed (*A. ovalifolia* Decne.) (Luna and Dumroese 2013), common milkweed (Bhowmik 1994; Polowick and Raju 1982), butterfly milkweed (*A. tuberosa* L.) (Luna and Dumroese 2013), and

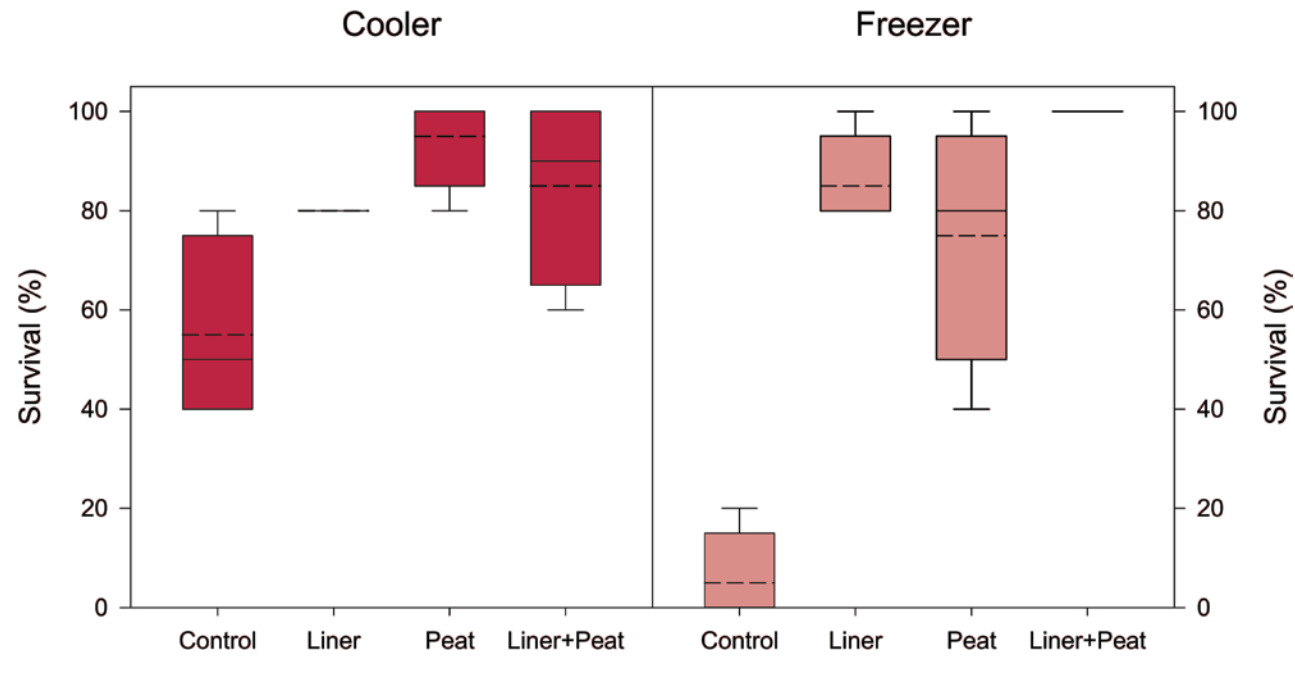


Figure 5. Survival rate distributions for taproots stored in cooler or freezer conditions with (plastic and/or) peat moss (or without (control) protection. For box plots, means are represented by the dashed, horizontal lines; medians by the solid, horizontal lines; boxes are 50% of the values, and whiskers the top and bottom 25%.

showy milkweeds (Stevens 2000; Luna and Dumroese 2013) are known to regularly spread by taproots. Unfortunately, research on milkweed taproot growth for multiple years is lacking; although, studies do focus on different aspects of milkweed growth and propagation. Growing milkweed from seeds and root cuttings has been described in a variety of studies (such as, Jeffery and Robison 1971; Luna and Dumroese 2013; Landis and Dumroese 2015), and other new research focuses on fertilizing milkweed and its response to container size (Hanson and others 2017).

The use of cold storage is fairly well understood for commercial tree species. Landis and others (2010) suggest that cooler storage is satisfactory for durations less than 2 mo, while freezer storage is optimal for longer durations. It is important to note that post-storage survival varies among species, and our understanding of how native plants respond requires more work (Landis and others 2010). Recently, Overton and others (2013) focused on the native shrub Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle & Young [Asteraceae]) and found reduced outplanting survival for freezer-stored seedlings compared to those cooler-stored. For the rhizomatous grass species Giant Miscanthus (*Miscanthus xgiganteus* J.M. Greif & Deuter ex Hodkinson & Renvoize [Poaceae]), Davies and others (2011) found it could be successfully stored for 6 wk at 2 °C (35.6 °F) without any loss of rhizome viability. To the best of our knowledge, our study is the first to assess long-term milkweed storage, and our results indicate that taproots can be successfully stored chilled or frozen for at least 4.5 mo with little difference in survival.

While storage temperature alone did not affect outplanting survival of our taproots, we found that level of protection during storage is a key factor to outplanting success. The addition of protection, in the form of peat or a plastic liner, increased survival rates, indicating that either method would be a viable way to store taproots. Protection is important, especially for root systems, against desiccation (Landis and Luna 2009). Plastic liners are the least expensive option of the two, and provide an easy, and perhaps less messy, way to significantly increase survival during cold storage.

In our study, we outplanted our taproots onto a nursery site. Despite no additional irrigation or weed control, our use of a mulch may have affected survival compared to conditions more typical of restoration sites. Certainly additional research would be prudent to ascertain the viability of using taproots across a variety of sites, because, even if properly stored, vagaries on outplanting sites can affect survival of outplanted milkweed taproots. Landis and Savoie (2018) reported poor survival of milkweeds established from seeds and taproots on a hot, droughty site in southwestern Oregon. More evaluations of outplanting performance of taproots, and comparisons using taproots, seedlings, and direct seeding, are required to inform land managers in their decision-making processes.

Although we counted stems on the outplanted milkweed taproots as a possible way to quantify plant quality after storage, we were unable to relate this to our treatments. Anecdotally, it may be that stem number better reflects taproot age and (or) size before storage; follow-up work on this metric may still have value toward better understanding the vegetative propagation of milkweed.

In this study, milkweed taproots collected from seed increase beds were harvested, stored, and outplanted the following spring. As expected, taproots stored during the winter with minimal protection experienced the lowest percentage survival regardless of storage temperature. Except for taproots stored without a liner or peat, outplanted taproots grew successfully. We noted that some of these plants flowered and set seeds the first year (personal observations), and early growth during the second field season was robust (Figure 6). Collecting and storing milkweed taproots alongside traditional seed propagation could be another way to produce plants efficiently for restoration projects and further conservation efforts to restore pollinator habitat.



Figure 6. Robust growth from an *Asclepias speciosa* taproot early in the second growing season following cold storage treatment. Photo by Thomas D Landis

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