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Experimental Cultivation of Eastern North America's Lost Crops: Insights into Agricultural Practice and Yield Potential

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Stratification Methods.

If no previous research has been conducted, inferring the seed dispersal mechanism can help narrow down the possible requirements for germination (Galloway 2005; Silvertown 1984), as can the life cycle of the plant (winter annual, summer annual, perennial) (Baskin and Baskin 2014). But regardless of dispersal mechanism, summer annuals (plants that germinate in the spring and produce seeds in the fall, including sumpweed, goosefoot, and erect knotweed) in temperate climates often require simulated winter to germinate. Winter annuals (plants that germinate in the fall and produce seeds in the spring, including maygrass and little barley) are more diverse in their temperature requirements, but frequently germinate after being exposed to low temperatures (Baskin and Baskin 1988). Mueller's previous experiments with erect knotweed showed that cold stratifying seeds in moist soil at 4° C for 6 weeks resulted in up to 100% germination, and that no seeds germinated without stratification (Mueller 2017). One previous experiments with goosefoot indicated stratification directly in the soil for six months resulted in greater germination rates, whereas cold dry stratification was not as successful (Halwas 2017). Another study found that wet stratification was not necessary for germination in *C. missouriense* Aellen, but that neither was it detrimental to germination rates (Williams 2019).

We needed to prepare batches of seed for spring planting over the course of a single winter, so we opted to investigate the effects of cold stratification rather than other possible seed treatments because we judged that it was the most likely treatment to increase germination in all five species. There may also be differences between seeds that were harvested early in the season and those that were harvested late in the season in terms of germinability requirements, a circumstance which would affect both the planning of future seed collection trips and harvests, and interpretations of ancient agricultural scheduling. We attempted to investigate this possibility in two of the lost crops, sumpweed and goosefoot, since Mueller had made collection of these species over the course of several weeks in 2017. We did not conduct germination experiments with erect knotweed, since Mueller had already developed methods that result in acceptable rates of germination for this species (Mueller 2017).

SI Table 1 reports the provenience of the seeds we used, and summarizes the results. We cold stratified beginning on January 5, 2018. Seeds were pressed into moist soil in 72-cell flats, covered, and stored in a 4° C refrigerator in the dark. Each "batch" consisted of 20 seeds. A control batch was immediately placed on the bench with no treatment. Every two weeks, a batch of each species was taken out of the refrigerator, uncovered, and placed on a bench where they were automatically misted and exposed to light (both natural and artificial) 12 hours a day. For little barley and maygrass we selected the single free-living population for which we had the most seed and that would be used

for the main experiment for this preliminary study. For sumpweed and goosefoot, we created two batches from the earliest and latest harvests taken by Mueller in the fall of 2017, providing a 4-week spread for sumpweed and a 3-week spread for goosefoot (SI Table 1). The mid-winter temperature in the heated greenhouse was approximately 20° C. Every week, the number of seedlings that had emerged from each batch was recorded. After four weeks, all seedlings were transplanted and moved off the mist bench. The experiment was terminated after ten weeks, in mid-March, when it became necessary to begin preparing larger batches of seeds for the summer experiments. Unfortunately, this meant that we did not collect data beyond 2 weeks on the mist bench for the 8-week stratification treatment. Figure 1 shows the results of this preliminary experiment for the other three species. Constraints on time, available seed, and woman-power prevented us from replicating this experiment, so our results should be seen as preliminary but can inform more intensive studies of germination requirements in the future.

Stratification Results

In sumpweed, this experiment revealed differences between seeds harvested in early October and those harvested in early November (hereafter, early and late harvest). In general, the early harvest germinated after fewer weeks of stratification and in less time on the mist bench. Even with no stratification at all, 10% of seedlings emerged. This suggests that at least some sumpweed seeds produced in late summer are not dormant and may germinate immediately if they fall or are removed from the mother plant. There was not much difference between the germination rate for no treatment, 2 weeks, and 4 weeks of stratification for the early harvest, whereas after 6 weeks of cold stratification, germination increased from a maximum of 15% to a maximum of 60%. After 8 weeks of stratification, germination began much more rapidly, with 25% of seedlings emerging in the first week, and 65% after two weeks. It is likely that if we had continued taking data for another two weeks, we would have achieved even higher germination rates with this treatment on the early harvest seeds.

None of the late harvest seeds germinated without stratification or after two weeks of stratification. They responded almost identically to 4-6 weeks of stratification as the early harvest and received a similar bump in germination after two weeks on the bench from the 8-week stratification. In general, the late harvest seeds took longer to emerge (none were ever observed after only one week on the bench), so it is probable that the 8-week stratification would have resulted in the highest germination rate for this batch, too, had we continued to make observations.

Although this was a small-scale, exploratory experiment, these results suggest that germination inhibitors may develop by late fall that are absent or reduced earlier in the season. Another possible explanation is local adaptation. The late harvest population comes from western Illinois, approximately 2 degrees of latitude further north than the early harvest population, which is from west-central Kentucky (SI Table 1). Northerly populations of sumpweed may have evolved greater dormancy in places where early spring freezes could kill seedlings that emerge too early. Either way, if seeds are somehow dispersed in late summer or early fall (for example, by people), this could allow two generations per growing season. We observed sumpweed seedlings emerging as late as early September in our experimental garden, and these rapidly developed flowers and fruits (Figure 2f). The sumpweed seedlings from this experiment that were raised in the greenhouse similarly started to produce seeds in a matter of weeks, rather than growing for several months first, as is typical of natural populations.

Little barley is the only one of the five lost crop progenitors that does not need

any seed treatment to germinate. Given four weeks in consistently damp and warm soil, and plenty of light, 80% of the untreated seeds germinated, a rate which was not exceeded by any of the cold treatments (Figure 1). Exposure to cold does not increase the percent germination, but it does result in quicker and more uniform emergence of seedlings. For all four of the cold treatments, all of the seedlings that emerged did so within one week, instead of gradually emerging over the course of several weeks as in the untreated batch. This makes sense for a species that germinates in the late fall or during the winter: exposure to cold, wet conditions trigger germination. But our results also suggest that if they are exposed to enough water and light, little barely seeds are capable of germinating in the summer of the same season they are produced. The fact that they are not observed to do so is probably because of shady conditions as summer annuals become established, and intermittently dry conditions during the summer. Little barely seeds could also be inhibited by high temperatures, as has been observed in some winter annuals (Baskin and Baskin 1988).

Maygrass did not respond well to cold stratification, but it also did not germinate at all with no treatment. The highest germination rate that we achieved was 20% after 6 weeks of cold stratification. Like little barley, these seedlings emerged immediately after stratification, within the first week on the bench. We did not have time to test other methods of seed treatment but we suspect that maygrass seeds need a period of after-ripening in hot temperatures to break dormancy (Baskin and Baskin 1988), a possibility that we are currently investigating.

For unknown reasons, none of the goosefoot germinated. For the main experiment, we used *Chenopodium berlandieri* from the United States National Germplasm system, which had been grown out and harvested in the Plum Bayou Garden, near Little Rock, Arkansas, in 2017 (SI Table 2). Although the results of Williams (Williams 2019) suggest that stratification is not necessary for some species of goosefoot, neither did he find that it was detrimental, and Halwas'(2017) experiments suggested that cold/wet stratification could increase germination rates.

Chenopodium caveats.

In the case of *Chenopodium*, some complications with species identification were inevitable. A high-resolution molecular phylogeny of North American *Chenopodium* species in badly needed. Closely related species of *Chenopodium* have been distinguished by an array of characteristics, including subtle differences in leaf shape, the color of the leaves, stems, and nodes, the orientation and arrangement of the inflorescences, degree to which the calyx encloses the fruit at maturity, flowering and fruiting time, and the texture of the pericarp (fruit coat). The two most important flora for the study area do not agree on the species level taxonomy of *Chenopodium*, either in terms of which species are supported, or in terms of how to distinguish them (Clemants and Mosyakin 2003; Yatskievych 2006). In his treatment of *Chenopodium*, Yatskievych (2006:872) cautions "Immature specimens of *Chenopodium* are often difficult or impossible to identify and should not be collected," mainly because flower and fruit morphology are the most useful distinguishing characteristics. For both the *Flora of Missouri* and the *Flora of North America*, the distinguishing characteristic of *Chenopodium berlandieri* is its honey-comb pitted or net-like pericarp texture, which of course cannot be observed until the seeds are mature. *Chenopodium album* L. is a particularly common field weed that we knew would be present in the seed bank in our experimental areas. It is distinguished from *C. berlandieri* most consistently by having fruits that are smooth or roughened. Our experiment included the seed that we

planted (USDA *C. berlandieri* after a year of cultivation in Arkansas) and *Chenopodium album* present in the soil seed bank, which could not be distinguished until harvest.

We include harvests from all *Chenopodium* that emerged in our plots in the following analyses for three reasons. First, checking the seed morphology of every plant at harvest would have been prohibitively time consuming. Second, if we had excluded *C. album* plants from our analysis based on fruit morphology at the end of the season, it would have prevented us from studying the effects of plant density on yield, which was the main aim of our study. And third, we are not convinced that ancient people could or would have distinguished between plants or populations based on a trait as difficult to observe as fruit coat texture, meaning that they also might have cultivated both species. With respect to this hypothesis, we must consider 1) if plants bearing both fruit types were available to ancient people; and 2) if both fruit types appear in the archaeological record. Current taxonomies include a purported native species (or sub-species of *C. album* depending on the taxonomist) that has smooth to roughened fruits (*C. missouriense* Aellen), which might have been available to ancient people. Our study also made us doubt that fruit coat texture is a true synapomorphy of a single species, rather than a trait that is variable or plastic within populations. We randomly sampled 1/32 of each harvest using a riffle splitter in order to examine seed morphology. Although we planted hundreds of thousands of *C. berlandieri* seeds, we only observed a few honey-comb pitted fruits in these samples. This could be due to a misidentification or contamination from field weeds in the USDA's seed bank, or contamination during cultivation in Arkansas (unfortunately, we did not check the fruit morphology before planting), or it could be because this morphology is not stable or heritable.

Regarding the archaeological record, the morphometric analyses of ancient *Chenopodium* fruits that let to their identification as *C. berlandieri* were conducted on rare uncarbonized assemblages where the pericarp was preserved (Smith 1984, 1985, Fritz and Smith 1988), but most archaeobotanical assemblages are carbonized and the pericarp is not preserved. While the testa does preserve a poorly defined imprint of pericarp texture (Gremillion 1993a), it is unclear whether most analysts look for this trait. The focus within archaeobotany has been on distinguishing between domesticated, wild, and weedy populations, which involves observations of testa thickness and seed shape (Gremillion 1993b). Where texture is noted, it is usually to observe the proportion of smooth tests, which can be used as a rough proxy for testa thickness. There may be many ancient *Chenopodium* assemblages that do not exhibit the honey-comb pitted texture characteristic of *C. berlandieri* as currently defined, or where such a feature is not observable one way or the other. We offer these caveats and justifications in order to be explicit about the composition of our experiment in the context of current taxonomies and the archaeobotanical literature, with the hope that remaining questions about the phylogenetic significance of *Chenopodium* phenotypes, both modern and ancient, will soon be resolved by our ongoing research.

SI Table 1: Germination experiments: seed provenience and summary of results

Species	Common name used in text	Type	Population Code	Provenience	Habitat	Lat	Long	Date harvested	Max % germination	Most successful cold stratification duration
Hordeum pusillum	Little barley	Winter annual	HP008	Washington County, Mississippi	Field margin	33.35	-91.10	23 May 2016	80	4 weeks
Phalaris caroliniana	Maygrass	Winter annual	PC005	Arkansas County, Arkansas	Field margin	34.45	-91.59	25 May 2016	20	6 weeks
Chenopodium berlandieri	Goosefoot	Summer annual	CB002	Jefferson County, Missouri	Sandy riverbank	38.09	-90.68	17 Oct 2017	---	---
			CB004	Madison County, Illinois	Sandy riverbank	38.75	-90.17	6 Nov 2017	---	---
Iva annua	Sumpweed	Summer annual	IA002A	Pulaski County, Kentucky	Woods, creek bank	37.06	-84.74	9 Oct 2017	65	8 weeks
			IA008	Calhoun County, Illinois	Slough	38.94	-90.50	5 Nov 2017	55	6 weeks
Polygonum erectum	Erect knotweed	Summer annual	See Mueller 2017a							6 weeks

SI Table 2: Provenience, seed treatment, and planting date

Species	Populations sampled	Seed weight (g)	Provenience	Cold treatment	Planting date
Hordeum pusillum	PBG2017		Lonoke County, AR	None	May 2
	PC005		Arkansas County, AR		
	PC008		Saline County, AR		
	HP007		Bolivar County, MS		
	Total	45.5			
Phalaris caroliniana	PC004		Bradley County, AR	5 weeks	May 2
	PC005		Arkansas County, AR		
	PC007		Arkansas County, AR		
	PC008		Saline County, AR		
		31.5			
Chenopodium berlandieri	PBG2017 (USDA)	45	Lonoke County, Arkansas	2 weeks	May 17
Iva annua	IA002		Nancy County, KY	7.5 weeks	May 18
	IA003		Henry County, KY		
	IA005		Desha County		
	IA006		Tichner County, KY		
	IA007		Desha County, KY		
	IA008		Calhoun County, IL		
		38.5			
Polygonum erectum	Tyson2016	263.5	Various	7.5 weeks	May 17

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