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A Technique for Germinating Seeds of Invasive Japanese Hop (*Humulus japonicus* Cannabaceae) and Indication of Seed Dormancy Characteristics

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ABSTRACT

Japanese hop (*Humulus japonicus* Sieb. & Zucc.), an invasive herbaceous vine from eastern Asia, is a threat to river bottom and moist soil communities throughout eastern North America. This disturbance fugitive is difficult to control due to rapid vegetative growth rates and copious supplies of highly dispersible achenes ("seeds"). Herbicide and cutting treatments have only temporary effects, indicating a new approach should be developed. A reliable means for growing research plants is therefore needed. We investigated a technique for germinating seeds collected from nature and subsequently stored under dry conditions. Hop seed was collected from four sites in southern Illinois, allowed to air dry one month and then subjected to dry after-ripening treatments of 2, 6, and 12 months at 4 °C. Seeds were subsequently sterilized and subjected to cold, moist stratification until germination was detected. Germination was ca. 50% after two months after-ripening but rose to ca. 80% at 6 and 12 months. Germination rates at two months seemed to vary between sites, but this was not statistically significant. Synthesizing this result with other literature, it appears that hop can germinate immediately if it is cold stratified for 30 days but requires more than two months after-ripening and cold stratification if it is allowed to dry. Given high germination rates and lack of contamination seen here, we recommend our sterilization protocol. Possible variations in seed maturity between collection sites should be considered when planning studies.

INTRODUCTION

The flood of invasive plant and animal species creates an ongoing challenge for ecologists seeking to limit their negative effects. Japanese hop (*Humulus japonicus* Siebold & Zucc., syn. *Humulus scandens* (Loureiro) Merrill, Cannabaceae) is among the worst invaders and has emerged as a substantial threat to plant communities of sunlit moist soil environments in eastern North America and Europe (Swearingen et al. 2010; EMPPO 2019, Schulz and Clark, pers. obs.). Originally native to eastern China, Japan, and Korea (EMPPO 2019), it is currently present in the eastern U.S. and Canada, reaching North Dakota south to Kansas and Arkansas (USDA 2022). Its habitat preferences in its invaded range are different from its native habitat, humid subtropical and subtropical dry winter zones of eastern Asia (EMPPO 2019). In Korea it is an indicator species for riverine forests (Cho et al. 2015), in Japan it is found in moist grasslands (Washitani and Masuda 1990), and in Inner Mongolia it is a species of river terraces (Xia et al. 2018). Predictably, it is a particular problem on the floodplains of large rivers in America and Europe (APWG 2010, NGRREC 2013, EMPPO 2019, MIPN 2018, Guyon and Cosgriff 2021).

Japanese hop is a disturbance fugitive, readily colonizing forest openings and disturbed soils. It climbs young trees, creating mounds of overtopping foliage (> 2 m) which stifle small tree growth (EMPPO 2019, Guyon and Cosgriff 2021, Schulz and Clark, pers. obs.). On the Upper Mississippi River (north of St. Louis, MO) the normal spring flood naturally creates establishment sites. This has been augmented by sporadic extreme floods and midsummer water releases from navigation dams that kill trees and expand hop habitat. Hop, in turn, perpetuates open canopies by preventing forest regrowth (Guyon and Cosgriff 2021, Schulz and Clark, pers. obs.)

Japanese hop is an annual in the northern parts of its adventive range, germinating in late spring, and continuing germination through the summer if conditions are suitable (Swearingen et al. 2010). Hop disperses seeds as achenes, which we will refer to as seeds to be consistent with the literature. A few gray literature sources (Meyers-Rice 1999, Pannill and Cook 2008) state without citation that seeds persist 3 years or more in the soil, but we have found no corroboration in the refereed literature. Krauss (1931), cited in EMPPO (2019), stated that seeds remain viable for three years, but we were unable to obtain the primary source to determine storage conditions. In the southern US hop behaves as a perennial (e.g., Keener et al. 2021), although the boundaries of its hardiness zone do not appear in the literature. Pannill and Cook (2008) suggest it is a weak perennial in Maryland. This may be the case in southern Illinois, the locale of this project.

Japanese hop is difficult to eradicate. Mechanical control and pre- and post-emergent herbicide applications are temporary remedies because survivors recolonize quickly, and a ready supply of seed allows reestablishment the following year (Guyon and Cosgriff 2021). These characteristics allow hop to occupy sunny locations for years at a time. We have observed sites in Illinois that have remained in hop cover for over a decade. Resource managers (e.g., Pannill and Cook 2008, Guyon and Cosgriff 2021) propose that the best way to reduce hop populations

is to maintain continuous tree cover. Strategies for reforesting infested areas will depend on the establishment of a tree canopy tall enough to escape hop overgrowth (Guyon and Cosgriff 2021).

Although the conservation aspects of Japanese hop invasion have been clear since at least 2008, the North American literature is primarily web or gray literature (e.g., Pannill and Cook 2008, Swearingen et al. 2010, MIPN 2018, USDA 2019). Much of this reiterates what is already known. EMPPO (2019) speaks to the problem in Europe but provides limited management advice. Given the scope and immediacy of the hop problem, investigators are likely to want to grow hop for use in research. The species has medicinal and other uses in China (Zheng et al. 2004) and is probably domesticated, however we have little practical information concerning its propagation from wild stock. Washitani and Masuda (1990) examined the germination requirements of hop and other warm grassland species in Japan by manipulating stratification conditions (moist/dry) and temperature pattern (cooling vs. warming between 4 and 36 °C). Dormancy was broken in hop after 5 months dry chilling followed by one-month moist chilling. After one-month initial moist chilling, with no dry chilling, it germinated in response to both warming (from 20 °C) and cooling (from 36 °C) conditions.

The purpose of this communication is to report one approach which quickly and easily produced vigorous seedlings of Japanese hop from seed after varying periods of cold storage. In addition, we report germination rates and potential variation in germination rates between different populations found in the central US.

METHODS and MATERIALS

Japanese hop is dioecious, flowering in early summer and producing seed (achenes) in late summer/fall. We collected seed from four widely spaced sites (at least 1.8 km apart) in Madison County, IL (ca. 38.8° N, 90.0° W) during October. Inflorescences were taken from four widely spaced plants in each site. Seed was easily collected by stripping ripe inflorescences and working dried material off the seed by hand. (Care should be taken because some individuals exhibit an allergic reaction to hop.) Seeds were stored one month dry in paper envelopes at 23 °C, then transferred to closed, sterile petri dishes stored at 4 °C. Germination tests were conducted 2, 6, and 12 months after harvest.

Before each test, seeds were sterilized by agitating them in a solution of 95% ethanol, 5% sodium hypochlorite (commercial bleach), and deionized water (1: 2: 5) for 5 minutes, then rinsed thoroughly in sterile deionized water. This protocol follows Haunold and Zimmerman (1972), who used it on brewers' hop (*Humulus lupulus* L.). For each round of germination tests, six petri plates consisting of 10 seeds each were prepared per population ($n = 60$). The germination substrate was 20 g heat-sterilized silica sand with 10 mL sterile deionized water. Plate preparation and planting took place under a laminar flow hood. Plates were organized at random in a 4 °C refrigerator and stratified for 6 weeks, when we noted the first radicle emergence. Plates were transferred to a light table (Cool White fluorescent tubes, ca. 125 uE m^{-2} s^{-1}; 14/10 light/dark, ca. 23 °C.). Germination (radicle emergence) was monitored daily until no new germinants were observed for 14 days. Untransformed, arc sine square root, and logit-transformed germination proportions were compared by 2-way model III ANOVA and Tukey's HSD (SYSTAT 13 Systat Software Inc., San Jose, California). All transformations of the data gave the same statistical results. We provide data for untransformed germination proportions here (Warton and Hui 2011).

RESULTS and DISCUSSION

Germination rates varied substantially and significantly between dry storage periods (Figure 1). After 2 months storage 40-65% of seeds germinated, depending on source population. Two populations had conspicuously, but not statistically lower ($p = 0.17$; $1 - \beta = 0.6$) germination rates (1/3 less) compared to the other two (c.f. Goodman 2016 regarding presenting pertinent but non-significant data). In trials after 6- and 12-months storage germination rates for all sites were much higher (80-89%). This represents a ca. 50% increase. Individual populations showed less difference between each other in the 6- and 12-month trials, which contrasts with an apparent performance differential between sites in the 2-month trial.

Lower rates of germination exhibited by seeds stored 2 months as opposed to 6 or 12 months suggest that not all seeds are physiologically ready to germinate until several months after dispersal. The apparent differences between seeds from different collection sites may be due to differences in the timing of seed development between sites. Site differences are unlikely due to genetic differences between sites because each of the multiple widely spaced parent plants was established from a large, naturally occurring pool of small, highly dispersible seeds. Differences in soil temperature or microclimate between sites could readily affect seed maturity. Notably, soil moisture differences in lowland habitats play a meaningful role in soil temperature (Brady 1974).

It is interesting to note that in our study dry Japanese hop seed requires a protracted period of after-ripening preceding cold stratification for germination.

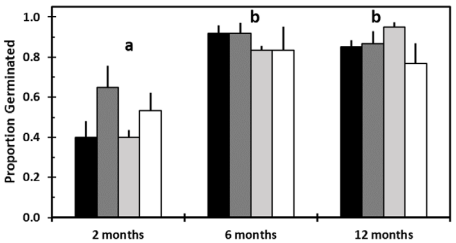

Figure 1. Mean (SE) germination rates of Japanese hop after three periods of after-ripening. Differently shaded bars are data from collections taken at four different sites. Significant differences ($p < 0.0001$) between after-ripening periods are indicated by different letter notations. Effects of individual sites ($p = 0.3064$) and the site by after-ripening interaction ($p = 0.1706$) were not significant.

In contrast, Washitani and Masuda (1990) demonstrated cold moist stratification could stimulate germination, with or without after-ripening. This situation prompts us to wonder why both germination controls are present. In American habitats after-ripening would have little role because conditions for cold/moist stratification are commonplace throughout the current range of hop. In the large range hop occupies in Asia, it is possible that the dry dormant season in northeast China has selected for a requirement for moist stratification after dry storage to prevent germination miscues. Both our data and the work of Washitani and Masuda (1990) are consistent with this.

Throughout the study only a few seeds (overall N = 180) experienced any form of contamination, suggesting our protocol of seed sterilization and aseptic technique was effective. An effective sterilization protocol and high germination rates (> 85%) are prerequisites to efficiently raise plants for research purposes. The sterilization regime used here was rather severe, employing both ethanol and sodium hypochlorite. We adopted this approach because it was recommended for domestic hop by Haunold and Zimmerman (1972) and we expected considerable contamination of seeds in the wild. It is likely less rigorous techniques are sufficient, but the technique used here *will* yield positive results.

In conclusion, we make these recommendations for germinating Japanese hop:

1. A seed sterilization procedure is desirable *pro forma*. While we suspect the hypochlorite/ethanol combination used here is perhaps extreme, it does give good results.
2. Seeds which have dried require a period of cold after-ripening of greater than two months, followed by cold stratification for best germination
3. Seed maturation rates at various sites probably vary appreciably. For dried seed, an after-ripening period greater than two months eliminated this issue.
4. Given the ease with which Japanese hop can be germinated, manipulative experiments using plants raised from seeds are feasible.

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