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Phenology of seed maturation in babysbreath (*Gypsophila paniculata*) in northwest Michigan, USA, and its relation to glyphosate efficacy

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Abstract

Babysbreath or perennial babysbreath (*Gypsophila paniculata* L.) is an aggressive invasive plant in large parts of southern Canada and the northern and western United States. It reproduces and disperses by seed, so the phenology of seed maturation is important in designing management programs. The present study provides the first quantitative assessment of *G. paniculata* seed-maturation phenology in a field population, as well as the first quantitative assessment of how the efficacy of herbicide treatment in preventing production of germinable seeds depends on the timing of treatment in relation to this phenology. Seeds were collected from untreated plants on five dates during July and August in both 2016 and 2017 and tested for germinability. Percent germination increased from 20% to 81% between July 22 and 28 and exceeded 90% by August 4, 2016. The seed-maturation phenology in 2017 was similar but delayed by about 4 d. On a growing degree-day scale, seed-maturation phenologies for the 2 yr were nearly identical. We also tested germinability of seeds from plants sprayed with glyphosate (23.4 ml ae L^{-1}) on July 11, 18, and 25, 2016 (one date per plant). Percent germination increased from 0% to 13% to 20% over successive treatment dates, highlighting the importance of completing treatment early in the growing season.

Note

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Introduction

Babysbreath or perennial babysbreath (*Gypsophila paniculata* L.) is a herbaceous perennial plant in the family Caryophyllaceae that is native to central and eastern Europe; the Black Sea region; and western portions of Russia, China, and Mongolia (Barkoudah 1962). It was introduced into North America at least as early as the 1880s (Darwent 1975), probably as an ornamental, and has established adventive populations in many locations across the continent. Populations are most common in southern Canada and in northern and northwestern parts of the United States (Darwent 1975; EDDMapS 2019) but extend southward into southern California in the west and the Florida Panhandle in the east (Hartman and Rabeler 2012; Wunderlin et al. 2019). The native range of the entire genus *Gypsophila* is geographically associated with dry steppe biomes in Europe and Asia, radiating outward from the Black Sea region, mainly between the latitudes of 30°N and 60°N (Barkoudah 1962). In both its native and introduced ranges, *G. paniculata* is primarily associated with sandy soils and with climate types characterized by hot or warm summers and cold winters (Barkoudah 1962; Darwent 1975).

Barkoudah (1962) and Darwent (1975) summarize much of the available biological information about *G. paniculata*. The plant has a long woody taproot (up to 4-m long) that provides access to water and nutrients deep in the soil during dry summer conditions and, together with the caudex (rootstock), serves as the plant's perennation organ. Aboveground portions of the plant dry and break off from the caudex in late summer, becoming tumbleweeds. Plants resprout from buds on the caudex in spring. While many other invasive plants, such as Japanese knotweed [*Fallopia japonica* (Houtt.) Ronse Decr.] (Hollingsworth and Bailey 2000) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Smith and Barko 1990), propagate mainly vegetatively in introduced areas, sexual reproduction by seed is the only known mode of reproduction in wild populations of *G. paniculata*; no form of asexual reproduction (e.g., apomixis, autofragmentation, turions, bulbils) or colonial expansion of individual plants (e.g., rhizomes, stolons, suckers) has been reported. Flowers begin to open in late June, and fruits begin to form in mid-July. Mature plants bear hundreds to thousands of perfect flowers at different stages of development (stamens developing before pistils), and both cross- and self-fertilization are thought to occur. Seeds are reniform or cochleate, roughly 1.5- to 2-mm long, have a thin black or dark-brown seed coat, and are orthodox (Royal Botanic Gardens Kew 2019). Darwent (1975) notes that the seeds exhibit "little or no dormancy," while a more detailed account by Geneve (1998)

Management Implications

Gypsophila paniculata (babysbreath, perennial babysbreath) is a problem invasive in a significant portion of Canada and the United States, but relatively little is known about the phenology of seed maturation in its introduced range. From a management perspective, it is important to understand the phenology of seed maturation of invasive plant populations that propagate by seed to determine the time frame in which plants can be treated or removed while also preventing reproduction and dispersal. Seed maturation of *G. paniculata* exhibits a consistent phenology from year to year, with a rapid increase in germinability in late July. The phenology is even more consistent between years on a growing degree-day scale, with the rapid increase in germinability occurring between 600 and 700 C d. The timing of glyphosate application relative to this phenology is important. Glyphosate prevents the production of germinable seeds when applied before the appearance of mature seeds but becomes less effective the later it is applied.

indicates that they exhibit non–deep physiological dormancy and require a short period of dry storage (to achieve dry afterripening) but not cold stratification in order to break dormancy and germinate. Absence of a moist chilling requirement to break dormancy may partially account for the ability of *G. paniculata* to maintain wild populations in areas with warm summers, such as Southern California and Florida. Seeds that have not fallen from plants by the time tumbleweeds form in late July and August are dispersed in "salt-shaker" fashion by the windblown tumbleweeds, facilitating spatial expansion of populations as well as reseeding of areas previously treated by managers. Seeds germinate mainly in midspring. Juveniles are thought to mature and begin flowering in their third year of growth but may do so earlier in areas with warm winters. We know of no studies that demonstrate how long *G. paniculata* seeds remain viable in the soil seedbank, or even whether they form a transient versus persistent bank (sensu Thompson and Grime 1979; Fenner and Thompson 2005). Based on counts in soil cores taken in late spring, Clements et al. (2007) estimated the density of *G. paniculata* seeds in the seedbank of a sandy semidesert shrub–steppe habitat in southern British Columbia, Canada, to be 391 m^{-2} (3.91 million ha^{-1}), but the distribution of seed residence times in the soil was unknown.

Available management methods for controlling *G. paniculata* are summarized by Darwent (1975) and DiTomaso et al. (2013). The most useful methods in nonagricultural areas are mechanical control (especially manual removal by cutting the taproot with a spade, just below the caudex) and chemical control with herbicides, both of which also can and should be used to prevent seed production. A variety of herbicides have been used, including 2,4-D, aminopyralid plus metsulfuron-methyl, chlorsulfuron, dicamba, glyphosate, imazapic, mecoprop, metsulfuron-methyl, and picloram. Regardless of the control method, *G. paniculata* infestations often are difficult to extirpate and may require multiple years of treatment (Emery et al. 2013; TNC 2013).

Coastal dune habitats of northwest Michigan are part of the largest freshwater dune system in the world. They are characterized by sandy soils and a warm-summer, cold-winter, continental climate (Köppen-Geiger climate type Dfb) that matches the predominant climate type in eastern and central European portions of *G. paniculata*'s native range (Barkoudah 1962; Beck et al. 2018; Jalas and Suominen 1986). *Gypsophila paniculata* commonly occurs in these dune habitats in dense stands of 50% to 80% ground cover that are associated with reduced native species abundance and increased abundance of other nonnative species (Emery et al. 2013; Karamanski 2000). Management is critical in this area, as it is home to several endemic, threatened, and endangered plant and animal species (Albert 2000).

Gypsophila paniculata infestations in northwest Michigan typically are managed with a combination of manual removal and directed spray-to-wet foliar application of glyphosate to individual plants using backpack sprayers, with glyphosate being the preferred treatment, except under windy conditions or when state or federally protected plant species are present (TNC 2013). Emery et al. (2013) found that manual removal of *G. paniculata* from 20 by 50 m marked plots significantly reduced its percent cover after 3 yr of treatment but did not extirpate the plant. We know of no comparable assessment of herbicide effectiveness. Regrowth commonly occurs in treated areas (S Howard, personal communication), though the relative importance of different sources of regrowth (resprouting of missed or partially treated plants versus germination of seeds from the soil seedbank) is unknown.

The phenology of seed maturation is important for managing invasive weeds that propagate by seed. Plants that are manually removed or treated with herbicide after seeds have matured (which is not unusual in summer removal projects that employ students or use volunteers) will leave behind large numbers of germinable seeds by which they can maintain or enhance populations. More subtly, a significant proportion of seeds produced by herbicide-treated plants may still mature successfully if seed development has proceeded far enough by the time plants are sprayed (Clay and Griffin 2000; Egley and Williams 1978; Jeffery et al. 1981; Klingman and Murray 1976). Because *G. paniculata* plants produce large numbers of seeds annually (ca. 14,000 $plant^{-1}$: Stevens 1957), even a 90% reduction in the number of mature seeds can be expected to leave behind more than 1,000 germinable seeds $plant^{-1}$. Given the average *G. paniculata* density of 2 plants m^{-2} in infested areas at our study site (see "Study Site" section), this translates to roughly 30 million germinable seeds ha^{-1}.

Despite the clear importance of seed production and dispersal in the population biology of *G. paniculata*, little information is available for this species regarding seed germinability or the phenology of seed maturation in wild populations. To our knowledge, only a single study of the germinability of *G. paniculata* seeds from a wild population in North America has appeared in the literature (Darwent and Coupland 1966), and it is restricted to seeds from a location near Saskatoon, Canada. We are also aware of no study that quantitatively assesses the phenology of seed maturation in a wild population or that assesses the efficacy of herbicide application in preventing seed maturation in *G. paniculata* plants that have already begun the process of seed maturation by the time they are sprayed.

The present study was conducted to provide new information on these important aspects of the biology and ecology of *G. paniculata*. Specifically, the purpose of the study was to answer two questions: What is the phenology of *G. paniculata* seed maturation in a coastal dune habitat in northwest Michigan? How is seed germinability affected by the timing of glyphosate application relative to this phenology?

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Materials and Methods

Study Site

This study was part of a collaborative project involving the Robert B. Annis Water Resources Institute and the Nature Conservancy. The larger project included four additional components: large-scale restoration of infested areas within Sleeping Bear Dunes National Lakeshore (SBDNL) in northwest Michigan, USA (44.872°N, 86.057°W); field experiments designed to assess the efficacy of management methods for *G. paniculata* that are currently in widespread use in Great Lakes coastal dune habitats; development and application of methods for determining the genetic structure of *G. paniculata* populations in the region; and development of a stochastic individual-based eco-evolutionary management model that allows incorporation of a high level of biological detail (including genetics), plausible representation of alternative management methods, and assessment of predicted long-term outcomes. The study by Emery et al. (2013) was conducted at different sites in the same general area.

All seeds were collected from a 40-ha section of the plateau region of SBDNL that had no history of prior management. Percent cover by *G. paniculata* ranged from 25% to 50%, with an average density of 2 plants m^{-2} (Rice 2018). Average annual temperature (2016: 10 C; 2017: 9 C; historical: 9 C) and average annual precipitation (2016: 81 cm; 2017: 84 cm; historical: 81 cm) were similar between 2016 and 2017 and also similar to historical averages (Supplemental Table S1; MRCC 2018b).

Phenology of Seed Maturation

The phenology of seed maturation at SBDNL was characterized using the method of Bram and McNair (2004), which exploits fundamental properties of the maturation process in orthodox seeds. A general acquaintance with these properties is necessary for understanding the biological basis of the method of Bram and McNair (2004), so we briefly outline them before describing the specific field and laboratory methods used.

The overall process of seed development and maturation in orthodox seeds consists of a complex but highly organized sequence of steps. Physiological details of this process (summarized by Angelovici et al. 2010; Bewley et al. 2013; Leprince et al. 2017) have been studied in only a small number of species (not including *G. paniculata*), but the major steps are thought to be similar for most orthodox seeds and are as follows: embryogenesis or histogenesis (development of the embryo, endosperm, and seed coat), seed filling (reserve deposition), attainment of physiological maturity, attainment of desiccation tolerance, maturation drying, attainment of harvest maturity, entry into non–deep physiological dormancy (in *G. paniculata*), quiescent overwintering, dry afterripening, and germination (imbibition, renewed metabolism and growth, radicle emergence). In natural environments, *G. paniculata* seeds must pass through all of these steps in order to overwinter and complete germination in the spring. However, as Angelovici et al. (2010) emphasize, the attainment of desiccation tolerance is a key milestone: this step must be completed by orthodox seeds to allow them to survive the natural process of maturation drying (seed moisture content is reduced from roughly 60%–80% to <20%), which in turn is a necessary step for entering the physiologically quiescent state in which they overwinter. Seeds that have attained desiccation tolerance, and only those seeds, are also able to survive *artificial* drying (e.g., with silica gel beads) and, after a suitable period of dry storage, germinate normally when exposed to water. Therefore, orthodox seeds that are removed from a plant, artificially dried, stored dry, and exposed to water in a germination test will germinate normally if they were removed from the plant after attaining desiccation tolerance but will not germinate if they were removed before attaining desiccation tolerance. In this way, the proportion of seeds in a wild population that have attained the desiccation-tolerance stage of maturation can be determined at different times during the growing season, providing an easily measured index of collective progress in acquiring the ability to overwinter successfully and germinate the following spring.

Seed Collections

To determine the phenology of seed maturation at SBDNL, seeds were harvested from approximately 100 untreated plants on each of five dates between July 22 and August 23, 2016. Plants from which seeds were collected on each date were scattered around the perimeter of a 15-ha rectangular area in the plateau region of SBDNL. All plants sampled were located outside this area but within 15 m of its boundary. (Seeds were not harvested inside this area, because it was treated during the field season.) Based on a visual survey, more than 75% of *G. paniculata* plants throughout the study area bore abundant seeds on these dates (and in 2017; see "Phenology of Seed Maturation" subheading in "Results and Discussion"), with most plants that lacked seeds likely being juveniles. Approximately 150 seeds were collected from each plant (about 15,000 total seeds collected on each date), taking care to sample fruits over the entire crown and sides of each plant to obtain a representative sample. Some of these seeds (and leaves collected from the same plants) were used in the population genetics component of the larger project. Of the roughly 500 untreated plants sampled over the five collection dates, 16 plants distributed around the perimeter of the 15-ha rectangular area were sampled on all five collection dates, making it possible to eliminate differences in maternal origin between collection dates as a potential source of variation in maturation phenology and overall germinability of seeds.

To assess the efficacy of glyphosate treatments applied at different times during the growing season, seeds were collected on August 23, 2016, from four to five previously treated plants scattered throughout each of nine 32 by 32 m marked treatment plots that were distributed over the interior of the same 15-ha rectangular area. A total of 38 plants were sampled (ca. 150 seeds $plant^{-1}$). All *G. paniculata* plants in each of the nine treatment plots had been individually treated by directed spray-to-wet foliar application of glyphosate (Roundup ProMax®, Monsanto, 23.4 ml ae L^{-1}) using backpack sprayers on one of three dates in July 2016 (July 11, 18, and 25), with three of the plots treated on each date. Plants bore flowers but not well-developed fruits on July 11, bore both flowers and well-developed fruits (white in color) on July 18, and had completed flowering by July 25 and bore fruits that were visually more mature (mostly orange-red in color) than on July 18.

In 2017, seed collections for the phenology study were repeated to assess potential year differences in phenology and, methodologically, to determine whether within-year variability in the phenology pattern (as measured by point-wise 95% confidence [CI_{95}] intervals) would increase noticeably if seed collections were not restricted to the same individuals on each date. Based on our experience in conducting the germination tests in 2016, fewer plants were sampled on each date in 2017. Specifically, seeds were collected from 20 untreated plants (ca. 150 seeds $plant^{-1}$) on each of five dates between July 26 and August 22, 2017, with no attempt

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to sample the same plants each time. Plants from which seeds were collected were scattered throughout four 1-ha areas, each located in one of the corners of a 16-ha rectangular area of the plateau region of SBDNL that was roughly 300 m north of the 2016 collection area and had similar topography and a similar plant community. (This area served as an untreated reference area in another component of the larger project that assessed treatment efficacy.)

Seeds from each plant in each collection date or treatment date group were placed in a separate paper coin envelope, which was then sealed. Coin envelopes from the same collection date were placed together in a sealed plastic bag containing silica gel beads and stored in the dark at ca. 20 C until the germination experiments were run. Dry storage is a standard practice for orthodox seeds (Bewley et al. 2013) and also satisfies the dormancy-breaking requirement for dry afterripening of *G. paniculata* seeds (Geneve 1998).

To ensure that our seed storage procedure was adequate, we assessed it as part of a pilot study conducted during summer 2016 using fully mature seeds collected in fall 2015. Groups of seeds from eight different plants were tested for germinability in July 2016 (ca. 9 mo of dry storage) without cold stratification and exhibited 92% to 98% germination when tested using the methods described in the following section. These results confirm the suitability of our seed storage method, adequacy of dry storage alone as a dormancy-breaking treatment, and efficacy of our germination test procedure.

Germination Experiments

The germination experiments were designed specifically to determine the proportion of seeds on each collection date that had matured sufficiently to attain desiccation tolerance and therefore would be able to undergo the natural process of maturation drying required for successful overwintering and subsequent germination in the spring. With this goal in mind, we define germinability in this study as the ability of intact *G. paniculata* seeds to complete germination successfully after natural or artificial drying has occurred, the requirement for dry afterripening has been satisfied, and conditions appropriate for imbibition have been provided. The distinction between germinability and viability is important in assessing seed maturation. By a viable seed, we mean a seed that is alive, or equivalently, metabolically competent (Elias et al. 2012). Thus, a healthy immature seed is viable but not germinable, while a healthy, fully mature seed is both viable and germinable. In the phenology study, we are concerned specifically with the seasonal acquisition of germinability.

Seeds collected in July and August 2016 were stored as described earlier until October 2016 (ca. 2 to 3 mo of dry storage, depending on collection date), when the first germination tests were run. Seeds for the phenology of maturation experiment and the maturation with glyphosate experiment were tested simultaneously. To eliminate differences in maternal origin between collection dates as a source of variation, we restricted germination tests for the phenology of maturation experiment to seeds from the 16 plants that were sampled on all five collection dates. To ensure that all seeds tested in both experiments could be checked for germination daily within a 3-h window, we further restricted the germination tests to seeds from a random subset of the plants sampled for each experiment. Thus, we tested seeds from 5 of the 16 untreated plants that had been sampled on all five collection dates in the phenology of maturation experiment (25 groups of seeds tested) and tested seeds from 12 of the 38 treated plants in the maturation with glyphosate experiment (4 different plants for each of the three treatment dates).

The phenology of maturation experiment was repeated in 2017 using seeds from different plants on each collection date. As in the 2016 experiment, seeds were collected in July and August, and the germination test was conducted in October. For each of the five collection dates, seeds from 12 of the 20 untreated plants sampled (a total of 60 different plants) were tested.

In each germination experiment, 100 dry-stored seeds collected from each parental plant in each collection date or treatment date group were placed on the surface of moist filter paper in 94-mm (diameter) by 16-mm (depth) petri dishes and incubated in a growth chamber at 20 C and 112 μmol m^{-2} s^{-1} photosynthetically active radiation from fluorescent bulbs with a 12:12-h light:dark cycle. Each day for 12 (2016) or 14 (2017) d, the same individual checked the petri dishes and counted and removed newly germinated seeds. Germination was operationally defined as radicle emergence (Baskin and Baskin 2014). Petri dish positions in the growth chamber were randomized daily to prevent potential biases in temperature or light exposure. In 2016, 37 petri dishes containing 3,700 seeds were processed, with 25 petri dishes and 2,500 seeds from the phenology of maturation experiment (100 seeds $plant^{-1}$ by 5 plants per collection date by 5 collection dates) and 12 petri dishes containing 1,200 seeds from the glyphosate experiment (100 seeds $plant^{-1}$ by 4 plants per treatment date by 3 treatment dates). In 2017, 60 petri dishes containing 6,000 seeds from the phenology of maturation experiment (100 seeds $plant^{-1}$ by 12 plants per collection date by 5 collection dates) were processed.

The germination test protocol is based on the protocol used by Darwent and Coupland (1966) and on protocols for *G. paniculata* promulgated by the International Seed Testing Association and the Association of Official Seed Analysts as stated and compared by Stephenson and Mari (2004). Choice of an incubation temperature of 20 C was based mainly on results of Darwent and Coupland (1966), who tested a range of temperatures and found that percent germination was maximal and statistically indistinguishable at 10, 20, and 28 C but that the maximum was achieved roughly 8 d sooner at 20 C. We assessed our protocol in a pilot study conducted with fully mature dry-stored seeds during summer 2016, before the experiments reported here, and obtained germination percentages of 92% to 98%, as noted earlier.

Statistical Analysis

For each collection date of untreated plants (phenology of maturation experiment) and each glyphosate treatment date of treated plants (maturation with glyphosate experiment), the observed temporal pattern of germination was quantified by fitting a nonparametric Kaplan-Meier time-to-event curve (McNair et al. 2012). Germination patterns for different collection dates were compared using pairwise log-rank tests with Holm correction for multiple comparisons (McNair et al. 2012), as were germination patterns for different glyphosate treatment dates. Statistical significance was assessed at the $\alpha = 0.05$ level. All statistical analyses were performed using the R programming language and computing environment, v. 3.4.1 (R Core Team 2018), and its survival package, v. 2.38 (Therneau 2015; Therneau and Grambsch 2000).

Growing Degree-Day Calculation

Rates of plant growth and development are temperature dependent and typically become negligible when ambient temperature falls below a threshold, usually taken to be 10 C unless species-specific information is available. Growing degree-days (GDDs) are a measure of how much time, and by how much, temperature exceeds

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this threshold over a specified period of time. Because they partially account for the effects of within- and between-year temperature variation on rates of physiological processes, GDDs are a better predictor of plant phenology than calendar date or day of the year (Soltani and Sinclair 2012). We therefore calculated seasonal patterns of GGD accumulation for 2016 and 2017 to aid in interpreting our phenology results.

GDD accumulation between an initial day t_0 and subsequent day t in the same growing season, denoted GDD(t_0, t), is defined by (units: C d)

$$GDD(t_0, t) = \int_{t_0}^{t} [T(\tau) - T_\theta]_+ \, d\tau \quad (1)$$

where $T(\tau)$ is ambient temperature (C) at time τ (d), T_θ is the threshold temperature (C) above which development proceeds and below which it does not, and $[x]_+ = \max(x, 0)$. We set $T_\theta = 10$ C and calculated GDD(t_0, t) as a function of t for the entire growing season in each year, using three different choices of t_0 (corresponding to January 1, March 1, and April 1). Because the three resulting GDD curves for each year were nearly identical, we present only the curves with t_0 chosen as March 1. On-site temperature time series are not available for SBDNL, so we used hourly data recorded at a nearby monitoring station (Dow Memorial Airport in Frankfort, MI; 44.625°N, 86.201°W), which we downloaded from the Midwest Regional Climate Center (MRCC 2018a). GDD(t_0, t) values with a 1-h time step were calculated by numerical integration (Simpson's 1/3 rule; Hoffman 2001) using a custom computer program written in R.

Regression Model of Seed Phenology

As an additional aid to interpreting the phenology results, a nonlinear regression model was fit to data on percent germination versus day of the year at collection as well as percent germination versus GDDs at collection. Because seeds were collected on only five dates each year, we restricted attention to regression models with no more than three parameters when assessing alternative models. A logistic growth model was found to describe the temporal trend of seed maturation adequately, as Bram and McNair (2004) also found in a similar study of seed maturation in *R. japonica*. The following parameterization was used:

$$y(x) = \frac{a}{1 + e^{-b(x-h)}} \quad (2)$$

where y is mean percent germination, x is day of the year or GDDs at seed collection, a is the asymptotic value that mean percent germination approaches as x becomes large, h is the half-saturation constant (the value of x at which $y(x) = a/2$), and b is the maturation rate parameter, which determines how rapidly $y(x)$ increases near $x = h$ for any given value of a (Seber and Wild 1989). We are mainly interested in the value of h, which serves as a location parameter for comparing the timing of phenologies in different years. The model was fit using the R programming language and computing environment, v. 3.4.1, and its *nls()* function (R Core Team 2018), which computes ordinary least-squares estimates of parameter values using a Gauss-Newton algorithm (Bates and Watts 1988).

Results and Discussion

Phenology of Seed Maturation

Results for seeds from untreated plants show increasing germinability with successive collection dates during late July and early August in both 2016 and 2017 (Figure 1). Differences between germination patterns of seeds from different collection dates were detected for 9 of the 10 pairs of dates each year (log-rank test; largest $P < 0.001$), the sole exception being the final pair of dates in August ($P = 0.71$ in 2016, $P = 0.093$ in 2017) (Supplemental Tables S2 and S3). The results show that in both years, cumulative germination at the end of the germination tests (day 12 in 2016, day 14 in 2017) increased from roughly 20% to roughly 80% during a 1-wk period near the end of July and exceeded 90% by the final collection date in August (Figure 2A).

The point-wise 95% confidence intervals in Figure 2 show no evidence that variability about the sigmoid trend in seed maturation was higher in 2017 than in 2016. Though the larger number of plants sampled in 2017 likely played a role in reducing variability, results for the 2 yr suggest there is no meaningful statistical advantage in sampling the same plants on each collection date, as in 2016, compared with sampling from the general population each time, as in 2017. Because of the greater simplicity of sampling from the general population each time, we recommend doing so in future studies.

Several potentially useful changes in the appearance of fruits on different collection dates were noticed in both 2016 and 2017. Fruit color had changed from white to orange-red by the first collection date, then darkened to brown by the second and subsequent collection dates. Seeds were dark brown or black by the final two collection dates in August but were lighter in color when the earlier collections were made. Some of the July seeds that failed to germinate were hollow or collapsed, and many were a red or light brown color instead of dark brown or black. Darwent and Coupland (1966), who studied a population of *G. paniculata* just south of Saskatoon, Canada (ca. 7° north of SBDNL but with the same Köppen-Geiger climate type, Dfb) noted that fruits "matured and began to split open by late July," which was also true at our study site.

The rapid increase in seed maturation observed in both study years occurred slightly later on a day of the year scale in 2017 than in 2016, but the rate of this increase once it began was very similar. Least-squares estimates of half-saturation constant h in the fitted logistic models for the 2 yr indicate a delay of 3.6 d in the phenology for 2017 compared with that for 2016 (Figure 2), while estimates of maturation rate parameter b were nearly identical (Figure 2). Several environmental factors are known to influence plant phenology (notably temperature, photoperiod, soil nutrients, and drought conditions), with temperature and photoperiod commonly being the most important (Soltani and Sinclair 2012). Air temperatures were slightly lower during the growing season in 2017 than in 2016, and GDDs consequently accumulated more slowly (Supplemental Figure S1). Because we are comparing phenologies at the same site in consecutive years (so photoperiod is the same), neither of which was a drought year, it is likely that the lower rate of accumulation of GDDs in 2017 was the main factor responsible for the slight delay in the phenology of seed maturation. Consistent with this hypothesis, the maturation patterns for 2016 and 2017 coincide when percent germination is plotted against GDDs instead of day of year (Figure 2B).

The sigmoid phenological patterns of seed germinability versus day of year or GDDs observed in our study are similar to

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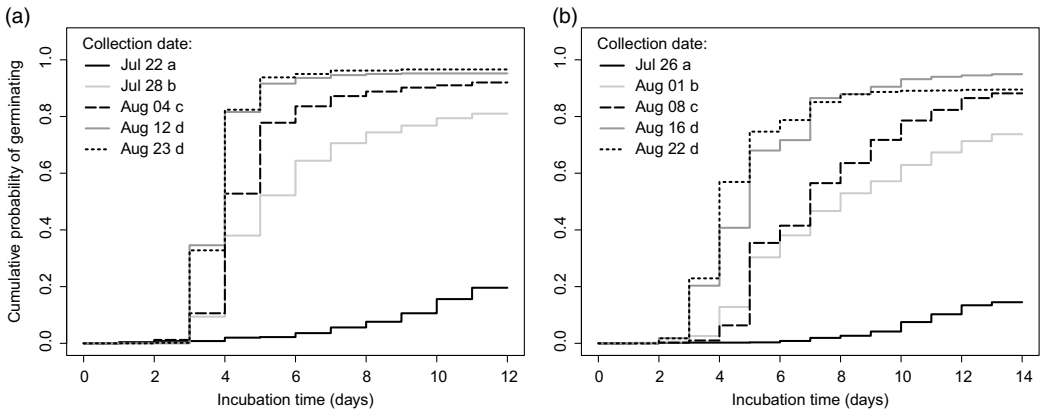

Figure 1. Kaplan-Meier time-to-germination curves for *Gypsophila paniculata* seeds collected from untreated plants at Sleeping Bear Dunes National Lakeshore near Empire, MI. To determine the phenology of seed maturation, seeds were collected at weekly intervals on five dates in July and August of 2016 (A) and 2017 (B). They were then stored dry until being tested for germinability at 20 C with a 12:12-h light:dark cycle in October of the same year. All pairs of germination patterns for different collection dates were different in each year (largest $P < 0.001$ in 2016 and 2017) except the final two collections in August ($P = 0.71$ in 2016, $P = 0.093$ in 2017). Distinct lowercase letters following dates in the legends identify collection dates for which germination patterns showed statistically significant differences.

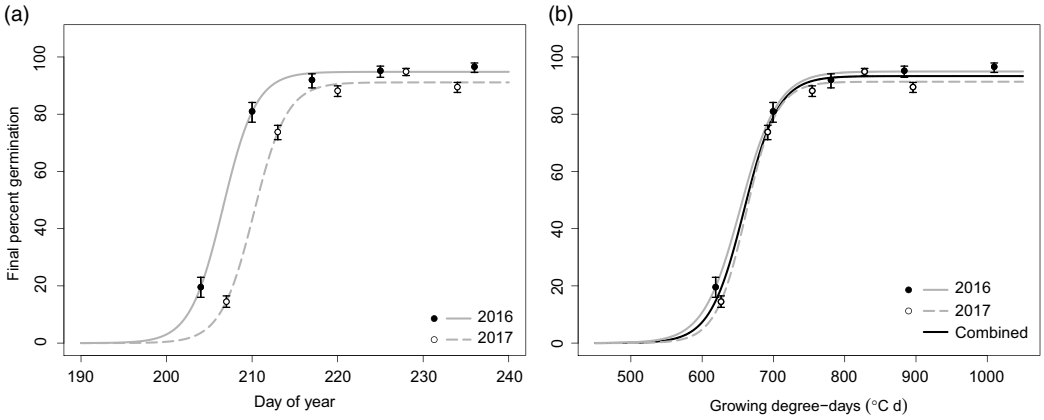

Figure 2. Final percent germination of *Gypsophila paniculata* seeds from untreated plants (day 12 in Figure 1A, day 14 in Figure 1B) as a function of day of the year on which seeds were collected (A) and the corresponding growing degree-days (C d) at collection (B). Bars represent 95% confidence intervals. Day 200 is July 18 in 2016 and July 19 in 2017. Curves shown are least-squares fits of the logistic model given by Equation (2) with parameter values as follows: (A) solid gray curve, year 2016, $a = 94.80$, $b = 0.5156$, $h = 206.6$; dashed gray curve, year 2017, $a = 91.12$, $b = 0.5150$, $h = 210.2$. (B) solid black curve, years 2016 and 2017 combined, $a = 93.32$, $b = 0.04178$, $h = 659.0$; for reference, fitted curves for 2016 (solid gray) and 2017 (dashed gray) are also shown.

phenological patterns documented in other studies of plants in nonagricultural systems. For example, Bram and McNair (2004) found that seed germinability in *R. japonica* exhibited a sigmoid increase from roughly 20% to roughly 80% over periods of 1 to 2 wk in late September at different study sites in southeastern Pennsylvania. Wagner and Mitterhofer (1998) found that average seed and embryo lengths in *Gentianella germanica* (Willd.) W.F. Warb., expressed as percent of mature length, exhibited a similar increase over a period of about 1 wk in September at a site in the Austrian Alps. Quantitative characterization of such phenological patterns based on GDDs or similar temperature-based measures of physiological time has previously been advocated as a useful tool in weed management (Ghersa and Holt 1995) and conservation biology (Baumgärtner and Hartmann 2000) and is also an important tool in modern agriculture (Soltani and Sinclair 2012).

Maturation with Glyphosate

Seeds from plants individually sprayed with glyphosate on three different dates in July 2016 showed a trend of increasing germinability with later treatment date (Figure 3). Differences were detected between all three pairs of treatment dates (largest $P < 0.008$; Supplemental Table S4), with 0% germination in seeds from plants treated in early July, 13% (CI_{95}: 10% to 16%) in plants treated in mid-July, and 20% (CI_{95}: 16% to 23%) in plants treated in late July.

Our results show that herbicide treatment was more effective in preventing production of germinable seeds when glyphosate application occurred earlier in the growing season. This outcome is consistent with studies of other plant species that found that when glyphosate is applied during early phases of seed maturation, the herbicide accumulates in the seed and results in incomplete development of the endosperm and embryo, causing reduced seed germinability (Baig et al. 2003; Cessna et al. 2002). Glyphosate application by summer crews at SBDNL ended on July 25 in 2016, by which time seed development had proceeded far enough so that roughly 20% of the seeds were germinable. Thus, the effectiveness of glyphosate appears to decline as seed maturation progresses, a trend seen in other plant species receiving late glyphosate application (Bennett and Shaw 2000; Clay and Griffin 2000).

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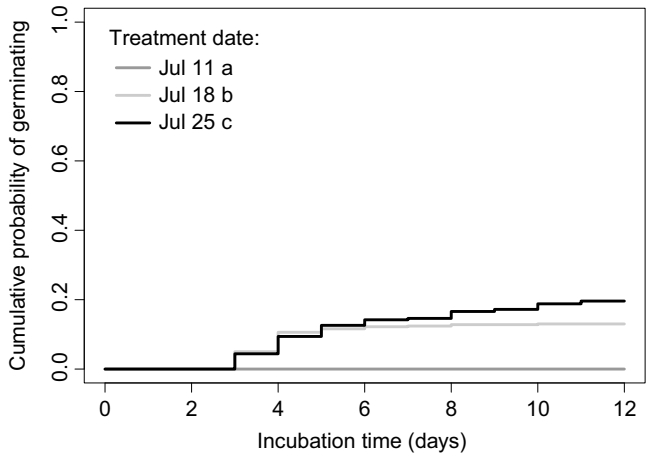

Figure 3. Kaplan-Meier time-to-germination curves for *Gypsophila paniculata* seeds collected from glyphosate-treated plants at Sleeping Bear Dunes National Lakeshore near Empire, MI. All seeds were collected on August 23, 2016, from plants, each of which had been sprayed with glyphosate on one of three dates in July 2016. Germination patterns for all three pairs of treatment dates were different (largest $P < 0.008$). Distinct lowercase letters following dates in the legend indicate treatment dates for which germination patterns were statistically significantly different.

In summary, our results suggest that seed maturation of *G. paniculata* in northwest Michigan has a consistent phenology from year to year, with a rapid increase in germinability near the end of July (between 600 and 700 C d) and attainment of maximum germinability by the end of August. To prevent production of germinable seeds and thus optimize control efforts, manual removal and glyphosate treatment of *G. paniculata* should be completed by mid-July. As is well known for cultivated plants, the phenology of seed maturation in other geographic regions may differ somewhat from the pattern in northwest Michigan, due to differences in the temporal pattern of GDD accumulation, photoperiod, and perhaps other factors. It will therefore be advisable to conduct studies like the present one in additional geographic regions where *G. paniculata* is invasive in order to characterize the phenological patterns for use in optimizing local management. These studies should include phenologies based on GDDs, which we think will be more consistent between years and therefore more reliable for prediction. We also recommend that, unless maternal effects or genetically based differences among seeds from different parents are specifically of interest, seeds on each collection date should be collected from as many plants as feasible and mixed when tested, with 100 seeds per petri dish and a minimum of 400 seeds per collection date (ISTA 1999). If detailed characterization of phenology patterns like those in Figure 2 is of interest, we recommend collecting seeds every 3 to 4 d starting ca. July 21 or about 610 C d and ending ca. August 21 or about 900 C d.

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Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/inp.2019.21

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