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ARTICLE

Phenology, dichogamy, and floral synchronization in a northern red oak (*Quercus rubra*) seed orchard

Lisa W. Alexander and Keith E. Woeste

Abstract: We developed a novel scoring system to assess spring phenology in a northern red oak (*Quercus rubra* L.) clonal seed orchard. The system was used to score from 304 to 364 ramets for three reproductive seasons and to place clones into early, intermediate, and late phenology classes. Although the absolute number of clones in each phenological class changed from year to year, the overall order of clonal flowering was highly stable ($r_s = 0.67$, $p < 0.001$). Early-flowering clones flowered significantly longer than later flowering clones in all 3 years. Dichogamy was present in the orchard, with male flowers of a clone emerging from 1.4 to 3.0 d sooner than its female flowers. Mean dichogamy values for individual clones ranged from 0.0 to 4.9 (± 1.3) d. Year strongly influenced a clone's dichogamy value ($F = 6.0$, $p = 0.004$), whereas genotype had no influence. The mean overall phenological synchronicity for the 3 years of observations was 0.30 ± 0.01 or about 30% overlap between the time when females were receptive and males were shedding pollen. This study represents the first effort to quantify phenology in an artificial population of northern red oak, and it provides a snapshot of the current relationship between temperature, phenology, and floral synchronization.

Key words: Quercus, seed orchard, genetic management, phenology.

Résumé : Nous avons développé un nouveau système de pointage pour évaluer la phénologie printanière dans un verger à graines clonal de chêne rouge (*Quercus rubra* L.). Le système a été utilisé pour noter entre 304 et 364 ramets pendant trois saisons de reproduction et assigner les clones à un groupe phénologique, soit hâtif, intermédiaire ou tardif. Même si le nombre absolu de clones dans chaque groupe phénologique changeait d'année en année, l'ordre dans lequel les clones fleurissaient est demeuré très stable ($r_s = 0{,}67$, $p < 0{,}001$). Les clones à floraison hâtive ont fleuri significativement plus longtemps que les clones à floraison plus tardive durant les trois années. La dichogamie était présente dans le verger alors que les fleurs mâles d'un clone apparaissaient entre 1,4 et 3,0 jours plus tôt que les fleurs femelles. La valeur moyenne de dichogamie des clones individuels variait de 0,0 à 4,9 ± 1,3 jours. L'année avait une grande influence sur la valeur de dichogamie des clones ($F = 6{,}0$, $p = 0{,}004$) tandis que le génotype n'avait aucune influence. La valeur moyenne du synchronisme phénologique pour l'ensemble des trois années atteignait 0,30 ± 0,01, soit environ 30 % de recouvrement entre toutes les fleurs femelles réceptives pendant que les fleurs mâles émettaient du pollen. Cette étude constitue la première tentative pour quantifier la phénologie dans un peuplement artificiel de chêne rouge et fournit un instantané de la relation actuelle entre la température, la phénologie et la synchronisation florale. [Traduit par la Rédaction]

Mots-clés : Quercus, verger à graines, gestion des ressources génétiques, phénologie, changement climatique.

1. Introduction

The primary objective of a seed orchard is to provide high-quality seed for reforestation. To help ensure maximum seed crop diversity and genetic efficiency, seed orchards are usually designed to facilitate panmixis and the minimization of self-fertilization. Studies, mostly on conifers, have shown, however, that seed orchards are rarely ideal panmictic populations. Phenology is often the single most important influence on outcrossing patterns in seed orchards (Slavov et al. 2005; Burczyk and Prat 1997; Eriksson and Adams 1989; El Kassaby et al. 1988). Phenology affects the frequency of gene exchange among clones and the genetic composition of the seeds derived from the seed orchard (Kang and Lindgren 1998).

Phenological differences between families or clones are often visually apparent; however, there are few studies quantifying the timing of bud break, leafing out, male and female flowering, and pollen shed in seed orchards (Askew and Blush 1990; Zas et al. 2003). Often, phenological data are presented qualitatively in the form of a phenogram, where the proportion of flowers in a given phenological stage is represented by bands on a timeline. Askew and Blush (1990) proposed the phenological overlap index, PO_{ij}, as a quantitative measure of the proportional symmetry of male and female phenograms. The index PO_{ij} for two comparison trees is the ratio of the common area to the maximum area between the male and female phenograms summed across all days. A SAS program became available in 2003 (Zas et al. 2003) that uses raw phenological scores to draw phenograms and calculate phenological synchronization indices.

Northern red oak (*Quercus rubra* L.) is monoecious, dichogamous, wind pollinated, and self-incompatible (Cecich 1993; Wiersma 2003). Staminate flowers are borne in catkins that develop from buds in the axils of the previous year's growth and

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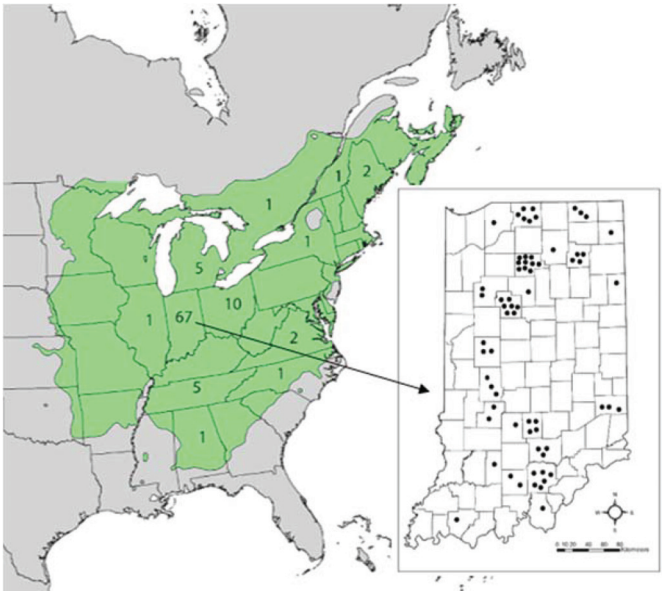
L.W. Alexander.* Department of Forestry and Natural Resources, Purdue University, 715 W. State Street, Pfendler Hall, West Lafayette, IN 47907, USA.
K.E. Woeste. Department of Forestry and Natural Resources, Purdue University, 715 W. State Street, Pfendler Hall, West Lafayette, IN 47907, USA; USDA Forest Service Northern Research Station, Hardwood Tree Improvement and Regeneration Center, Purdue University, 715 W. State Street, Pfendler Hall, West Lafayette, IN 47907, USA.
Corresponding author: Lisa W. Alexander (emails: Lisa.Alexander@ars.usda.gov, lworthen@purdue.edu).
*Present address: USDA Agricultural Research Service, U.S. National Arboretum, 472 Cadillac Lane, McMinnville, TN 37110, USA.

Fig. 1. Geographic source of 89 northern red oak ortets grafted into the Vallonia Seed Orchard. Shaded area shows the range of northern red oak. Numbers represent the number of ortets located in a specific state. Inset panel of Indiana shows the number of ortets from each county; dots do not correspond to a specific location within a county. Figure is provided in colour online.



emerge before or at the same time as the current leaves. As the catkins elongate and mature, the pistillate flowers emerge in the axils of expanding leaves of the new shoot. The pistillate flowers are solitary or occur in two- to many-flowered clusters. Pollen shed begins 1–2 weeks after the appearance of catkins and continues for 3–4 d. Cecich and Haenchen (1995) concluded that pistillate flowers were receptive for no more than 1 week based on the flexibility and bright red coloration of the stigmas. The fruit, an acorn or nut that occurs singly or in clusters of two to five, is partially enclosed by a scaly cup, and it matures in 2 years.

Little information exists on the floral phenology of northern red oak, so in this study, we had four objectives: (*i*) create a scoring system representing phenological stages and associated pollen shedding or female receptivity for *Q. rubra* male and female flowers, (*ii*) determine the degree of dichogamy in the seed orchard population, (*iii*) determine relative influences of genetics and environment on the variation in phenology and dichogamy, and (*iv*) quantify the phenological synchronization within the seed orchard. This will constitute one of only a handful of studies quantifying floral phenology in an artificial population of hardwood trees and will provide a snapshot of the current relationship between phenology, temperature, and provenance in the midst of a warming climate.

2. Materials and methods

A northern red oak seed orchard at the Indiana State Tree Nursery in Vallonia, Indiana (38.847, −86.098), served as the study population. The Vallonia Seed Orchard (VSO) was established between 1986 and 1988 using grafts from trees selected across the native range of northern red oak (Fig. 1). Scion wood was collected from visually favorable trees in wild populations, plantations, or from trees in visually favorable stands and grafted to seedling root stocks of northern red oak. Healthy trees with relatively long, straight, unbranched boles and a large stem diameter were considered visually favorable. The grafts (ramets) are genetically identical clones of the individual tree (ortet) from which they came. The seed orchard was designed be a heterogeneous, unstructured population because the ramets were copies of genotypes from many provenances and stands throughout eastern North America.

The VSO consists of five sublines, or smaller orchards, separated by between 150 and 1100 m. Each subline contains a unique set of genotypes. The orchard was designed to minimize differences in ortet origin location between sublines by distributing multiple genotypes from a single location across all sublines. For example, if scion wood from five genotypes was collected at a single stand, each genotype would be replicated in a different subline. At the time of establishment, each subline contained 30 clones with 8 ramets·clone^{-1} for a total of 240 trees·subline^{-1} or 1200 total trees in the orchard. Initial spacing was 8 m × 8 m. High mortality occurred due to delayed graft incompatibility, so at the time of evaluation, each subline contained from 19 to 27 clones with a mean of 4.6 ± 1.7 ramets·clone^{-1}.

2.1. Phenology data collection

A rating system for phenology of northern red oak was developed in 2007 by observing the phenological phases of trees in the seed orchard. The length of the reproductive period and each phenological stage of a clone were used to derive a numerical rating system for male and female floral phenology. Male flowers (recorded for all ramets) were rated on a 1 to 8 scale as follows: 1, bud break; 2, new shoots emergent; 3, compact catkins visible; 4, catkins pendulant; 5, catkins fully extended, 5% pollen shed; 6, 40% pollen shed; 7, 100% pollen shed; 8, after pollen shed (Fig. 2). Female flowers on selected ramets were rated on a 1 to 4 scale as follows: 1, flower visible; 2, pistils emergent, light red, straight, 10% receptive; 3, pistils halfway to full length, dark red, recurved, 100% receptive; 4, pistils fully emerged, black, brittle, 5% receptive (Fig. 3). Hourly weather data was collected from the Brownstown, Indiana, national weather station (MADIS A1114) located 6.4 km from the seed orchard.

Phenology data was collected in the spring of 2008, 2009, and 2010. Trees ranged in height from 4.2 to 26 m. All variables were assessed in the top 50% of the tree crown. Trees were observed beginning on or near 1 April each year, and phenology data collection began when the earliest individual(s) reached bud break stage. Phenology data were collected every 2–3 d in 2008 and 2009 and every 4 d in 2010. Data collection continued until the phenologically latest individual(s) completed pollen shed. Julian dates for bud break, beginning, peak, and ending pollen shed dates, and leaf out date were collected for each tree.

Bud break was recorded on an individual when the scales covering the leaf bud were visibly opened, just before the new shoot began growth. Onset of pollen shed was recorded when observed catkins had at least one pollen sac opened with extended anthers; peak pollen shed was when observed catkins had 75%–100% of their pollen sacs open, and the end of pollen shed was when catkins were dark brown, dry, and no longer dehiscing pollen. Leaf out was recorded at the appearance of the first fully expanded green leaves. The rating system developed for this project was used to score bud break and leaf out for an average of 99 clones and 329 ramets each year (Table 1).

Male flowers were scored from the ground using binoculars and female flowers were assessed by bending down branches, climbing trees, or from an aerial lift. The beginning, end, and duration of male and female flowering was determined for an average of 90 clones and 251 ramets (males) and 86 clones and 238 ramets (females) each year (Table 1). Clones were grouped into early, middle, and late phenological classes based on the average timing of beginning pollen shed of their ramets. Early (E) clones began pollen shed within 7 d of the earliest clone, late (L) clones ended pollen shed within 7 d of the latest clone, and all other clones were classified as intermediate (M).

Fig. 2. Male floral phenological stages and associated rates of pollen shed for northern red oak trees in Indiana: 1, bud break; 2, new shoots emergent; 3, compact catkins visible; 4, catkins pendulant; 5, catkins fully extended, 5% pollen shed; 6, 40% pollen shed; 7, 100% pollen shed; 8, after pollen shed. Figure is provided in colour online.

2.2. Data analysis

All data analysis was performed using SAS statistical analysis software (SAS v. 9.3, Cary, North Carolina). Measurements describing the seed orchard population include standard deviations; all other means are presented with standard errors. We performed all analyses of variance using the GLM procedure. "Clone'" was nested within "subline" for all analysis of variances (ANOVAs), as clones are not repeated across sublines. Separate ANOVAs for leaf out and bud break were performed for each year; ANOVA models included "subline", "clone(subline)", and "ramet(clone)". Within-year clonal heritability (H^2), or repeatability, for bud break and leaf out was calculated using Type III mean squares as follows: $H^2 = MS_{clone}/MS_{total}$. The ANOVA model for dichogamy included "year", "subline", "clone(subline)", and "ramet(clone)". Separate ANOVAs for clonal phenological overlap (PO) values were performed each year for male and female flowers; the ANOVA models contained the single-factor "phenology group". Least-squared means were compared using Tukey's studentized range distribution at the $\alpha = 0.05$ level of significance.

Spearman rank correlation was performed using PROC CORR to assess the stability of clonal ranks for leaf out over the three study years. Latitude of ortet origin (based on collection records) was regressed on mean leaf out date, pollen shed onset date, and female flowering onset date for each clone to determine the relationship between ortet location and leaf out date of seed orchard clones. We performed a χ^2 test using PROC FREQ with the "chisq" option to determine the relationship between year and distribution of clones into phenology groups.

Floral phenology was analyzed using SYNCHRO, a SAS program for the analysis of floral phenology in seed orchards (Zas et al. 2003). SYNCHRO was used to generate male and female phenograms based on input from the floral phenology scoring system and to calculate orchard-wide floral synchronicity. Floral synchronicity was also calculated for each pair of clones in the seed orchard where both male and female flowering data were available. Synchronicity was quantified using the PO_{ij} index (Askew and Blush 1990). PO_{ij} uses the proportional symmetry between male and female phenograms to determine the number of days where clone *j* is receptive while clone *i* is shedding pollen for every *ij* pair where both male and female flowering data are available. We calculated PO_{mean}, PO_{min}, and PO_{max} for each clone in the seed orchard. PO_{mean} represents the mean overlap of a given clone's pollen shedding period with all other clones' receptive female flowers. Thus, the mean of PO_{mean} for all clones represents the orchard-wide synchronicity in a given year. PO_{min} and PO_{max} represent the smallest and largest observed overlap, respectively, between a pair of clones in a given year.

3. Results

3.1. Bud break and leaf out

The onset of bud break in the seed orchard occurred between Julian days 85 and 120 and averaged about day 106 (15 April) across the study years (Table 2). The onset and ending of leaf out occurred about 15–16 d after bud break, beginning on day 101 and ending on day 135. On average, leaf out was about 11 d later than bud break (Table 2). For each study year, the variation of bud break and leaf out date was significantly influenced by subline location and genotype. ANOVA models containing subline, clone nested within subline, and ramet nested within clone explained from 84% to 88% of the variation in bud break (Table 3) and from 78% to 81% of the variation in leaf out date (Table 4) each year. Within-year clone mean heritabilities for bud break and leaf out averaged 0.25 and 0.43, respectively.

The Spearman rank correlation for clonal leaf out date over the three study years averaged 0.67, indicating a moderate association between the ranks of clonal leaf out between years. The regression of the latitude of ortet origin onto the mean Julian day of leaf out

Fig. 3. Female phenological stages and associated rates of stigmatic receptivity for northern red oak trees in Indiana: 1, flower visible; 2, pistils emergent, light red, straight, 10% receptive; 3, pistils halfway to full length, dark red, recurved, 100% receptive; 4, pistils fully emerged, black, brittle, 5% receptive. Figure is provided in colour online.

(across years) was not significant. When the same regression was performed using only clones representing ortets from natural stands, however, the result was marginally significant ($p < 0.03$), although the explained variation was low ($R^2 = 0.18$). Clones tended to break bud and leaf out later with increasing latitude of ortet origin. Relationships between latitude and pollen shed onset and latitude and female flower receptivity onset were not significant.

Daily temperatures averaged 13.7 °C and 18.3 °C for the onset of bud break and leaf out over the 3 years, respectively (Table 2). There were no severe frosts during the three observed reproductive periods. One-factor ANOVA showed that the beginning of the reproductive period in 2010 was significantly warmer than the same period in 2008 and 2009 ($F = 23.42$, $p < 0.001$). By the end of the reproductive period, temperatures among years were not significantly different ($F = 1.76$, $p = 0.184$).

3.2. Pollen shed

The mean onset of pollen shed was on Julian day 109 or about 4 d after bud break (Table 5). On average, the earliest clones started shedding pollen on Julian day 103, whereas the latest clones started shedding on Julian day 114. Variation among clones resulted in a spread of 13–17 d in the onset of pollen shedding. Similarly, the earliest clones, on average, ended pollen shedding about 11 d before the latest clones, i.e., on day 114, compared with day 125 for the latest clones. The earliest clones also had a greater spread of days, 18 (day 103 to day 121), in completing pollen shed compared with the spread for the latest clones, 8 d (day 120 to 128). A regression of date of pollen shed onset against pollen shed duration shows that early-blooming clones shed pollen significantly longer than later shedding clones in all 3 years of the study (Fig. 4).

Table 1. Flowering observations in 2008, 2009, and 2010 at the Vallonia northern red oak clonal seed orchard.

Subline	Ramets (*n*)	Clones (*n*)	Trees that flowered (*n*)	Clones that flowered (*n*)
2008				
A	84	26	69	26
B	50	20	42	17
C	108	29	89	29
E	122	29	108	29
Total	364	104	308	101
2009				
A	79	25	72	24
B	50	20	50	20
C	87	27	82	27
E	104	25	75	24
Total	320	97	279	95
2010				
A	77	25	71	23
B	45	20	37	18
C	84	27	55	22
E	98	25	45	21
Total	304	97	208	84

Note: The orchard consisted of five spatially separated blocks or sublines (A–E). Subline D was distant from the others and was not included in the study.

Table 2. Mean, minimum, and maximum values for the date of onset of bud break and the onset of leaf out in a northern red oak clonal seed orchard in 2008, 2009, and 2010.

Phenology point	Temperature (°C)	*N*	Mean	SEM	Minimum	Maximum
2008						
Bud break	13.4	364	109.8	0.3	100	120
Leaf out	16.7	364	120.7	0.3	109	135
2009						
Bud break	12.4	320	113.3	0.3	101	117
Leaf out	19.9	320	120.3	0.3	115	128
2010						
Bud break	15.0	304	93.6	0.3	85	102
Leaf out	18.4	304	107.2	0.2	101	113

Note: *N*, number of ramets scored. SEM, standard error of the mean; temperature, average daily temperature on mean phenology point date. Values are Julian dates.

Table 3. Nested analysis of variance for bud break in a northern red oak seed orchard in 2008 and 2009.

Source	df	Type III SS	Mean square	*F* value	Pr > *F*	H^2
2008 (R^2 = 0.88)						
Subline	3	218.4	72.8	33.6	<.0001	0.22
Clone (subline)	103	2328.1	22.6	10.4	<.0001	
Ramet (clone)	177	1378.9	7.8			
2009 (R^2 = 0.84)						
Subline	3	142.1	47.4	19.8	<.0001	0.27
Clone (subline)	96	1727.4	18.0	7.5	<.0001	
Ramet (clone)	210	501.9	2.4			

Note: Bud break data were not captured in 2010. Within-year clonal heritabilities were calculated as $H^2 = MS_{clone}/MS_{total}$. df, degrees of freedom.

3.3. Female flowers

The mean onset of female flower receptivity occurred on Julian day 112, which was nearly midway through the period during which pollen was shed (Table 5). Again, clonal variation was observed in the mean onset of flower receptivity, with the average Julian day of 107, 113, and 117 for E, M, and L clones. The mean end of female flower receptivity occurred on Julian day 117, 124, and 126 for E, M, and L clones, respectively. Thus, the total length of female flower receptivity averaged 10.5 d for E and M clones, and 8.4 d for L clones.

Table 4. Nested analysis of variance for leaf out in a northern red oak seed orchard 2008–2010.

Source	df	Type III SS	Mean square	*F* value	Pr > *F*	H^2
2008 (R^2 = 0.78)						
Subline	3	45.2	15.1	1.9	0.13	0.70
Clone (subline)	103	5060.2	52.7	6.63	<.0001	
Ramet (clone)	177	1406.8	8.0			
2009 (R^2 = 0.80)						
Subline	3	61.2	20.4	5.4	0.003	0.49
Clone (subline)	96	1119.6	23.3	6.16	<.0001	
Ramet (clone)	210	287.6	3.8			
2010 (R^2 = 0.81)						
Subline	3	739.8	246.6	62.8	<.0001	0.10
Clone (subline)	96	2694.1	27.8	7.07	<.0001	
Ramet (clone)	213	836.4	3.9			

Note: Within-year clonal heritabilities were calculated as $H^2 = MS_{clone}/MS_{total}$. df, degrees of freedom.

Table 5. Yearly mean flowering onset dates and dichogamy values in a northern red oak seed orchard in Indiana, USA.

Year	Pollen shed onset (Julian day of year)	Female receptivity onset (Julian day of year)	Dichogamy (95% CL)
2008	113.6±0.4	116.6±0.3	3.0 (2.2, 3.7) a
2009	115.9±0.3	117.7±0.2	1.8 (1.3, 2.3) ab
2010	100.9±0.2	102.3±0.3	1.4 (0.9, 1.9) b

Note: Dichogamy was calculated as the difference in number of days between onset of pollen shed and onset of female flower receptivity for a single ramet. Clonal dichogamy values are means over all ramets of a clone. Values are given as mean ± standard error. Yearly mean dichogamy values with different lowercase letters are significantly different at the 0.05 level. CL, confidence limits.

3.3. Dichogamy

Mean pollen shed onset occurred on day 114, 116, and 101 in 2008, 2009, and 2010, respectively, whereas onset of female receptivity in the same years occurred on day 117, 118, and 102 (Table 5). The difference between the within year means represents a quantification of dichogamy in the seed orchard. Differences between mean day of onset of pollen shed and mean day of onset of female receptivity ranged from 1.4 to 3.0 d, a two-fold difference between years. Mean dichogamy values for individual clones ranged between 0.0 and 4.86 (± 1.26) d. An ANOVA containing year, subline, and clone (R^2 = 0.62) showed that year strongly influenced a clone's dichogamy value (F = 6.0, p = 0.004) and clonal genotype had no influence (F = 1.2, p = 0.26; Table 6). There was no significant relationship between a clone's phenological class (E, M, or L) and the mean or variance of its dichogamy.

3.4. Phenological synchronization

An average of 11.3, 64.0, and 17.7 clones were in the E, M, and L phenological classes, respectively, each year (Table 7). In 2008 and 2009, the percentage of clones in the three phenology classes was quite similar; an average of 69% of the clones was in the M phenology class, followed by 25% in the L class, and 8% in the E class. In 2010, however, the percentage of clones in the E class increased to 22%, whereas the percentage of clones in the L class decreased to about 10%. These differences across years were reflected in the significant association between year and distribution of clones across the phenology groups (χ^2 = 16.53, p < 0.005).

Phenological synchronicity averaged 0.29, indicating a 29% overlap in the time of female flower receptivity and pollen shedding by male flowers (Fig. 5). Synchronization was remarkably

Fig. 4. Date of pollen shed onset versus duration of pollen shed in a northern red oak clonal seed orchard for (*a*) 101 clones in 2008, (*b*) 95 clones in 2009, and (*c*) 83 clones in 2010. Pollen shed data was collected every 2–3 d in 2008 and 2009 and every 4 d in 2010. Values for a clone represent the mean of its ramets. Each square corresponds to multiple clones with the same value.

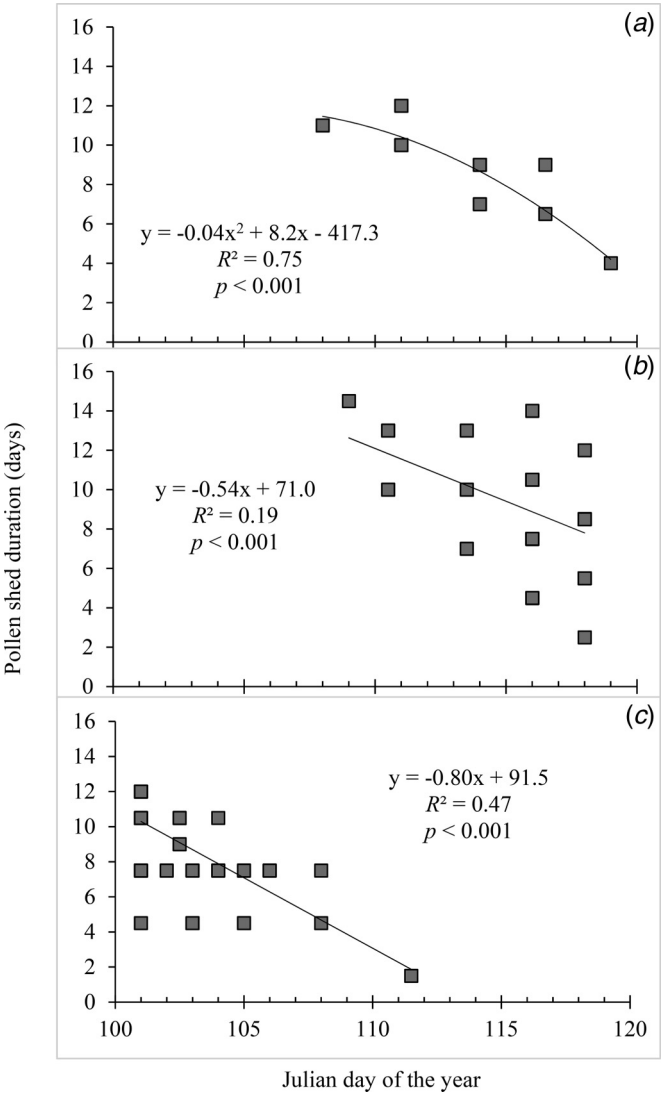


Table 6. ANOVA for dichogamy in a northern red oak seed orchard in Indiana, USA.

Source	df	Type III SS	Mean square	*F* value	Pr > *F*
Year	2	78.7	39.4	6.0	0.0036
Subline	3	15.9	47.7	2.4	0.12
Clone (subline)	83	624.4	7.5	1.2	0.26
Year × subline	4	70.1	17.5	2.7	0.037
Ramet (clone)	84	548.0	6.5		

Note: 84 clones had > 1 ramet with both male and female flowers in all three study years (2008, 2009, and 2010). Dichogamy was calculated as the difference in number of days between onset of pollen shed and onset of female flower receptivity for a single ramet. Clonal dichogamy values are means over all ramets of a clone. df, degrees of freedom.

Table 7. Number (percentage) of northern red oak clones placed in early, intermediate, and late phenological groups over 3 years.

		Phenological group		
Year	*N*	Early	Intermediate	Late
2008	101	9 (8.9)	73 (72.3)	19 (18.8)
2009	95	7 (7.4)	62 (65.3)	26 (27.4)
2010	83	18 (21.7)	57 (68.7)	8 (9.6)
Mean	93	11.3 (12.7)	64 (68.8)	17.6 (21.3)

Note: Clones were grouped by date of first pollen shed. *N*, number of flowering clones. There was a significant association between year and distribution of clones across the phenology groups ($\chi^2 = 16.53$, $p < 0.005$).

stable from year to year (Table 8). The range of PO_{min} values for male and female flowers included zero each year. A $PO_{min} = 0$ indicates that at least one clone had no overlap with any other clone in a given year. The mean PO_{max} was significantly greater for male flowers than female flowers ($F = 3.25$, $p = 0.0415$), indicating that more female flowers were available when males were at peak pollen shed than males were available when females were at peak receptivity. In all years, there was an inverse relationship between PO_{mean} as a female and PO_{mean} as a male; clones with a high PO_{mean} as a male had a lower PO_{mean} as a female.

For both male and female flowers, clones in E, M, and L phenology classes differed significantly in PO averages in nearly all years (Table 9). Specifically, for female flowers, the E phenology group had the highest PO means, followed by the M group, and then the L group. For the male flowers, the opposite trend was observed (Table 9). The PO patterns in 2010 were unlike the other study years. Males in the E phenology group had significantly higher PO in 2010 than those in 2008 or 2009, and males in the L phenology class had a strikingly lower mean PO value in 2010 relative to the other study years.

4. Discussion

We developed a numerical rating system for male and female floral phenology that was simple to use in the field, fast to input in any spreadsheet program, and compatible with the allowed input ranges of the selected phenological analysis software. By using this system, we determined that in the VSO, year influenced leaf out date more than subline location or genotype, indicating that environmental factors at the flowering time strongly regulated absolute flushing date. In all 3 years, the same two sublines flushed earlier than the remaining two, indicating that these microsites may experience favorable temperatures in the spring. It is unlikely that differences in flushing date among sublines were caused by nonrandom allocation of clones, as efforts were made to randomize clones within sublines at the time of planting. The importance of genetic differences among the clones for date of leaf out was reflected in the significance of genotype in the ANOVA model, the moderate within-year clonal heritability (i.e., repeatability), and by the constancy among years of the order in which the clones leafed out within years. These findings are in agreement with studies that found genotype and population ranks for timing of bud break were stable from year to year in *Quercus petraea* (Matt.) Liebl. (Ducousso et al. 1996; Vitasse et al. 2009*a*). Studies of genetic variation in phenology have shown moderate to high heritabilities (from 0.16 to 0.70) for bud break and bud set (Howe et al. 2003).

On average, pollen shed occurred over 22 d, corresponding closely with observations in wild populations of *Quercus alba* L. and *Quercus velutina* Lam. in Missouri (Cecich and Haenchen 1995). Cecich (1993) stated that individual *Q. alba* trees release pollen for 3–4 d; we found that the mean duration of pollen shed for northern red oak clones was between 3 and 11 d. Similarly, we found that an individual tree may have receptive female flowers for

Fig. 5. Overall phenological synchronicity for each year showing the mean pollen shedding rate and mean female receptivity rate among all northern red oak clones across the reproductive period.

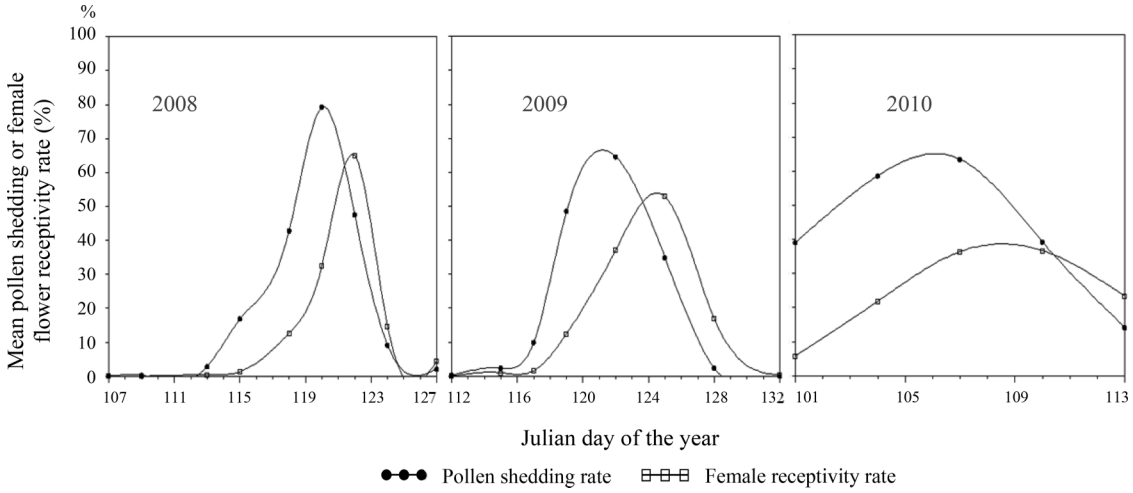


Table 8. Phenological synchronicity in a northern red oak seed orchard in Vallonia, Indiana, USA, for 3 years for male and female flowers.

	PO_{mean}			PO_{min}			PO_{max}		
Year	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Male flowers									
2008	0.05	0.51	0.30±0.02	0	0.04	0.013±0.002	0.36	0.94	0.74±0.02
2009	0.06	0.48	0.30±0.02	0	0.13	0.049±0.003	0.41	0.97	0.74±0.01
2010	0.03	0.39	0.27±0.01	0	0.07	0.017±0.002	0.31	0.98	0.74±0.01
Female flowers									
2008	0.04	0.52	0.30±0.02	0	0.10	0.024±0.002	0.15	0.94	0.68±0.02
2009	0.06	0.49	0.30±0.01	0	0.19	0.032±0.004	0.21	0.97	0.68±0.02
2010	0.02	0.50	0.27±0.02	0	0.03	0.063±0.001	0.09	0.98	0.60±0.02

Note: Means are followed by standard errors. PO_{ij} uses the proportional symmetry between male and female phenograms to determine the number of days where clone *j* is receptive while clone *i* is shedding pollen; values for each year are means of all seed orchard clones. PO_{min} is the smallest overlap observed for an *ij* pair, and PO_{max} is the largest overlap observed for a pair of clones.

Table 9. Mean phenological overlap (PO) values for female and male northern red oak flowers in three phenology classes over 3 years.

	2008		2009		2010	
Phenology group	PO_{mean}	SEM	PO_{mean}	SEM	PO_{mean}	SEM
Male flowers						
Early	0.16b	0.03	0.15c	0.03	0.21b	0.03
Intermediate	0.31a	0.03	0.30b	0.02	0.28a	0.01
Late	0.36a	0.03	0.41a	0.02	0.29a	0.02
Female flowers						
Early	0.45a	0.02	0.40a	0.02	0.36a	0.03
Intermediate	0.29b	0.02	0.32b	0.01	0.30b	0.02
Late	0.21c	0.03	0.19c	0.02	0.17c	0.03

Note: Means within year and sex with different lowercase letters are significantly different at the 0.05 level. SEM, standard error of the mean.

8.5–10.5 d, which is a much longer period than previously reported for other oaks (Cecich and Haenchen 1995).

Clones in the E phenology class shed pollen longer than late-blooming clones. A regression of date of pollen shed onset against pollen shed duration showed that early-blooming clones shed pollen significantly longer than late-shedding clones in all 3 years of the study. These results indicated that the initiation and duration of flowering were influenced by weather conditions. We observed that late-blooming clones, however, often experienced daytime high temperatures >18 °C soon after the first (topmost and distal) flower buds opened. When this occurred, lower and more proximal flower buds either did not open or catkins emerged and abscised before maturity. Other oak species have displayed similar responses. A high daily maximum temperature had a hastening effect on the emergence of staminate and pistillate *Q. alba* flowers (Sharp and Chrisman 1961; Wolgast and Stout 1977) but a negative effect on *Q. alba* and *Q. velutina* flower survival (Cecich and Sullivan 1999).

The results of our regression analysis indicated a weak but significant association between latitude and flushing date ($R^2 = 0.183$, $p < 0.029$). Clones representing ortets of more northerly origins flushed later than clones representing ortets of more southerly origins when only clones from natural populations were considered. Other studies of latitudinal clines in oak species based on common-garden experiments have reported conflicting results. Some researchers found a clinal pattern of variation in bud break where trees from stands in the northern part of a species' range broke bud earlier than trees from more southerly stands (Daubree and Kremer 1993; Kriebel et al. 1976). Conversely, Gall and Taft (1973) and Ducousso et al. (1996) found that trees from populations from higher latitudes broke bud later than trees from populations from southern latitudes. Reasons for conflicting information may include the latitudinal range represented in each study and the species studied (Ducousso et al. 1996). In this study, we used grafted ramets (clones) of naturally occurring trees rather than genetically variable seedlings. Thus, each clone had a relatively narrow phenotypic variance for any phenological trait. In contrast, seedling phenotypic variances are expected to be much higher than those for clones, resulting in less precise family or population means.

High PO is desirable for successful seed production in an orchard, assuming other barriers are minimal. Our analyses showed that PO was remarkably stable across years, even though weather conditions (as indicated by temperature) differed and clones were distributed across different phenological groups. Moreover, orchard-wide phenological synchronization revealed that about 30% of all pollen was shed during receptivity of the female flowers. Somewhat higher POs were found in an orchard of 10 *Pinus pinaster* Aiton clones (PO_{ij} = 0.48) (Zas et al. 2003) and in a *Pinus sylvestris* L. orchard (PO_{ij} = 0.41) (Burczyk and Prat 1997). Because dichogamy lowers synchronicity values, in our study, the low PO of early-blooming males and later blooming females likely resulted in lower orchard-wide PO compared with those studies.

In addition to the need for PO in an orchard, a manager is also interested in having the seed produced result from outcrossing, not inbreeding. In this study of northern red oak, there were different POs for early- and late-flowering clones, and the trend was not the same for male and female flowers. Practically, this means that trees cannot have high PO for both male and female flowers. Because female flowers mature about 1–3 d later than male flowers on the same tree, pollen from late-blooming trees is more likely to pollinate female flowers on a different tree. It has been suggested that a gametophytic self-compatibility system most likely exists in *Quercus* (Wiersma 2003), so the protandry we observed may promote outcrossing by keeping stigmatic surfaces clear of self-pollen. However, some clones had a dichogamy value of 0, indicating that dichogamy is a weak barrier to self-pollination. Further, the strong environmental influence on flowering indicates that dichogamy values may be altered by a changing climate, so that late-blooming individuals may have no dichogamy and very little chance to pollinate female flowers or to receive pollen.

Oak phenology has been shown to be especially sensitive to temperature increases when compared to other wide-ranging temperate species (Vitasse et al. 2009*b*), indicating that current oak ranges and patterns of long-distance pollen dispersal may be affected by a warming climate (Vitasse et al. 2011, 2009*b*; Moran and Clark 2012). Clear records related to phenology will help future researchers measure climate shifts. The phenology data presented here can be combined with existing meteorological data to evaluate effects of winter temperatures on budburst relative to proximal (spring) temperatures.

The rating system proposed here was useful for assessing spring phenology in northern red oak. It provides a quantitative approach for characterizing male and female flower phenology, including dichogamy and phenological synchronization. Because it is easy to use, we think that this rating system and the analysis software of Zas et al. (2003) could be widely adopted by red oak seed orchard managers to facilitate management, add to basic understanding of red oak phenology, and permit comparisons among seed orchards in space and time. The quantification of phenological synchronization is also fundamental in making decisions regarding orchard rouging, supplemental mass pollination, or controlled pollinations within an orchard managed to attain high levels of genetic diversity in seed orchard progeny.

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