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Shifts in pollen release envelope differ between genera with non-uniform climate change

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PREMISE OF THE STUDY: Plant phenological responses to climate change now constitute one of the best studied areas of the ecological impacts of climate change. Flowering time responses to climate change of wind-pollinated species have, however, been less well studied. A novel source of flowering time data for wind-pollinated species is allergen monitoring records.

METHODS: We studied the male flowering time response to climatic variables of two wind-pollinated genera, *Betula* (Betulaceae) and *Populus* (Salicaceae), using pollen count records over a 17-year period.

KEY RESULTS: We found that changes in the pollen release envelope differed between the two genera. Over the study period, the only month with a significant rise in temperature was April, resulting in the duration of pollen release of the April-flowering *Populus* to shorten and the start and peak of the May-flowering *Betula* to advance. The quantity of pollen released by *Betula* has increased and was related to increases in the previous year's August precipitation, while the quantity of pollen released by *Populus* has not changed and was related to the previous year's summer and autumn temperatures.

CONCLUSIONS: Our findings suggest that taxa differ in the reproductive consequences of environmental change. Differing shifts in phenology among species may be related to different rates of change in climatic variables in different months of the year. While our study only considers two genera, the results underscore the importance of understanding non-uniform intra-annual variation in climate when studying the ecological implications of climate change.

KEY WORDS Betulaceae; climatic change; flowering duration; flowering time; Ottawa, Ontario, Canada; phenology; pollen count; pollen limitation; reproductive success; Salicaceae.

Recent interest in phenological responses to climate change has resulted in substantial use of herbarium specimens and field observations to study the impacts of climate change on the timing of flowering, fruiting, and leaf out (Calinger et al., 2013; Everill et al., 2014; Panchen and Gorelick, 2017; Willis et al., 2017). The response of wind-pollinated species to climate change has been less well studied. Herbarium specimens of wind-pollinated species, although often collected with flower structures, are rarely collected when actually in anthesis (Munson and Long, 2017; Primack and Gallinat, 2017). In addition, detecting flowering times of wind-pollinated species in the field through observations, or determining whether a herbarium specimen is in flower, is more challenging than for showy animal-pollinated species. A novel approach to determining flowering times of wind-pollinated species is the use of pollen counts from allergen monitoring stations. The general findings of previous studies using these stations suggest earlier release of pollen and increased pollen levels in recent years (Emberlin et al., 1997, 2002; van Vliet et al., 2002; Ziska et al., 2011; Ziello et al., 2012; Newnham et al., 2013; Zhang et al., 2014). However, the focus of these phenology studies analysing pollen counts has been on allergens and human health impacts. Thus, there is a gap in our knowledge of the ecological implications of climate change with respect to pollen release timing.

The timing of flowering, and hence of pollen release, is often related to the accumulation of temperatures above a threshold (referred to as growing degree days or heat sum), such that warmer temperatures generally result in earlier or advanced flowering (Rathcke and Lacey, 1985; Bernier and Périlleux, 2005). Temperatures in the month or months just prior to the start of flowering generally have the strongest influence on the timing of flowering (Fitter et al., 1995; Primack et al., 2004; Panchen and Gorelick, 2017). The plant's flowering time sensitivity to temperature can be quantified in days per degree Celsius, where a negative value indicates an advance in flowering with warming temperatures. Precipitation can also have an effect on flowering time, although its effect is most pronounced in regions with wet–dry seasons (Rathcke and Lacey, 1985; Zhang et al., 2015; Matthews and Mazer, 2016; Munson and Long, 2017). Day length can also influence the time of flowering but temperature has the stronger influence for most non-tropical species (Rathcke and Lacey, 1985; Thórhallsdóttir, 1998; Keller and Körner, 2003; Bernier and Périlleux, 2005; Hülber et al., 2010). Warmer temperatures within the timeframe of flowering duration can also reduce the duration of flowering (Ellebjerg et al., 2008; Høye et al., 2013). Studies in Europe and the United States have found that in recent years the timing of pollen release of *Betula* L. and *Populus* L. has advanced, remained the same, or been delayed depending on the location, and that the timing of pollen release of these two genera is related to monthly temperatures before flowering or to growing degree days (Frei, 1998; Emberlin et al., 2002; van Vliet et al., 2002; Damialis et al., 2007; Newnham et al., 2013; Zhang et al., 2014, 2015).

Phenological sensitivity to temperature affects reproductive success, fitness and survival (Chuine, 2010), such that species abundance has been linked to phenological sensitivity to temperature where species that are less sensitive to temperature have become less abundant as a result of contemporary climate change (Willis et al., 2008; Cleland et al., 2012). Monoecious and dioecious wind-pollinated species have been shown to have flowering time sensitivities to temperature that differ between male and female flowers (Jones et al., 1997; Stenström and Jónsdóttir, 2004). Selection may therefore act differently on male and female flowering times as the climate warms. Plant species, including wind-pollinated species, often exhibit pollen limitation in the production of seed (Knight et al., 2005). Therefore, if pollen production increases with a warming climate (Ziello et al., 2012; Zhang et al., 2014), the potential for increased seed production in pollen-limited species will depend on the flowering time sensitivity of both male and female flowers.

As with herbarium specimens, the use of pollen counts in phenology–climate change studies utilise historical records for a purpose they were not originally collected for and introduces some challenges. A herbarium specimen in flower records a single flowering date for a species but we do not know at what point along the continuum of flowering duration this date represents and can introduce considerable variation into the analysis, particularly for species with long flowering durations (Miller-Rushing et al., 2008; Panchen et al., 2012; Panchen and Gorelick, 2017). In contrast, pollen counts can provide a very accurate record of the time of flowering, and in particular the time of anthesis. However, pollen counts do not differentiate between locally released pollen and pollen transported from locations potentially hundreds of kilometres away that was released by plants that may have flowered earlier or later than local plants (Estrella et al., 2006; Mahura et al., 2007; Siljamo et al., 2008; Karlsen et al., 2009; Varis et al., 2009). The amount and timing of long-distance pollen collected locally depends on climatic factors at the distant location and larger-scale atmospheric air movement that exhibits intra- and inter-annual variation. In addition, the morphology of pollen grains restricts identification to the level of genus or family. Thus, the pollen envelope for a genus or family (Fig. 1) consists of a series of peaks, where each peak could represent a different species (Frei, 1998) or potentially a long-distance pollen transport event. Since species tend to flower and leaf out in the same order across years (Panchen et al., 2014; Panchen and Gorelick, 2016), we can hypothesise that the species producing the first peak in the pollen envelope in one year will be the species producing the first peak in other years and similarly for subsequent peaks. The sensitivity of flowering time to temperature differs among species as well as intraspecifically across a species' range (Panchen et al., 2012; Calinger et al., 2013; Panchen and Gorelick, 2017; Prevéy et al., 2017).

Studies of pollen count trends analyse changes in start, peak, end and duration of the pollen envelope (Fig. 1; Damialis et al., 2007; Zhang et al., 2014; Jato et al., 2015). Pollen envelope parameters have been defined in a number of ways. For example, the start of flowering has been variously defined as the first grain detected (García-Mozo et al., 2006), when a cumulative total of 50 or 75 grains has been detected (van Vliet et al., 2002; Myszkowska, 2014), or when 1%, 2.5% or 5% of the annual cumulative pollen count has been detected (Emberlin et al., 1993; González-Parrado et al., 2014). Similarly, the end of flowering has been variously defined as when 95%, 97.5%, or 99% of the annual cumulative pollen count has been detected, and the duration of flowering as 1–99%, 2.5–97.5% or 5–95% of the annual cumulative pollen count. In this study, we use the 5% and 95% annual cumulative pollen count to define the start and end of flowering to reduce the effects of long-distance pollen transport and variation in start and end dates (Miller-Rushing et al., 2008; Zhang et al., 2015). The peak is usually defined as the day on which the maximum amount of pollen was

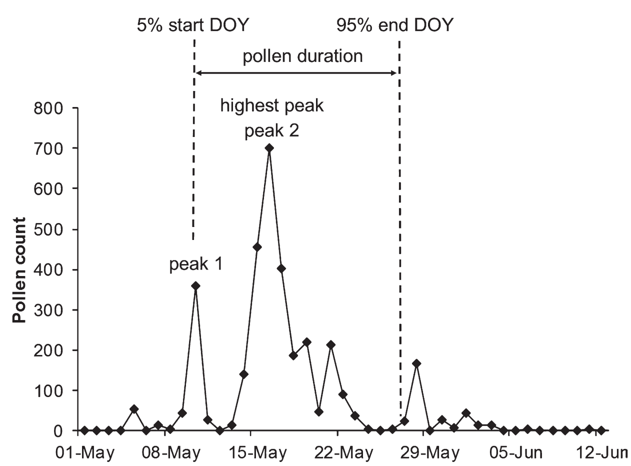

FIGURE 1. *Betula* pollen count in 2003, illustrating the pollen envelope parameters, where 5% start DOY is the day of the year on which 5% of the annual cumulative pollen count is reached; highest peak DOY is the day of the year on which the maximum amount of pollen is recorded; peak 1 DOY and peak 2 DOY are the first two pollen count peaks; 95% end DOY is the day of the year on which 95% of the annual cumulative pollen count is reached; and pollen duration is 95% end DOY minus 5% start DOY. The highest peak DOY can coincide with peak 1, peak 2 or a subsequent peak.

detected but, for the reasons described above, this may represent a different species in different years. The annual cumulative pollen count (Ziello et al., 2012) and the highest daily pollen count (Frei, 1998) are two further pollen envelope parameters of particular interest in allergen studies.

Here, we take an in-depth look at the pollen release phenology of two wind-pollinated genera, *Populus* and *Betula*, from Ottawa, Ontario, Canada, over a 17-year period to compare and contrast the climate related responses in pollen production of these two genera. Our overall objective was to better understand potential ecological implications of climate change in wind-pollinated species. In Ottawa, *Populus* typically flowers in April, while *Betula* typically flowers in May. The spring season for wind-pollinated species, where flowering occurs before leaf out (Newnham et al., 2013), ranges from April to June in Ottawa (Panchen et al., 2014). Thus, we can compare early- vs. mid-spring flowering genera. Specifically, we asked: (1) Have the pollen envelope parameters of the two genera shifted over time? and (2) How sensitive to climatic variability are the pollen envelope parameters of each genus?

MATERIALS AND METHODS

Study genera and site

Daily pollen counts for the genera *Betula* and *Populus* for 17 yr (March–November, 1995–2011) in Ottawa (45°N, 76°W) were obtained from Aerobiology Research Laboratories, Ottawa (Aerobiology, 2018). *Betula* and *Populus* were chosen for this study because the pollen counts were high in comparison to other spring-flowering genera whose pollen counts were only trace amounts or very low. Species of *Betula* and *Populus* found in the Ottawa area and throughout Ontario, Quebec and New York (i.e., within several hundred kilometers of Ottawa) are *Betula alleghaniensis*, *B. papyrifera*, *B. populifolia* and *Populus balsamifera*, *P. deltoides*, *P. grandidentata* and *P. tremuloides* (Gleason and Cronquist, 1991; USDA, 2018). Pollen was collected using Aerobiology's aeroallergen rotation impaction sampler. For each year, the daily counts were used to calculate the following pollen envelope parameters for each genus (Fig. 1): the day on which 5% of the cumulative annual pollen was reached (5% start day of year [DOY]); the day on which the maximum amount of pollen was recorded (highest peak DOY); the first two pollen count peaks (peak 1 DOY and peak 2 DOY) where the pollen count was at least twice the count of the previous or following three days; the day on which 95% of the annual cumulative pollen count was reached (95% end DOY); the 5–95% pollen duration (95% end DOY minus 5% start DOY); annual cumulative pollen count; and highest peak pollen count. Monthly mean temperatures, monthly total rainfall, monthly mean wind speed and daily maximum wind speed (1994–2011) at the McDonald-Cartier International Airport, Ottawa, weather station were obtained from Environment Canada's Historical Climate Data records (Environment Canada, 2017).

Pollen envelope change over time (years)

To determine, for each genus, whether the timing and quantity of pollen released have changed during the 17-year period (1995–2011), simple linear regressions were conducted for each pollen envelope parameter described above, with year as the predictor variable. To compare the year-to-year variation in pollen envelope parameters, we calculated the coefficient of variation for each pollen envelope parameter for each genus. We used the Student's *t*-test to determine if there was a significant difference between the two genera in annual cumulative pollen produced or in highest peak pollen produced.

Current year temperature and precipitation effects on flowering time

Timing of pollen production is expected to depend on current-year temperature and rainfall. For each genus, we regressed each pollen timing variable separately on March, April and May mean temperatures together in a multiple regression and March, April and May total monthly rainfall amounts together in another multiple regression. These multiple regressions showed the importance of each month to pollen release timing, and the coefficients can be interpreted as flowering time sensitivity to temperature or rainfall expressed as change in days per degree Celsius or millimetres rainfall, in both cases holding the effects of other months constant. To test whether the two genera differed in flowering time sensitivity to temperature, we conducted two-tailed bootstrap tests (10,000 iterations) of the difference between the genera in the regression slope for each pollen envelope parameter vs. temperature. The mean temperature chosen as the predictor variable for *Populus* or *Betula* in each bootstrap test was from the month that had the strongest effect on the time of flowering in the multiple regressions described above.

Previous year temperature and precipitation effects on pollen quantity

Pollen quantity is expected to depend on the previous year's summer and autumn temperature and rainfall because flower buds and catkins of spring-flowering species are formed in the previous year's summer and autumn (Sørensen, 1941). To determine whether the amount of pollen released was related to climatic variables in the previous year's summer and autumn, we conducted both simple and multiple regressions of annual cumulative pollen count or highest peak pollen count vs. the previous year's June–October mean temperature or the previous year's June–October total rainfall.

Climatic effects on pollen detection

To determine whether current-season rainfall dampened or high winds amplified the amount of pollen detected, simple regressions of peak or annual cumulative pollen counts vs. monthly (April or May) total rainfall and vs. daily maximum or monthly (April or May) mean wind speeds were run, respectively.

Temperature and precipitation change over time (years)

To determine whether there has been a change in temperature or precipitation from 1995 to 2011, we performed simple regressions of monthly (January–December) mean temperature or total precipitation vs. year.

All statistics were run using JMP version 13 (SAS Institute, Cary, North Carolina, USA). Bootstrap analyses were conducted using programs written in Mathematica version 11.2 (Wolfram Research, Champaign, Illinois, USA).

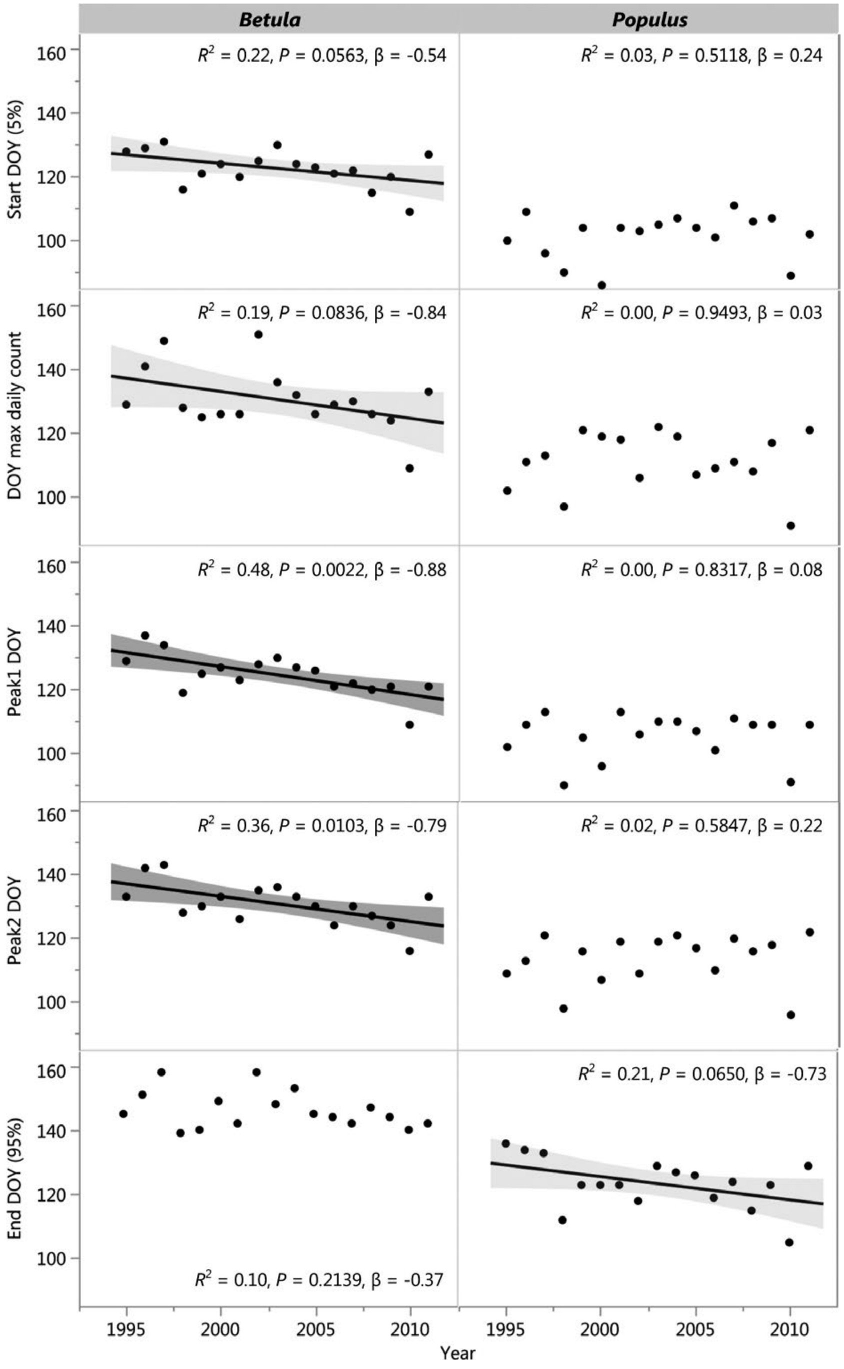

FIGURE 2. Regression of start (start day of year [DOY] (5%)), peak (DOY maximum daily count, Peak1 DOY, and Peak2 DOY), and end (End DOY (95%)) pollen release envelope parameters vs. year, showing that the start and peak of pollen release of *Betula* and the end of pollen release of *Populus* have advanced significantly (α = 0.1) over the 17 years of the study (1995–2011). Shading indicates 95% confidence intervals, with dark gray representing a significant trend (α = 0.05) pale gray representing a trend approaching significance (α = 0.1).

RESULTS

Pollen envelope change over time (years)

Over the 17 years (1995–2011), *Betula* exhibited a significant (α = 0.05) trend toward an earlier peak 1 and peak 2, a trend toward an earlier start and highest peak that approached significance (α = 0.1), but no significant trend in end or duration of pollen release (Figs. 2 and 3). In contrast, over the same period, *Populus* exhibited a significant trend toward a shorter duration of pollen release, a trend toward an earlier end that approached significance and no significant change in the timing of start or peak of pollen release (Figs. 2 and 3). Neither species showed a significant change in the amount of pollen released, although *Betula*'s annual cumulative pollen count showed an increase that approached significance (P = 0.053). The male flowering phenology of *Betula* was slightly more consistent from year to year than the male flowering phenology of *Populus*; that is, the *Betula* coefficients of variation of start, peak 1, peak 2, end and duration of pollen release were smaller than the *Populus* coefficients of variation for the same parameters (Appendix S1; see Supplemental Data with this article). *Betula* produced significantly more pollen annually than *Populus* (1995–2011 mean annual cumulative pollen count: *Betula*, n = 3524; *Populus*, n = 1526; t = 2.04, P = 0.0077; mean highest peak count: *Betula*, n = 568; *Populus*, n = 364; t = 2.04, P = 0.1361).

Current year temperature and precipitation effects on flowering time

Monthly mean temperatures had a significant effect on the pollen envelope timing and duration parameters of both genera (Table 1). For May-flowering *Betula*, April mean temperature had the strongest effect on all pollen release parameters except end DOY, where May mean temperature had the strongest effect. For April-flowering *Populus*, March mean temperature had the strongest effect on all pollen release parameters except end DOY and duration, where April mean temperature had the strongest effect. The results were similar using minimum or maximum monthly temperatures in the multiple regressions (data not shown). *Populus* and *Betula* differed in sensitivity to temperature for the end of pollen release (P = 0.046), with

Populus advancing more rapidly with warming temperatures than *Betula* (Appendices S2 and S3). Differences in sensitivity for duration of pollen release approached significance (P = 0.056), with *Populus* pollen duration shortening more rapidly with warming temperatures than *Betula*. Although *Betula* start and peak pollen envelope parameters were 1.5 to 2 times more negative than *Populus*, we detected no significant difference between the genera in sensitivity to temperature for start or peak pollen envelope parameters (P > 0.14; Appendices S2 and S3).

There was no significant overall relationship of timing and duration pollen envelope parameters with monthly total rainfall for either genus (Table 2). Although the overall models were not significant, higher March total rainfall was associated with earlier start DOY and peak 1 DOY of *Populus*. To determine whether temperature or rainfall had the strongest influence on the timing of pollen envelope parameters and whether there were any interactions between temperature and rainfall, we also ran multiple regressions for each pollen envelope parameter separately with March–May temperature and March–May precipitation as explanatory variables (i.e., six explanatory variables per multiple regression). These multiple regressions resulted in the same message as the multiple regressions with either monthly temperatures or total rainfall, that is, the same monthly temperatures, and not total monthly rainfall, explained the timing of the pollen envelope parameters (results not shown).

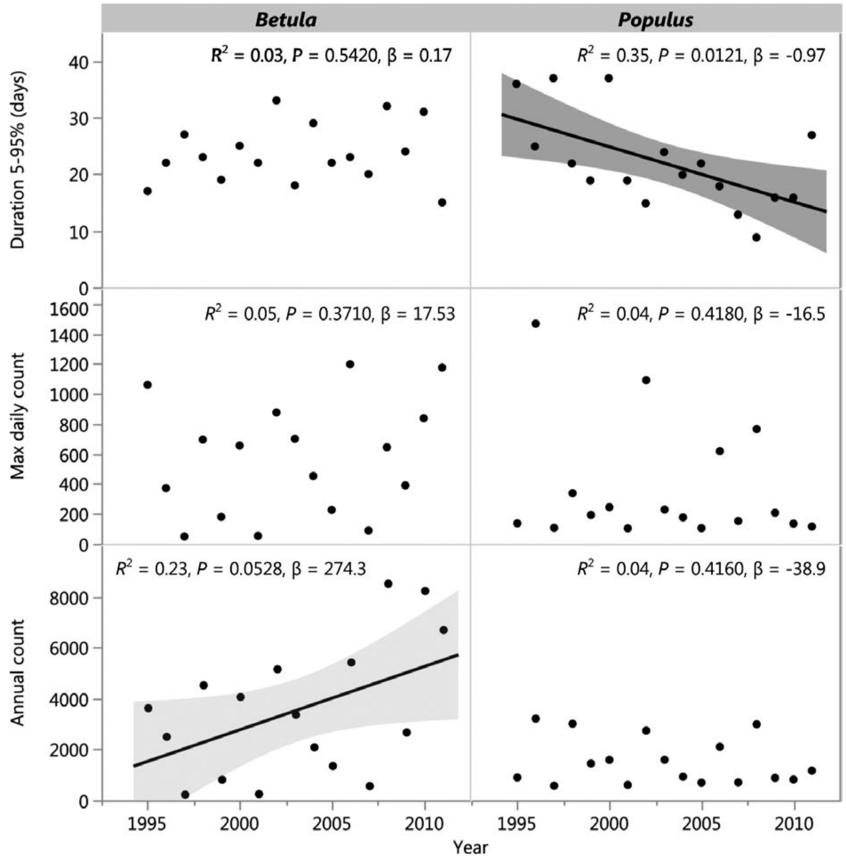

FIGURE 3. Regression of duration (Duration 5–95% (days) and pollen count (Max daily count and Annual count) pollen release envelope parameters vs. year, showing that the duration of pollen release of *Populus* has significantly shortened and that the annual cumulative pollen count of *Betula* has significantly increased over the 17 years of the study (1995–2011). Shading indicates 95% confidence intervals, with dark gray representing a significant trend (α = 0.05) and pale gray representing a trend approaching significance (α = 0.1).

Previous year temperature and precipitation effects on pollen quantity

There was no significant relationship between *Betula* annual cumulative pollen count or highest peak pollen count and any of the June–October mean temperatures from the previous year (simple regressions, P > 0.2; for multiple regression, see Appendix S4). For the *Populus* simple regressions, there was only a significant positive relationship between *Populus* annual cumulative pollen count or highest peak pollen count and June or October mean temperatures from the previous year (annual cumulative count: June: R^2 = 0.35, P = 0.012; October: R^2 = 0.26, P = 0.036; highest peak count: June, R^2 = 0.27, P = 0.034; October, R^2 = 0.43, P = 0.004). For the *Populus* multiple regression, annual cumulative count was most strongly influenced by the previous year's June mean temperature and highest peak count was most strongly influenced by the previous year's August, September and October mean temperature (Appendix S4). There was an approaching significant positive relationship between

TABLE 1. Multiple regression models for pollen release envelope parameters vs. March, April, and May mean temperatures over the 17 years of the study (1995–2011).

Pollen envelope parameter	*Betula*					*Populus*				
	Model					Model				
	R^2	P	March (β, P)	April (β, P)	May (β, P)	R^2	P	March (β, P)	April (β, P)	May (β, P)
Start DOY (5%)	**0.85**	**<0.0001**	−0.54, 0.0988	**−2.85, <0.0001**	−0.32, 0.4224	0.42	0.0606	**−1.86, 0.0270**	−0.90, 0.3948	0.00, 0.9994
Highest peak DOY	**0.58**	**0.0088**	−1.64, 0.0818	−2.22, 0.0852	−1.41, 0.2317	0.36	0.1130	−1.50, 0.1490	−2.80, 0.0565	0.81, 0.5312
Peak 1 DOY	**0.82**	**<0.0001**	−0.76, 0.0621	**−2.92, <0.0001**	−0.47, 0.3480	**0.70**	**0.0011**	**−1.89, 0.0037**	−1.14, 0.1440	−0.81, 0.2619
Peak 2 DOY	**0.73**	**0.0006**	−0.90, 0.0794	**−2.72, 0.0010**	−0.42, 0.5034	**0.55**	**0.0127**	**−1.94, 0.0175**	−1.42, 0.1710	−0.46, 0.6258
End DOY (95%)	**0.68**	**0.0016**	0.21, 0.6486	−0.57, 0.3776	**−2.37, 0.0014**	**0.81**	**<0.0001**	−0.69, 0.1697	**−4.12, <0.0001**	−0.14, 0.8175
Duration 5–95%	**0.56**	**0.0115**	0.75, 0.1424	**2.28, 0.0042**	**−2.05, 0.0056**	**0.46**	**0.0388**	1.17, 0.1813	**−3.22, 0.0140**	−0.14, 0.8956

Notes: Day of year (DOY) is the number of days from 1 January. Bold entries indicate statistical significance at α = 0.05. β is the pollen envelope parameter sensitivity to temperature in days/°C.

TABLE 2. Multiple regression models for pollen release envelope parameters vs. March, April, and May total rainfall over the 17 years of the study (1995–2011).

Pollen envelope parameter	*Betula* Model R^2	*Betula* Model P	*Betula* March (β, P)	*Betula* April (β, P)	*Betula* May (β, P)	*Populus* Model R^2	*Populus* Model P	*Populus* March (β, P)	*Populus* April (β, P)	*Populus* May (β, P)
Start DOY (5%)	0.33	0.1509	−0.11, 0.1181	0.02, 0.5726	0.08, 0.0913	0.30	0.1829	**−0.19, 0.0381**	0.03, 0.5048	0.00, 0.9517
Highest peak DOY	0.17	0.4663	−0.14, 0.2781	0.01, 0.9030	0.10, 0.2466	0.28	0.2155	−0.06, 0.5784	0.01, 0.8257	**0.15, 0.0477**
Peak 1 DOY	0.30	0.1886	−0.15, 0.0582	−0.01, 0.8535	0.05, 0.2887	0.43	0.0582	**−0.21, 0.0139**	0.02, 0.6101	0.06, 0.2333
Peak 2 DOY	0.13	0.5873	−0.09, 0.2783	0.01, 0.7597	0.05, 0.4223	0.31	0.1717	−0.16, 0.0857	0.04, 0.3470	0.08, 0.2170
End DOY (95%)	0.22	0.3465	−0.08, 0.2942	−0.01, 0.8755	0.07, 0.1419	0.31	0.1716	−0.19, 0.0531	0.03, 0.5252	0.06, 0.3128
Duration 5–95%	0.05	0.8853	0.03, 0.6740	−0.03, 0.5019	0.00, 0.9266	0.05	0.8756	−0.01, 0.9620	0.00, 0.9754	0.06, 0.4245

Notes: Day of year (DOY) is the number of days from 1 January. Bold entries indicate statistical significance at α = 0.05. β is the pollen envelope parameter sensitivity to rainfall in days/mm.

Betula annual cumulative pollen count or highest peak pollen count and August mean precipitation of the previous year only in the simple and multiple regressions (annual cumulative pollen count: R^2 = 0.21, P = 0.064; highest peak count: R^2 = 0.23, P = 0.050; Appendix S5) and a significant positive relationship between *Populus* highest peak pollen count and October mean precipitation of the previous year only in the simple and multiple regressions (R^2 = 0.28, P = 0.030; Appendix S5). That is, the amount of pollen released by *Betula* increased with precipitation but not with temperature in the previous summer, while the amount of pollen released by *Populus* increased with higher temperatures in the previous summer and autumn and with greater precipitation in the previous autumn.

Climatic effects on pollen detection

We detected no relationship between monthly [April or May] total rainfall and either annual cumulative pollen count or peak pollen count (highest, peak 1 or peak 2: R^2 < 0.15, P > 0.13), which suggests that the amount of pollen detected is not dampened by precipitation. Similarly, we detected no relationship between peak and annual cumulative pollen counts and wind speed (monthly [April or May]: R^2 < 0.04, P > 0.4; same day or day prior to peak: R^2 < 0.05, P > 0.2), which suggests that the amount of pollen detected is not amplified on windy days or months.

Temperature and precipitation change over time (years)

Over the 17 years, April was the only month with a significant trend towards warmer temperatures, and April and August were the only months with a significant trend towards greater precipitation (April mean temperature trend: R^2 = 0.29, P = 0.025, β = 0.17°C/yr; April total rainfall trend: R^2 = 0.24, P = 0.046, β = 3.82 mm/yr; August total rainfall trend: R^2 = 0.26, P = 0.036, β = 3.28 mm/yr; Appendices S6 and S7).

DISCUSSION

The results show a change in the pollen release envelope of *Betula* and *Populus* over the 17yr period (1995–2011) that is related to the month (April) where there have been significant changes in temperature. *Betula* start, peak and duration of pollen release have strong relationships with April temperatures, while *Populus* end and duration of pollen release have strong relationships with April temperatures. These are the pollen envelope parameters that have changed significantly (*Betula* peak pollen release timing and *Populus* pollen release duration) or approaching significance (*Betula* start pollen release timing and *Populus* end pollen release timing). This has resulted in two contrasting changes in the pollen envelope for these genera (Fig. 4). Our results highlight that the differing rates of change of temperature for different months of the year may impact study findings (Baker et al., 2016). Our results may also help to explain why the timing of pollen release of these two genera has advanced in some locations but not in others (Emberlin et al., 2002; Damialis et al., 2007; Newnham et al., 2013; Zhang et al., 2014, 2015).

The start, peak and end of pollen release timing of both *Betula* and *Populus* were most strongly influenced by the monthly temperatures just prior to the phenological event, which is consistent with the results of other studies of non-wind-pollinated species (Fitter et al., 1995; Sparks et al., 2000; Panchen et al., 2012; Panchen and Gorelick, 2017). Given that (1) species' flowering times are sensitive to temperatures in the month(s) prior to flowering, (2) the sensitivity to temperature varies among species and (3) monthly temperatures are not rising uniformly under climate change (Stocker et al., 2013), as seen in this study, the timing of pollen release across species may not alter uniformly under climate change and hence, there could be periods of increased or reduced airborne pollen compared to years past. The shortening of *Populus* pollen envelope duration without a significant increase in the amount of pollen released or a shift to earlier peak pollen release may have greater consequences for *Populus* seed production in comparison to *Betula*, whose pollen envelope has not significantly shortened over the 17-year period but whose peak pollen release is significantly earlier and whose amount of pollen released has increased slightly. In addition, given that we found a relationship between the amount of pollen released and climatic conditions in the previous summer and autumn, we suggest that the flower bud primordia of both *Populus* and *Betula* have differentiated to the level where the anthers are fully developed prior to the onset of winter (Sørensen, 1941). Total rainfall in the month prior to flowering had no significant effect on *Betula* pollen envelope parameters and only a significant effect on *Populus* start and the first peak pollen envelope parameters. However, the significant relationships for *Populus* may merely indicate that the proportion of March precipitation that falls as rain is greater in warmer years.

The end and duration of pollen release were both less sensitive to temperature in *Betula* than in *Populus*, while there was no difference between the two genera in sensitivity to temperature of start and peak of pollen release. For example, the duration of pollen release shortened by 0.84 days/°C rise in temperature for *Betula*, compared to 3.07 days/°C for *Populus*. Thus, as temperatures warm, and depending on the level of synchronicity between male and female flowers, the window of opportunity for pollination could shorten more dramatically for *Populus* than for *Betula*, differentially affecting the potential for pollen limitation and seed production. Future effects on reproduction in the two genera will depend, to some extent, on relative flowering time sensitivity to temperature of female flowers vs. male flowers, the sensitivity of which has been shown to differ for some dioecious and monoecious species, with male flowering times being more sensitive to temperature than female flowering times (Alatalo and Molau, 1995; Jones et al., 1997; Stenström and Jónsdóttir, 2004). Consistent with other studies (CaraDonna et al., 2014), start, peak, end and duration of flowering exhibited different sensitivities to temperature, particularly in the case of *Populus*, where the start of pollen release advanced by 1.86 days/°C rise in March temperatures but the end of pollen release advanced by 4.12 days/°C rise in April temperatures. Thus, we might expect to see the timing of start, peak and end of pollen release shifting by differing amounts as the climate warms.

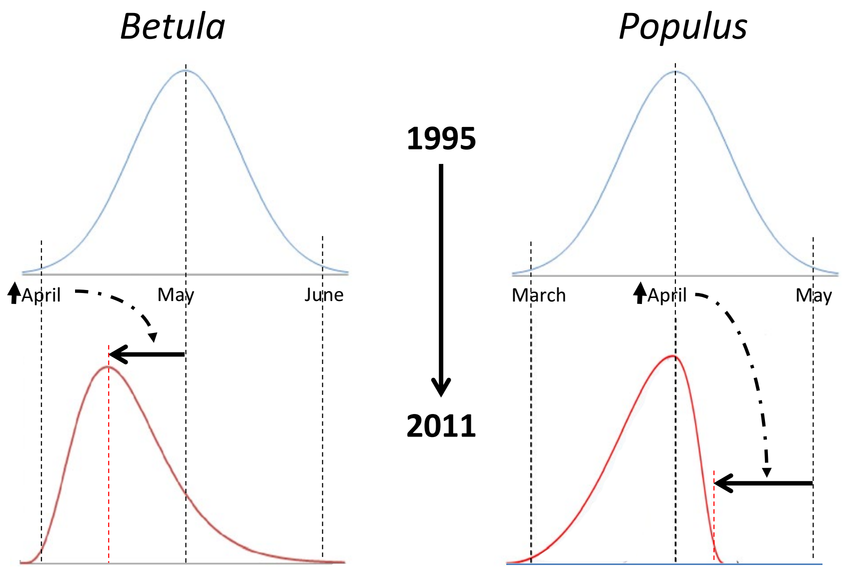

FIGURE 4. Conceptual illustration of how the trend over time (1995–2011) has changed the *Betula* and *Populus* pollen release envelopes in Ottawa, Ontario, Canada. The left-hand side of the curves represents the start of pollen release, the peak of the curves represents highest peak or peak 1 or peak 2 pollen release, and the right-hand side of the curves represents the end of pollen release. The top panel illustrates the original pollen release envelope, and the bottom panel represents how it has changed during the study period. April is the only month with a significant increase in mean monthly temperature over the 17 yr, and *Betula* start, peak, and duration pollen envelope parameters are related to April temperatures, while *Populus* end and duration pollen envelope parameters are related to April temperatures, resulting in *Betula* peak pollen release significantly advancing and *Populus* pollen duration significantly shortening. The changes are not shown to scale.

Reproductive success of plant populations is dependent on the quantity of pollen produced when seed production is pollen limited. *Salix* species (in the same family as *Populus* [Salicaceae]) and *Betula* species have been shown to be pollen limited (Weis and Hermanutz, 1993; Holm, 1994; Knight et al., 2005). Thus, assuming that pollen limitation is a phylogenetically conserved trait (Larson and Barrett, 2000; Knight et al., 2005; Johnston and Bartkowska, 2017), an increase in pollen production could result in higher seed production for both *Betula* and *Populus*. The amount of pollen released by each genus is dependent on different climatic variables in the previous summer and autumn. That is, *Betula* will release more pollen when the previous summer (August) is wetter, while *Populus* will release more pollen when the previous summer and autumn (June and August–October) temperatures are warmer and the autumn (October) is wetter. Our results also show that, over the study period, summer and autumn temperatures have not risen but August total precipitation has significantly increased. Thus, *Betula* could experience increased seed production compared to *Populus* if the climate continues to change in the same pattern as seen during the study period—that is, increased precipitation in August but no rise in summer and autumn temperatures. If, however, summer and autumn temperatures increase over the long term under a warming climate, *Populus* could increase the amount of pollen released in comparison to *Betula*. Thus, future increases or decreases in reproductive success are expected to differ among species and will depend on which months of the year experience changes in temperature or rainfall.

Peak 1 and peak 2 generally had stronger relationships with year, temperature or precipitation than the highest peak. We have suggested that peak 1 and peak 2 may represent different species. If the order of leaf out and flowering time of species in a plant community tends to be in the same order every year (Panchen et al., 2015; Panchen and Gorelick, 2016), then the highest peak may represent a different species each year and, thus, the amount of pollen a species releases may show some level of masting (Spieksma et al., 1995). However, this assertion may not hold if some of the peaks or some quantity of pollen in peak 1 and/or peak 2 is due to long-distance pollen transport. The day with the highest pollen count over the complete pollen release envelope may not necessarily be the same species each year. Hence, stronger relationships might be detected if the peaks could be associated with an individual species.

Our analysis did not attempt to incorporate particle trajectory and deposition models to compensate for the effects of long-distance pollen transport. It is thus possible that the pollen capture dates differed from the flowering phenology of the local plants (Estrella et al., 2006). However, the use of 5% and 95% of the cumulative annual pollen to represent the start and end of pollen release (as opposed to 1% and 99%) reduces the chances of the start or end date being the result of the effects of long-distance pollen transport (Zhang et al., 2015). In addition, the set of species present within the long-distance pollen transport range of Ottawa is relatively homogeneous, which substantially reduces the likelihood that species not found in the Ottawa area and with a very different pollen release envelope timing could have biased or invalidated the results. Furthermore, the peak parameter correlations over time and climatic variables were the strongest of all the pollen envelope parameters, which suggests that long-distance pollen transport did not affect the overall results because inter-annual variation in atmospheric conditions would likely have introduced randomness into the peak parameters.

In conclusion, this study has shown that the pollen release timing and the amount of pollen released by each genus depends on how climatic conditions change through the year. Differing shifts in phenology among species may be related to different rates of change in climatic variables in different months of the year. While our study considered only two genera, the results underscore the importance of understanding intra-annual (i.e., month-to-month) variation in climate change when studying the ecological implications of climate change.

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AUTHOR CONTRIBUTIONS

Z.P. designed the study and drafted the manuscript. Z.P. and M.O.J. contributed to data analysis, interpretation of the results, and revision of the manuscript.

DATA ACCESSIBILITY

Betula and *Populus* daily pollen counts recorded in Ottawa, Ontario, Canada, from 1995 to 2011 are provided in Appendix S8.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information section at the end of the article.

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