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ORIGINAL PAPER

The phenology of *Rubus fruticosus* in Ireland: herbarium specimens provide evidence for the response of phenophases to temperature, with implications for climate warming

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Abstract To date, phenological research has provided evidence that climate warming is impacting both animals and plants, evidenced by the altered timing of phenophases. Much of the evidence supporting these findings has been provided by analysis of historic records and present-day fieldwork; herbaria have been identified recently as an alternative source of phenological data. Here, we used *Rubus* specimens to evaluate herbaria as potential sources of phenological data for use in climate change research and to develop the methodology for using herbaria specimens in phenological studies. Data relevant to phenology (collection date) were recorded from the information cards of over 600 herbarium specimens at Ireland's National Herbarium in Dublin. Each specimen was assigned a score (0–5) corresponding to its phenophase. Temperature data for the study period (1852 – 2007) were obtained from the University of East Anglia's Climate Research Unit (CRU); relationships between temperature and the dates of first flower, full flower, first fruit and full fruit were assessed using weighted linear regression. Of the five species of *Rubus* examined in this study, specimens of only one (*R. fruticosus*) were sufficiently abundant to yield statistically significant relationships with temperature. The results revealed a trend towards earlier dates of first flower, full flower and first fruit phenophases with increasing temperature. Through its multi-phenophase approach, this research serves to extend the most recent work—which validated the use of herbaria through use of a single phenophase—to confirm herbarium-based research as a robust methodology for use in future phenological studies.

Keywords Climate warming · Flowering · Fruiting · Herbarium specimen · Ireland · Phenology · Rubus

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Introduction

The traditional approach to phenological research has relied on the analysis of historic records and present-day field observations of the timing of natural life-cycle events in animals and plants. Some historic records of phenology span centuries; in Japan, for example, records of the flowering of cherry trees exist from the eighth Century (Aono and Kazui 2007). Historical records have been provided not only by professionals in the scientific realm, but also by amateur naturalists for whom spending time observing animals and plants was a fashionable pastime in the eighteenth and nineteenth centuries in England (Sparks and Carey 1995). Indeed, tales recounting searches for elusive species exist for well over a century, as exemplified in this note by Hart

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and Barrington (1892) in the Irish Naturalists' Journal, "[*Rubus chamaemorus*] has been searched for in vain by many botanists during the past sixty-six years…" Such amateur recording continues today and has been termed 'citizen science'. Today, the general public can record their observations online at a variety of websites; this has the potential to make a substantial contribution to phenological research. Indeed, legacy records from both amateur and professional naturalists have provided us with a potentially robust source of data that can provide evidence of the impacts of temperature on phenology (Lawrence 2009; Sagarin and Pauchard 2010). Because many of these observed life-cycle events are dependent largely on temperature, current climate change research has utilized the analysis of such records as an indication of the impacts that climate warming has had on both plants and animals (Sparks and Carey 1995). Indeed, both historic and contemporary records have provided evidence from a host of species and locations that climate change is altering the phenology of both plants and animals across ecosystems (see Walther et al. 2002; Root et al. 2003; Parmesan and Yohe 2003; Menzel et al. 2006; Parmesan 2006; Thackeray et al. 2010 for reviews and meta-analyses). Such phenological research has contributed to the development of environmental policy. For example, the Intergovernmental Panel on Climate Change (2007) cites changes in phenology as an "observed climate change impact" (with high confidence) on terrestrial, marine and freshwater biological systems.

Although historic records and present-day fieldwork of phenological events have proven invaluable in climate change research, as we begin to exhaust known records, the necessity of sourcing alternative datasets has been recognised (Miller-Rushing et al. 2006; Sparks 2007). One such source of data is herbaria, of which there are over 4,000 worldwide, containing an estimated 350 million specimens (International Plant Science Center 2010). Herbaria are in effect plant museums, storing plant material and specimens that have been collected around the world. Herbaria are usually located in botanic gardens or institutions of higher learning, where the specimens are preserved in climate-controlled environments for future reference. Most of these specimens have a label card onto which the species name, the collector's name, and—most importantly for phenologists—the date and location of collection are recorded, thereby enabling spatial and temporal analyses of the data. As a result, in addition to their use in species distribution mapping and invasive species tracking (Crawford and Hoagland 2009), herbaria have been used in phenological studies since the mid-1980s (Borchert 1996). Several more recent studies have utilized herbarium specimens in climate change research, and preliminary methodologies have been developed (Primack et al. 2004).

Initially suggested for use in conjunction with either fieldwork or analysis of historic records, more recent research has demonstrated that herbarium specimens alone can provide a statistically robust dataset for analyses (Miller-Rushing et al. 2006; Robbirt et al. 2011). As a result, there has been an increased focus on the use of herbarium specimens within the field of phenological research, and in particular, the identification of appropriate phenophases with the potential to demonstrate climate warming impacts. An examination of the relevant literature indicates that, although many advantages to this method have been cited, it is an approach that is not without limitations (Borchert 1996; Lavoie and Lachance 2006; Miller-Rushing et al. 2006; MacGillivray et al. 2010). For example, although long time series may be available (e.g. spanning several centuries), there may be large gaps in the data series. This is due to a number of factors, including, for example, reduced collection during wartimes. Table 1 presents a comprehensive list of the potential advantages and limitations of using herbarium specimens in phenological research.

Miller-Rushing et al. (2006) suggest that most of these limitations can be countered through use of a sufficiently large sample size to ensure that uncertainty is minimized and, despite the noted limitations, all authors agree that herbaria remain excellent potential sources of phenological data. It is important to recognise that it is an approach for which the methodology continues to evolve and as such, the potential for modification and advancement of suggested methodologies exists. To date, the selection of phenophases has been somewhat limited; much of the research has focussed on the use of a single phenophase such as first flower. However, in general, herbaria contain specimens exhibiting a range of phenophases, which, despite the limitations outlined in Table 1, present an opportunity for interesting research. Such a multi-phenophase approach allows for evaluation of the extent to which a response to climate warming may differ between phenophases, thus giving a more accurate overall picture of the response of a species over the course of its lifetime.

To date, phenological research in Ireland has focussed on correlating the timing of spring events in plants and birds with temperature variables; this has been achieved through use of historic records and datasets (Donnelly et al. 2006, 2009). Between 1890 and 2004, mean annual air temperature in Ireland increased by 0.7°C (McElwain and Sweeney 2007). This has been correlated with the earlier arrivals of birds and earlier leafing of trees; however, these studies have been limited to more recent time periods (1969–1999 and 1960–2009, respectively). In their recent comprehensive review of 143 different phenological events from records in the Irish Naturalists' Journal between 1927 and 1947, Carroll et al. (2009) extended this time series

Table 1 Advantages and limitations of using herbarium specimens in phenological research

	Advantages	Limitations
Sample size	The number of specimens available is often greater than can be achieved with field observations	
Time series	A long time series is readily available (specimens collected over 100+ year periods can be accessed)	Large gaps resulting from sampling effort are common
Species availability	A large number of species are available, enabling assemblage studies	Certain species are poorly represented
Phenology	Specimens are often collected at a phenologically important time (e.g. first flower, first fruit, etc.) and can therefore be incorporated readily into phenological research	Herbarium specimens may provide less certainty with regards to true flowering phase dates than field observations Collection biases—both towards collecting specimens in the first flower phenophase and during the peak abundance of full flower—can complicate analysis
Geographic analysis	A large geographic area can be analyzed (specimens from a large geographic area – often worldwide – can be found in one herbarium)	Geographically large-scale studies may require the use of correction procedures to account for climatic variations, complicating analysis Relatively few large single-site collections exist, meaning that the potential for localized studies is limited
Other	Specimens are systematically stored for future reference	

backwards and concluded that most species analysed displayed responses to spring warming. Here, we evaluate the potential use of herbarium specimens as a method to extend this time series backwards even further, enabling the investigation of the impacts of climate warming on phenology in Ireland as far back as the nineteenth century. We do so through an analysis of *Rubus* spp. phenophases from specimens collected in Ireland over a period of 156 years (1852–2007). Because multiple-phenophase studies have been under-utilised to date in herbarium-based research, our study will serve to assess the validity of the proposed approach and help expand the use of herbarium specimens in future research.

Further to this, by using *Rubus* spp. as a study organism, we are also able to assess the robustness of herbarium-based approaches, given that *Rubus* spp. typically have a long flowering period. Whereas previous herbarium-based research has often deliberately selected species with short flowering periods, we were interested to evaluate whether or not we would be able to detect a signal (i.e., a response to temperature) using a species for which we would expect considerable noise. This allows us to infer the general validity of the approach—one that could then be used for other similar less than ideal species.

Materials and methods

Climatic variables

The 5° grid square (50–55°N, 10–5°W) covering Ireland was isolated from the CRUTEM3v gridded temperature dataset, which contains monthly temperature anomalies from the 1961–1990 average (sourced from the Climate Research Unit, University of East Anglia and available online at http://www.cru.uea.ac.uk). Temperature anomalies were selected as they give an indication of deviation from the long-term mean. We tested several time periods (including each month, 2-month, 3-month and 6-month mean within the period from January to June) to find that which best correlated with *Rubus* spp. phenology. The mean of January to June temperature anomalies was found to be the most appropriate, and was calculated for each year of the study period (1852–2007). These means were examined against year to determine trends in Irish temperature over the study period.

Herbarium data

Specimens used in this study are located in Ireland's National Herbarium (Index Herbariorum code DBN). Founded in 1847 at the National Museum of Ireland in Dublin (former Index Herbariorum code DUB), the herbarium is presently located within the grounds of the National Botanic Gardens in Dublin, and contains a collection of over 500,000 plant specimens from around the world.

Two factors contributed to our selection of *Rubus* as the focus of this investigation. Firstly, *Rubus* spp. are found throughout much of Ireland (National Biodiversity Data Centre 2010), and as a result, specimens are plentiful in the National Herbarium, providing a large dataset. Secondly, *Rubus* spp. have a relatively long flowering and fruiting period and, whereas most previous studies have selected species with short flowering periods (e.g. Gallagher et al. 2009), *Rubus'* long flowering period allows us to test the robustness of a herbarium-based approach to phenological studies in a less-than-ideal candidate species.

Over 600 herbarium specimens of *Rubus* spp. collected in Ireland were examined systematically. Collector, collection date, and location were recorded from the information cards of each specimen. Only specimens with a complete collection date (day, month, and year) were used in this study; each date was translated into day of year (days after December 31). Each specimen was then assigned a score from 0 to 5 corresponding to particular phenophases, with reference to the stage of development of the apical branch (Table 2). Flowering and fruiting definitions were developed with reference to Schmidt et al. (2001). The 50% threshold was selected after Primack et al. (2004), who use it as a benchmark for the 'full flower' phenophase. The scoring system that we developed is ideal for a herbarium-based study because the phenophases (and thresholds by which they are defined) are easy to identify and the scoring process is incredibly time efficient.

Statistical analysis

Due to previous research indicating species-specific variation in response to temperature within the *Rubus* genus (Fitter and Fitter 2002), the data were divided into five species groups: *R. caesius*, *R. fruticosus* agg., *R. idaeus*, *R. saxatilis*, and *R. spectabilis*. Within each species, where more than one specimen was collected in a particular phenophase within a given year, the records were averaged to give a single date for the occurrence of that phenophase in that year (number of specimens = sample size). Sample size was then used as a weight in weighted linear regression. Since records of the other four species covered only 5–12 years, only *R. fruticosus* (which comprised 86% of all records) was examined further.

Although traditionally used in phenological analyses, several authors have questioned the reliability of using the first appearance, or first flower phenophase; the full flower phenophase has been suggested as a more suitable alternative (Lavoie and Lachance 2006; Moussus et al. 2010; A.J. Miller-Rushing, personal communication). We therefore undertook weighted linear regression analysis of dates of first flower, full flower, first fruit and full fruit on mean January–June temperature anomalies to identify responses to temperature. Analyses were carried out using the SPSS statistical package.

Table 2 Phenophase scoring system for *Rubus* spp. herbarium specimens

Score	Phenophase	Defined by
0	No flowers	No flowers
1	First flower	< 50% flower buds open
2	Full flower	> 50% flower buds open
3	First fruit	< 50% fruits ripe (fruit colouration reached)
4	Full fruit	> 50% fruits ripe (fruit colouration reached)
5	End of fruiting	> 90% fruits ripe (most dispersed)

Results

Climatic variables

The January–June mean temperature anomalies (relative to 1961–1990), as calculated from the CRU dataset for each year of the study period, indicate a recent trend towards warmer temperatures. Indeed, positive temperature anomalies are evident in 19 of the last 20 years of the study period (Fig. 1).

Herbarium data

Of over 600 specimens examined, 540 were found to have complete information. Of these, *R. fruticosus* dominated (n=464); the other species were represented by significantly fewer specimens (*R. caesius*: n=40, *R. idaeus*: n=15, *R. saxatilis*: n=15, *R. spectabilis*: n=6). The time series was not continuous due to a decrease in collections between 1910 and 1960 (Fig. 2). Of the two primary collecting periods (pre-1910 and post-1960), the former represents that period during which a larger number of specimens were collected; leaving the lone specimen collected in 1852, an average of 8.3 specimens were collected per year pre-1910, 0.2 per year between 1910 and 1960 (inclusive), and 3.3 per year post-1960. As a result of the paucity of specimens between 1910 and 1960, no time-series analysis was undertaken; instead, we focussed on determining the extent to which temperature influenced phenophase dates in *R. fruticosus* using the day of year numbers calculated from the collection dates on the information cards. The earliest

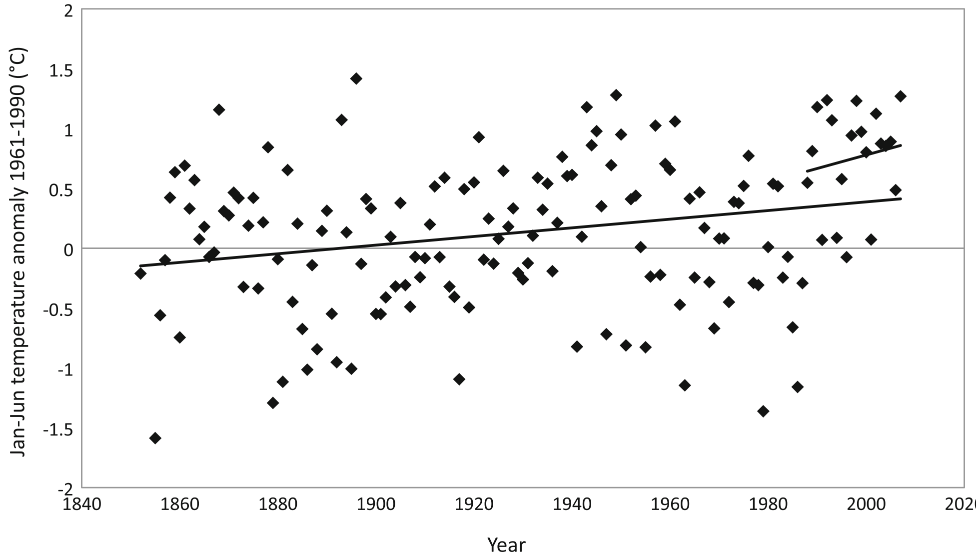

Fig. 1 January–June temperature anomalies 1852–2007 relative to the 1961–1990 mean (shown as the *horizontal line*). The trendlines facilitate comparison of temperature anomalies between the last 20 years of the study period (1988–2007) with the entire study period (1852–2007)

collection day of year was 111 (a specimen collected in the 'full flower' phenophase); the latest was 294 (a specimen collected in the 'end of fruiting' phenophase).

Analyses of the four phenophases in *R. fruticosus* found significant responses in first flower, full flower and first fruit ($P<0.05$) and a marginally statistically significant trend in the full fruit phenophase (Table 3, Fig. 3). All suggested earlier phases [as indicated by a negative regression slope (Table 3, column b)] of 7 to 13 days per 1°C of warming, with first flower and first fruit exhibiting the greatest responses.

Discussion

Rubus spp. phenophases response to temperature

Using herbarium specimens collected between 1852 and 2007 from across Ireland, we were able to demonstrate a statistically significant response to mean January–June temperature for first flower, full flower, and first fruit phenophases in *R. fruticosus*, and a marginally significant response in full fruit date. The other four *Rubus* species in this study were considered too sparse to reveal a response to temperature. The responses of *R. fruticosus* correspond to responses in flowering and fruiting demonstrated in recent meta-analyses on phenology and climate warming (Parmesan and Yohe 2003; Menzel et al. 2006). Our results indicate that the first flower phenophase exhibited the strongest response to temperature, providing evidence of the suggestion that early-season events are advancing the most (Fitter and Fitter 2002; Cleland et al. 2007). However, the scale of responses found here (7–13 days per 1 ° C) was somewhat greater than that yielded in other studies that focussed on flowering, such as the 4 days determined by Fitter et al. (1995) and the 2–10 days by Sparks et al. (2000). As is typical with much of the phenology research conducted to date using historic records or herbarium

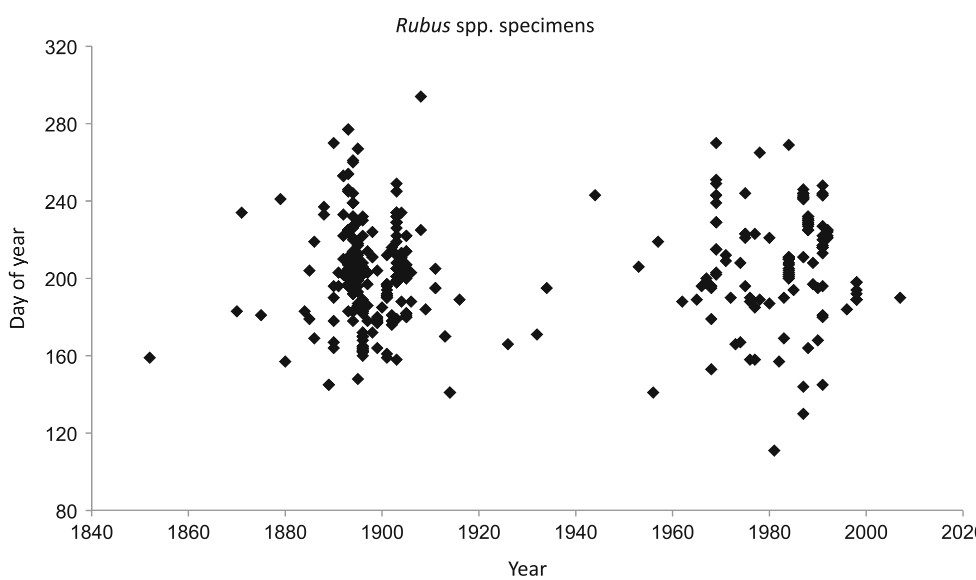

Fig. 2 Collection date (day of year) of all *Rubus* spp. specimens (*R. caesius, R. fruticosus*, *R. idaeus*, *R. saxatilis*, and *R. spectabilis*). Each point represents one specimen (n=540). Two main collecting periods are evident (~1880 to 1910 and ~1960 to 2000)

Table 3 Results of the weighted linear regression of the four *R. fruticosus* phenophases on January–June mean temperature anomalies. *n* Sample size, *b* slope of the regression, *SE* standard error, R^2 coefficient of determinant, *P* significance level

Phenophase	*n*	*b*	SE	R^2	*P*
First flower	24[a]	-13.094[a]	4.033[a]	0.226[a]	0.019[a]
Full flower	37[a]	-9.549[a]	3.292[a]	0.194[a]	0.006[a]
First fruit	21[a]	-11.761[a]	3.650[a]	0.353[a]	0.004[a]
Full fruit	19	-7.183	4.042	0.157	0.093

[a] Statistically significant responses

specimens, these studies focussed on flowering; thus, comparisons between our study and these with reference to fruiting phenology are not possible. Through our research we have demonstrated that changes in fruiting phenology can be investigated using herbarium specimens, confirming our multi-phenophase approach as one that can produce valid, comprehensive results.

The elucidation of phenological trends within *R. fruticosus* in our investigation was complicated by the taxonomy of *Rubus* spp., which has been treated in a variety of ways by different authors. This has resulted in thousands of species having been proposed and a taxonomy that has been described as 'challenging', 'difficult', 'controversial', and 'confused' (Blackman et al. 1977; Alice and Campbell 1999; Nybom and Kraft 1995; Kraft et al. 1996; Wada and Reed 2008). *R. fruticosus* serves as an 'umbrella group' into which many species of a questionable status are placed (Kraft et al. 1996). Indeed, this group represented the greatest proportion of our samples (86%). Given its status as an aggregate group, it is possible that unrelated species exist within it, thus contributing noise to our analysis. MacGillivray et al. (2010) suggest digitisation as a process through which errors in the scientific names on herbarium specimens could be corrected. This would serve to minimise potential error in future research. In addition, digitisation would increase the accessibility of herbarium specimens, facilitating remote research efforts—and could thus prove to be a cost-effective way of increasing sample size. As such, digitisation (including comprehensive taxonomic review processes) should be a priority for herbaria.

Because of a large gap in collections in the mid-twentieth century, our analysis did not take into account change over time. However, the advance in dates in response to increasing temperature anomalies that we found is consistent with the results of previous research in Ireland that have utilized conventional data sources (Donnelly et al. 2006; Carroll et

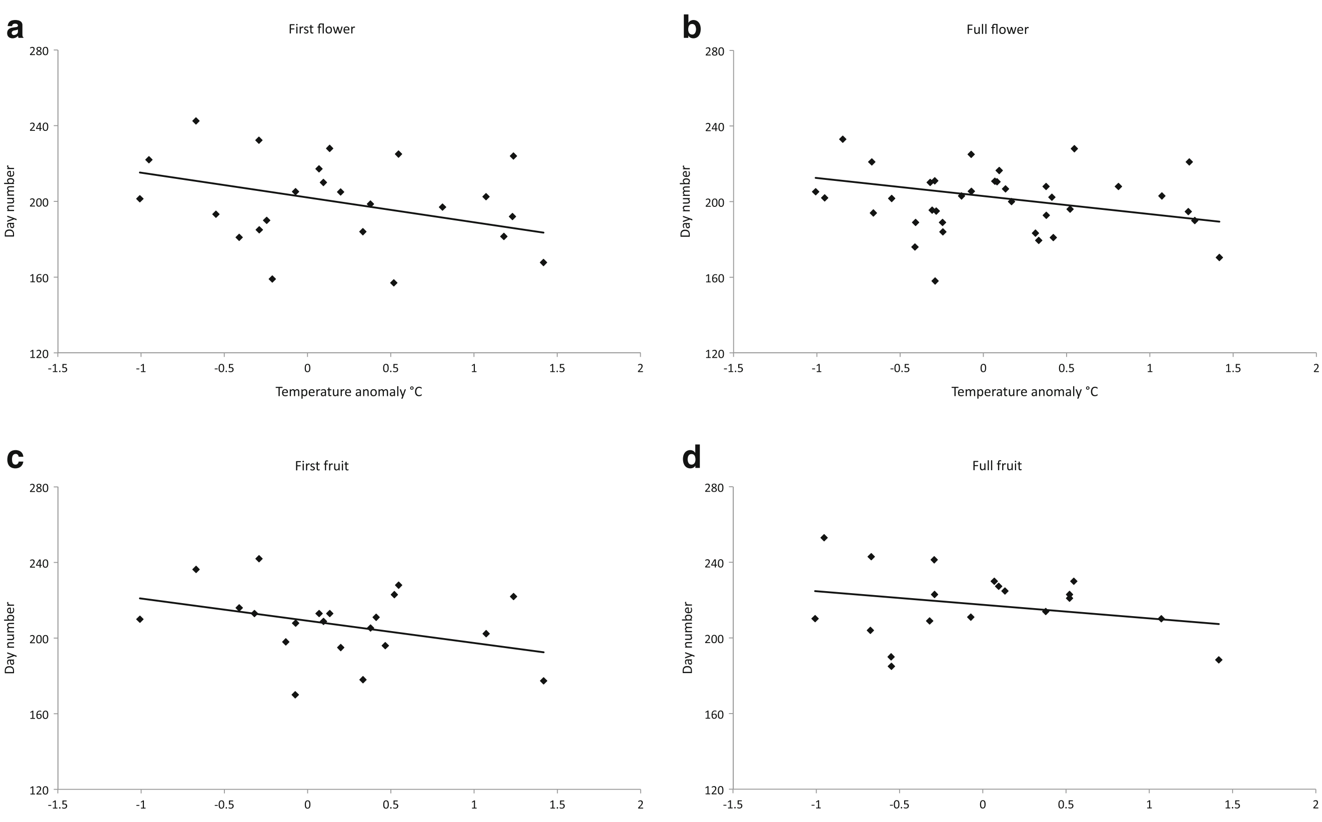

Fig. 3 Mean date (day of year) for first flower (**a**), full flower (**b**), first fruit (**c**), and full fruit (**d**) against January–June temperature anomaly in *R. fruticosus*, including weighted regression lines

al. 2009). Consequently, it can be postulated that, with a more complete dataset, a similar temporal trend would be revealed for *R. fruticosus*. With reference to research that has used future climate scenarios to make phenological projections, it is likely that given projected temperature increases in Ireland, trends towards earlier first flower, full flower, and first fruit in *Rubus* spp. will continue (McGrath et al. 2008; Lebourgeois et al. 2010). The result that these phenophases are linked intimately to temperature is of critical importance to our understanding of how future climate warming will impact ecological processes.

Indeed, this research is interesting in light of an emerging paradigm in phenological research in the past decade, in which a move from a single-species approach towards a more holistic approach has occurred through consideration of species interactions (Harrington et al. 1999; Sutherst et al. 2007; Thackeray et al. 2010). This is done with the aim of determining the potential for mismatches that may result where changes in a species at one trophic level are not matched by the changes in another dependent species at a different trophic level. Such disruption in synchrony is important to consider given its potential impacts on ecosystem function and services (Thackeray et al. 2010). It is important to consider the implications for the higher trophic levels that depend on *Rubus* spp., and the lower trophic levels upon which *Rubus* spp. itself depends. For example, it has been suggested that climate warming has the potential to adversely affect inter-dependent species such as plants and their insect pollinators due to phenological mismatches (Hegland et al. 2009). However, Jacobs et al. (2009) provide evidence that some *Rubus* spp. species may self-pollinate. Thus, it may be interesting in future research to monitor the extent to which species of *Rubus* spp. that do not rely upon insect pollinators are affected by climate warming in comparison to those that do rely upon insect pollinators. Rodents and frugivorous birds should also be included in these interactions given that *Rubus* spp. seeds and berries comprise important components of their respective diets (Schreiner et al. 2000; Jacobs et al. 2009). As demonstrated by Schreiner et al. (2000), these interactions can have important implications for habitat management; thus highlighting that it is critical that changes be monitored over time.

Methodological—herbarium based studies

In addition to its contribution to our understanding of the response of *R. fruticosus* phenology to temperature, this study also supports the potential validity of future herbarium-based phenology studies, despite our choice of a less-than-ideal subject species. Whereas Borchert (1996) proposed using herbarium specimens to supplement field-based studies, both Bolmgren and Lönnberg (2005) and Miller-Rushing et al. (2006) concluded that herbarium specimens alone could provide a sufficiently robust source of data. While our analysis yielded statistically significant temperature responses in phenology for *R. fruticosus*, some problems with the herbarium-based approach were noted. As a consequence, we could not extend this study in as many ways as we would have liked; for example, the inclusion of the additional *Rubus* species was not possible (as there were insufficient specimens). Indeed, in their analyses, Moussus et al. (2010) noted sensitivity to sample size, recommending a sample size of several hundred. Although a larger sample size would likely have allowed us to carry out more detailed analyses, we were able to draw several conclusions despite a sample size well under this recommendation. This suggests that future herbarium-based studies may also be successful where resources (i.e. specimens) are limited.

An additional limitation noted by others refers to the use of species with long flowering periods, given the uncertainty with which a 'true' flowering date (or fruiting date) can be determined from herbarium specimens (Miller-Rushing et al. 2006; Gallagher et al. 2009). Despite *Rubus* spp. having a long flowering period, we were able to obtain statistically significant results for *R. fruticosus*. Primack et al. (2004) similarly found long-flowering species to be as capable of producing results as those with a short flowering duration. In addition, although Miller-Rushing et al. (2006) cited long flowering periods as a potential limitation of herbarium-based studies, they too ultimately concluded that, with a large enough sample size, results comparable to those that would be obtained in a field study were possible.

Variable information on collection cards (such as incomplete dates) meant that not all of the *Rubus* spp. specimens available in the herbarium were used in this study. While the location on some of the information cards was very specific (e.g. "V.c.H27, Hedge of lane by bridge at Tawnynameeltoge, South of Knappagh"), on others it was only as specific as the county (e.g. 'Dublin'); this is a problem that has been noted elsewhere (MacGillivray et al. 2010). Interestingly, it would appear as though this vagueness was sometimes intentional—after giving a rather vague location for their self-described "triumphant" discovery of *Rubus chamaemorus* (following the aforementioned 66-year search), Hart and Barrington (1892) assert, "Notwithstanding the difficulty which it is believed any botanist would find in detecting the exact locality, it is prudent not to disclose it further. The species occurs so sparingly, and has apparently such a keen struggle for life… that no effort should be spared to protect [it]."

Many of the problems experienced with missing information are the result of the specimens not having been collected originally for the purpose of phenology-based climate change research. As a result, specimens were not always gathered according to proper scientific methodology (T. Sparks in Whitfeld 2001). Despite this, Schnelle (in Rutishauser et al. 2007) asserts that amateurs collecting specimens ‘for pleasure’ make the most precise observations. However, a decline in the number of herbarium specimens collected noted here (i.e. very few post-2000), has been reported elsewhere (Hedenäs et al. 2002; Prather et al. 2004; Lavoie and Lachance 2006). Rumpff et al. (2008) attributed this in part to restrictions on collecting imposed by environmental policies. In addition, limited space in herbaria has meant that there has been, at least amongst some, a reluctance to collect a large number of specimens (Rumpff et al. 2008). An associated problem—relating to both ‘missing information’ of herbarium specimens and to available space in herbaria—concerns the accuracy of using one branch (i.e. one specimen) as an indicator of what is occurring on the rest of the plant. Because it is not possible for herbaria to store large numbers of specimens from a given plant, it is necessary to make inferences using the available specimens. Given that there can be a variety of phenophases present on a given plant (particularly those with long flowering periods), it is possible that certain herbarium specimens (as only a part of a plant) may not entirely represent what is occurring on plant in total. Such specimens may show up as outliers. Although this can present a potential limitation, our study provides evidence that an adequately large sample size will overcome any such atypical samples and can thus result in a study with statistically significant findings.

To conclude, we have used herbarium specimens to demonstrate that three phenophases of *R. fruticosus* in Ireland exhibit a statistically significant response to temperature. As a result of this sensitivity to temperature, *R. fruticosus* may be particularly sensitive to climate warming. Our use of a herbarium-based methodology has served as an evaluation of an approach suggested by previous authors. In our selection of a species with long flowering and fruiting periods, we were able to fully assess the robustness of an approach that has commonly been suggested to be limited by duration of phenophases. In addition, through our use of multiple phenophases, we extend recent research that has evaluated herbaria as potential sources of phenological data—but has focussed only on flowering—to demonstrate that herbarium-based approaches can be used for a variety of phenophases.

We therefore recommend the use of herbarium specimens as a robust methodology for use in phenological research—with the caveat of selecting a species with a large sample size from which significant results can be elucidated. Given that herbaria worldwide contain an estimated 350 million specimens, representing an as yet largely untapped resource, the potential for future studies is enormous.

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