Drivers of leaf-out phenology and their implications for species invasions: insights from Thoreau's Concord

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Summary

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• To elucidate climate-driven changes in leaf-out phenology and their implications for species invasions, we observed and experimentally manipulated leaf out of invasive and native woody plants in Concord, MA, USA.
• Using observations collected by Henry David Thoreau (1852–1860) and our own observations (2009–2013), we analyzed changes in leaf-out timing and sensitivity to temperature for 43 woody plant species. We experimentally tested winter chilling requirements of 50 species by exposing cut branches to warm indoor temperatures (22°C) during the winter and spring of 2013.
• Woody species are now leafing out an average of 18 d earlier than they did in the 1850s, and are advancing at a rate of 5 ± 1 d °C^{-1}. Functional groups differ significantly in the duration of chilling they require to leaf out: invasive shrubs generally have weaker chilling requirements than native shrubs and leaf out faster in the laboratory and earlier in the field; native trees have the strongest chilling requirements.
• Our results suggest that invasive shrub species will continue to have a competitive advantage as the climate warms, because native plants are slower to respond to warming spring temperatures and, in the future, may not meet their chilling requirements.

Introduction

The connection between plant phenology and temperature is well established for temperate ecosystems; spring plant phenophases, such as leaf out and flowering, generally occur earlier in warmer years (Cleland *et al.*, 2007; Polgar & Primack, 2011). Across temperate regions of the world, woody species are advancing their timing of leaf out at an average rate of 4.6 d °C^{-1} in response to warming annual temperatures (Wolkovich *et al.*, 2012). However, the response of leafing out to warming is species specific (Lechowicz, 1984; Cleland *et al.*, 2007), and the magnitude of the response of individual species to temperature can affect species composition at the community level. Weak responses to temperature in flowering are linked to native species declines (Willis *et al.*, 2008) and strong responses are linked to increases in abundance of non-native species (Hulme, 2011). Strong responses of leafing out to increased temperature may increase the growing season length of a species and its ability to compete for local resources in spring (Fridley, 2012).

As spring temperatures have reached record highs, flowering and leaf-out dates continue to advance in many temperate areas (Wolkovich *et al.*, 2012; Ault *et al.*, 2013; Ellwood *et al.*, 2013). Leaf-out models that consider only heat sums, the accumulation of warm temperatures (or thermal units) that force bud burst, predict continued advances in leaf-out dates as winter and spring temperatures warm (Vitasse *et al.*, 2009). However, before the

accumulation of heat sums, temperate plants must accumulate a specific number of chilling units for the release from dormancy, referred to as the chilling requirement (Falusi & Calamassi, 1990; Faust *et al.*, 1997). As winter and spring temperatures continue to warm, the fulfillment of chilling requirements may be delayed or may go unmet for many species, possibly delaying spring onset or causing abnormal leaf-out events, such as extremely late bud burst or incomplete leaf development (McCreary *et al.*, 1990; Morin *et al.*, 2009; Fu *et al.*, 2012). In these situations, plants may need additional warming to overcome the unmet winter chilling requirement. In fact, delays in spring leafing out attributed to incomplete winter chilling have already been reported in some regions, such as the southeastern USA and the Tibetan Plateau (Zhang *et al.*, 2007; Yu *et al.*, 2010; Cook *et al.*, 2012).

Chilling requirements are believed to serve as a way to protect plants from leafing out too early in the spring in response to brief warm spells, when they would still be vulnerable to freezing events after the warm spell had passed (Gu *et al.*, 2008; Korner & Basler, 2010; Basler & Korner, 2012). Winter chilling, spring warming and photoperiod have all been identified as requirements necessary for spring leaf out. Although several studies suggest that chilling and warming have a stronger effect than photoperiod on leaf-out phenology, their relative importance and differences among species remain largely unknown (Vitasse *et al.*, 2009; Polgar & Primack, 2011; Laube *et al.*, 2013; Vitasse & Basler, 2013).

Invasive species often leaf out earlier than native species (Xu *et al.*, 2007), a difference that suggests that invasive species may have weaker winter chilling requirements or stronger responses to spring warming. If, in general, invasive species have significantly weaker winter chilling requirements or stronger responses to spring warming than native species, there is a risk that invasive species will gain an additional competitive advantage as the climate continues to warm. Differences in response rates of invasive vs native woody plants may have important implications for the future of temperate forests, altering species composition, ecosystem functions and feedbacks to the climate system.

In this study, we investigated the sensitivity of spring leaf-out phenology to temperature and the relative chilling requirements for a variety of woody species in Concord and nearby areas of Massachusetts, USA. Species included native trees and shrubs, non-native invasive shrubs and vines. We experimentally determined the duration of chilling required by each species before leaf out and explored differences between native and invasive species. To study sensitivity to spring and winter temperatures in the field, we used observational leaf-out data from Concord from two time periods, 1853–1860 and 2009–2013. We investigated the following questions. How have first leaf-out dates changed from the 1850s to the present? Do native trees, native shrubs and non-native invasive shrubs differ in the duration of chilling they require before leafing out? How do winter chilling requirements affect the timing of leaf out in the field and the phenological response of plants to warmer spring temperatures? The answers to these questions will ultimately help ecologists predict which woody species will benefit most and how the temperate forest community will change as temperatures continue to warm as a result of climate change.

Materials and Methods

Observational field study

The field study was conducted in Concord, MA, northeastern USA, for three reasons. First, this temperate forest ecosystem has four distinct seasons, and pronounced winters with multiple days below freezing and potential freezes into late spring. Second, the weather in this region of the USA is highly variable both among and within years. Third, we have extensive prior knowledge of the effects of climate change on the flowering phenology and abundance of plant species over the past 150 yr in this location (Miller-Rushing & Primack, 2008; Willis *et al.*, 2008, 2010; Primack *et al.*, 2009; Primack & Miller-Rushing, 2012; Ellwood *et al.*, 2013).

We obtained historical records of first leaf dates (FLDs) for woody plant species in Concord, MA, USA, recorded by the environmental writer and philosopher Henry David Thoreau from the Morgan Library in New York City. These observations were made from 1852 to 1860, with the most complete observations made in the springs of 1854, 1855 and 1860. From this list, we selected the 43 deciduous species that were still present in Concord and for which there were > 2 yr of leaf-out data from the 1800s (Table 1, Supporting Information Table S2). Thoreau's observations of flowering times have already been extensively studied and compared with modern observations (Primack & Miller-Rushing,

2012; Ellwood *et al.*, 2013), but this is the first scientific use of his leaf-out observations. Thoreau did not describe his field methods for recording leaf-out dates. However, Thoreau's detailed descriptions in his journals of the small size of the young leaves and his frequent journal entries suggest that his methodology was similar to ours. We also know that Thoreau was recording the first flowering date anywhere within Concord, and so it seems likely that he was recording the first leaf-out date of particular tree species anywhere in Concord, rather than focusing on specific individuals or plots of land, again just as we did. In any case, because of the extremely fast process of leaf flush in Massachusetts, even variations of Thoreau's method would almost certainly yield similar, if not identical, values to our methods.

To obtain the current FLDs of these same 43 species, we visited Concord twice a week throughout March, April and May of 2009–2013, and recorded the first time each of the study species was in leaf (Tables 1, S2). We considered a species to have leafed out when at least three branches on one individual plant anywhere in Concord had at least one fully unfolded leaf, the definition used by Project Budburst (www.budburst.org), and similar to that used by the USA National Phenology Network (www.usanpn.org). We obtained air temperatures from the Blue Hill Meteorological Observatory in Milton, MA (data available from the National Climatic Data Center, www.ncdc.noaa.gov), a site 33 km southeast of Concord, where temperatures are highly correlated with those in Concord (Miller-Rushing & Primack, 2008). Of the 43 species, 23 had complete data for the years 1854, 1855, 1860 and 2009–2013.

In 2013, we recorded the FLDs in the field for an additional 13 species from Concord, and also from Newton (22 km southwest of Concord) and the Arnold Arboretum of Harvard University (34 km southwest of Concord; Table 1).

Laboratory chilling study

In 2013, we collected dormant twigs of 50 species of woody plants, including trees, shrubs and vines. The species collected included 36 of the 43 species from the observational field study in Concord, plus four additional native species and 10 additional invasive species (Table 1). We collected most twigs from the wild in Newton and Concord, MA. In some cases, plant species were too rare locally for us to collect twigs from wild individuals; in those cases, we collected twigs from plants growing at the Arnold Arboretum (Table 1).

We collected twigs on 8–11 January, 14–20 February, 20–24 March and 18–20 April, 2013. In Table S1, we provide the chilling days and thermal units that plants had accumulated in the field before these collection dates. In the first collection period, we collected 48 species. In February, we did not collect the nine species that leafed out in fewer than 21 d after being collected in January. However, in February, we began collecting two additional species, witch hazel (*Hamamelis virginiana*) and spicebush (*Lindera benzoin*), that we had not collected in January. We collected a total of 41 species in February. In March, we collected all 50 species. In April, we collected only those species that took longer than 40 d to leaf out in the laboratory after the March collection – we wanted to confirm that they would be able to leaf out under laboratory conditions when collected as twigs. We collected 20 twigs from

Table 1 Species used in the study listed with their functional group, chilling requirement, days to leaf out after twig collection in March 2013, date of leaf out in the field in 2013 and collection site – Newton (N), Arnold Arboretum (A) and Concord (C)

Common name	Scientific name	Functional group	Chilling requirement	Days to leaf out in March	Date of leaf out in 2013	Collection site
Japanese barberry	*Berberis thunbergii*	Invasive shrub	Minimal	8	9 April	N
European barberry[1]	*Berberis vulgaris*	Invasive shrub	Minimal	16	16 April	A
Bittersweet	*Celastrus orbiculatus*	Invasive vine	Minimal	22	2 May	N
Silverling	*Eleagnus umbellata*	Invasive shrub	Minimal	9	14 April	A
Compact privet	*Ligustrum compactum*	Invasive shrub	Minimal	9	9 April	A
Ibota privet	*Ligustrum ibota*	Invasive shrub	Minimal	9	14 April	A
Late honeysuckle	*Lonicera maackii*	Invasive shrub	Minimal	9	9 April	A
Subsessilis honeysuckle	*Lonicera subsessilis*	Invasive shrub	Minimal	9	14 April	A
Multiflora rose	*Rosa multiflora*	Invasive shrub	Minimal	8	9 April	N
American elder[1]	*Sambucus canadensis*	Native shrub	Minimal	9	16 April	A
Apple[1]	*Malus domestica*	Non-native tree	Minimal	8	9 April	N
Winged euonymus	*Euonymus alatus*	Invasive shrub	Mild	15	24 April	A
Black chokeberry[1]	*Aronia arbutifolia*	Native shrub	Mild	9	16 April	A
Buttonbush[1]	*Cephalanthus occidentalis*	Native shrub	Mild	16	10 May	N
Sweet fern[1,2]	*Comptonia peregrina*	Native shrub	Mild	15	27 April	N
Silky dogwood[1]	*Cornus amomum*	Native shrub	Mild	15	24 April	N
Hazelnut[1]	*Corylus americana*	Native shrub	Mild	13	24 April	C
Sheep's laurel[1,2]	*Kalmia angustifolia*	Native shrub	Mild	22	27 April	N
Bayberry[1]	*Myrica pensylvanica*	Native shrub	Mild	16	10 May	A
Meadow sweet[1]	*Spiraea latifolia*	Native shrub	Mild	9	16 April	A
Early low-bush blueberry[1]	*Vaccinium angustifolium*	Native shrub	Mild	16	16 April	A
Arrowwood[1]	*Viburnum recognitum*	Native shrub	Mild	13	21 April	C
Grape[1]	*Vitis aestivalis*	Native vine	Mild	15	2 May	N
Paper birch[1]	*Betula papyrifera*	Native tree	Mild	9	24 April	A
Gray birch[1]	*Betula populifolia*	Native tree	Mild	15	27 April	N
American ash[1,2]	*Fraxinus americana*	Native tree	Mild	33	2 May	A
Buckthorn	*Rhamnus frangula*	Invasive shrub	Moderate	15	27 April	N
Wild alder[1,2]	*Alnus serrulata*	Native shrub	Moderate	20	18 April	C
Coast pepperbush[1,2]	*Clethra alnifolia*	Native shrub	Moderate	15	27 April	N
Huckleberry[1]	*Gaylussacia baccata*	Native shrub	Moderate	15	2 May	N
Witch hazel	*Hamamelis virginiana*	Native shrub	Moderate	15	27 April	C
Mountain laurel[1,2]	*Kalmia latifolia*	Native shrub	Moderate	33	7 May	A
Staghorn sumac	*Rhus typhina*	Native shrub	Moderate	15	2 May	N
Highbush blueberry[1]	*Vaccinium corymbosum*	Native shrub	Moderate	8	24 April	N
Late low-bush blueberry[1]	*Vaccinium pallidum*	Native shrub	Moderate	22	21 April	N
Red maple[1,2]	*Acer rubrum*	Native tree	Moderate	15	21 April	N
Black birch[1]	*Betula lenta*	Native tree	Moderate	22	27 April	N
Pignut hickory[1,2]	*Carya glabra*	Native tree	Moderate	16	2 May	A
Bigtooth aspen[1,2]	*Populus grandidentata*	Native tree	Moderate	32	2 May	N
Black cherry[1,2]	*Prunus serotina*	Native tree	Moderate	16	16 April	A
White oak[1,2]	*Quercus alba*	Native tree	Moderate	22	2 May	N
Red oak[1,2]	*Quercus rubra*	Native tree	Moderate	15	27 April	N
American elm[1,2]	*Ulmus americana*	Native tree	Moderate	23	24 April	A
Spicebush[2]	*Lindera benzoin*	Native shrub	Strong	42	2 May	C
Greenbriar[1,2]	*Smilax rotundifolia*	Native vine	Strong	42	10 May	N
Silver maple[1,2]	*Acer saccharinum*	Native tree	Strong	43	2 May	A
Sugar maple[1,2]	*Acer saccharum*	Native tree	Strong	43	27 April	A
American beech[1,2]	*Fagus grandifolia*	Native tree	Strong	42	27 April	N
Black gum[1,2]	*Nyssa sylvatica*	Native tree	Strong	42	10 May	N
Sassafras[2]	*Sassafras albidum*	Native tree	Strong	40	7 May	C

[1]Species that both we and Thoreau monitored in Concord.
[2]Species that did not leaf out within 40 d after the January collection.

each of the selected species at each collection time. For wild species, we collected the 20 twigs of each species from at least five individuals. For species collected at the Arnold Arboretum, we were only able to collect from one or two individuals per species. In the analysis described below, we used the twigs to generate a single leaf-out date for each species in each treatment, and did not treat them as replicates.

We brought the twigs into a laboratory at Boston University, where we re-cut the base of each one and placed them into containers with 500 ml of tap water. The twigs of each species were

evenly split into two groups of 10, and each group was randomly placed in one of two light banks fitted with plant grow lights (Sylvania 20 W T12 Florescent Bulb, Gro-Lux Wide Spectrum Phosphor; Osram Sylvania Ltd, Danvers, MA, USA). Plants in both light banks were exposed to 14 h of daylight, typical of mid-April in eastern Massachusetts, to standardize photoperiod throughout the experiment and to ensure that it was not a factor in leaf-out timing. The laboratory was maintained at a constant temperature of 22°C, a temperature selected to ensure rapid accumulation of thermal units and to be easily repeated by other researchers. Because the laboratory was well ventilated, the bulbs did not appreciably affect plant temperature.

We evaluated twigs once each week for leaf out. We considered a twig to have leafed out when at least one leaf on the stem was fully unfolded and the top surface of the leaf was visible. We considered a species to have leafed out when over half of the live twigs in one of the two light banks had leafed out. In general, species leafed out at the same time in each of the two light banks and all of the twigs in a light bank leafed out at about the same time. Occasionally, twigs of certain species wilted or stopped developing; however, this occurred rarely and only after they had met our definition of having leafed out, and so these species were still included in all analyses.

Each week, we re-cut the base of each stem to expose fresh tissue and maintain the ability of the twigs to take up water. We changed the water in each container weekly and washed the twigs to remove any mold growing on the stem. Weekly twig evaluations and washing continued for 4–6 wk following each collection date, after which we discarded the remaining twigs. We calculated the time between collection and leaf-out dates for each species after each collection period.

During the winter of 2011–2012, we carried out a pilot study to examine the feasibility of using cuttings from wild plants to test chilling requirements, as well as photoperiod requirements. During the pilot study, we used the same methodology as the 2013 main chilling study with the following exceptions. The pilot study included only 17 species, many of which were included in the main study (Table S2), and we collected twigs in November in addition to January–April. We exposed 10 twigs of each species to one of two light treatments: ambient day length or extended day length (14 h : 10 h, day : night). We identified FLD as the date on which at least one leaf on one twig was unfolded. American beech (*Fagus grandifolia*) was the only species for which photoperiod had an effect on leaf out. Thus, we did not test photoperiod requirements in the main study and focused only on chilling requirements.

Analysis

All analyses were performed using JMP Pro 10 statistical software (SAS Institute Inc., Cary, NC, USA) and an α value cut-off for significance of 0.05. Our statistical analyses are based primarily on direct measurements, such as the dates of collection, the number of days to leaf out and the average spring temperatures, rather than degree-day models, although the two are highly correlated (Archetti *et al.*, 2013; Ellwood *et al.*, 2013). We report chilling days and thermal units of warming in Table S1 for twig collection dates; we

found that thermal units that had accumulated by the date of leaf out in the field were strongly correlated with dates of leaf out in 2013 ($r = 0.97$; $P < 0.0001$).

Field study   For the 23 species for which we had complete data for the years 1854, 1855 and 1860, and 2009–2013, we used a two-way ANOVA to compare the differences in FLD among species and between time periods (Thoreau's years vs recent years). For each of the 43 species for which we had multiple years of observations, we used simple linear regression to test the relationship between FLD for each year with the mean March and April temperature for that year. We considered the slope of this relationship to be the 'sensitivity to temperature'. We then used linear regression to test the relationship between sensitivity to temperature and the mean FLD from 2009 to 2013 to determine whether sensitivity to temperature is related to the relative time during the spring in which a species typically leafs out (early or late in the season).

Laboratory chilling study   We assigned each species to one of four categories of chilling requirement: minimal, mild, moderate or strong. These groupings were based on the differences between the number of days it took for a species to leaf out after being collected and brought into the laboratory (Table 2). For each collection date, we calculated the number of chilling days that had occurred before that date – chilling days were equal to the number of days after 1 November on which the mean temperature was below 5°C (Murray *et al.*, 1989). In 2013, species in the minimal chilling requirement category required fewer than 49 winter chilling days before they were competent to leaf out, species in the mild category required fewer than 83 winter chilling days, species in the moderate category required fewer than 114 winter chilling days and species in the strong category required 114 or more chilling days before they became competent to leaf out (Table S1). For the pilot study of 2011–2012, winter temperatures were milder, and so species accumulated fewer chilling days in the field before they were collected.

We used Spearman rank correlations in analyses for some of our categorical data and for data that were not normally distributed. For example, we used Spearman rank correlations to determine the strength of correlation between chilling categories assigned in the pilot study of 2011–2012 and the main study in 2013 for overlapping study species.

Connecting field observations and chilling results   Based on their ecological characteristics, we assigned 46 of the 50 species used in the chilling study to a functional group category: invasive shrub (10 species), native shrub (20 species) or native tree (16 species) (Table 1). To characterize invasive shrubs, we used the current (as of August 2013) Invasive Plant Atlas of New England (IPANE) list (www.eddmaps.org/ipane). Although they are not on the IPANE invasive plant list, we classified two *Ligustrum* species (*L. compactum* and *L. ibota*) and one *Lonicera* species (*L. subsessilis*) as invasive, because of relatedness and morphological similarity to the many invasive species in the same genera. Four species did not fit into the three main functional group categories, and were omitted from the functional group analyses – two native vines, grape (*Vitis aestivalus*)

Table 2 Definitions of categories of chilling requirements in our pilot experiment and main experiment

Chilling requirement	Pilot experiment	Main experiment
Minimal	Days to first leaf after November collection ≤ double the days to first leaf after March collection[1]	Days to first leaf after January collection within 10 d of days to first leaf after March collection
Mild	Days to first leaf after January collection ≤ double the days to first leaf after March collection[1]	Days to first leaf after February collection within 10 d of days to first leaf after March collection
Moderate	Days to first leaf after February collection ≤ double days to first leaf after March collection	First leaf < 40 d after March collection
Strong	Days to first leaf after February collection more than double days to leaf out after March collection	First leaf > 40 d after March collection

[1]Also includes species that leafed out within 1 wk of collection in the pilot study for the November and January collections.

and greenbriar (*Smilax rotundifolia*), the invasive non-native vine bittersweet (*Celastrus orbiculatus*) and the non-native apple tree (*Malus domestica*).

We used one-way ANOVA to test for the effects of functional groups on leaf-out time in March in the laboratory and FLD in the field. We followed this test with a multiple range test, Tukey's honestly significant difference test, to determine which particular functional groups were different from each other. In order to separate the effects of functional group and chilling requirement category on days to leaf out in March and FLD in the field, we used two-way ANOVA to test for relationships of chilling categories and functional groups on the variables of days to leaf out in March, field FLD and sensitivity to temperature.

Results

Field study

Woody plants in Concord are leafing out significantly earlier now (2009–2013) than they were in Thoreau's years (1854–1855,

1860). In a two-way ANOVA, there was a significant difference in FLD among species ($P < 0.001$), as well as between time periods ($P < 0.001$). For the 23 species for which we have complete data for years 1854, 1855, 1860 and 2009–2013, the mean FLD in Thoreau's years was May 8 ± 7 d (± SD), whereas the mean leaf out in recent years was April 20 ± 7 d, a difference of 18 d. All species are leafing out earlier now than in Thoreau's time ($P < 0.001$; Fig. 1). In 2013, there were 31 d between the FLD of the earliest and latest of the 23 species (Table 1). We found a significant correlation between order of FLD in the two time periods, meaning that the species tended to leaf out in the same sequence in both time periods ($r = 0.76$; $P < 0.001$; Fig. 1).

Warmer late-winter and early-spring temperatures in March and April were associated with earlier FLDs. Winter temperature in January and February had no apparent effect on FLD, presumably because there were always sufficient chilling days in Concord by the time of spring leaf out. The mean March and April air temperature for Thoreau's years (1854, 1855, 1860) was 2.8°C; the mean for these years is representative of the mid-1800s overall, as the mean March and April temperature for the years 1845–1865 was 2.9°C
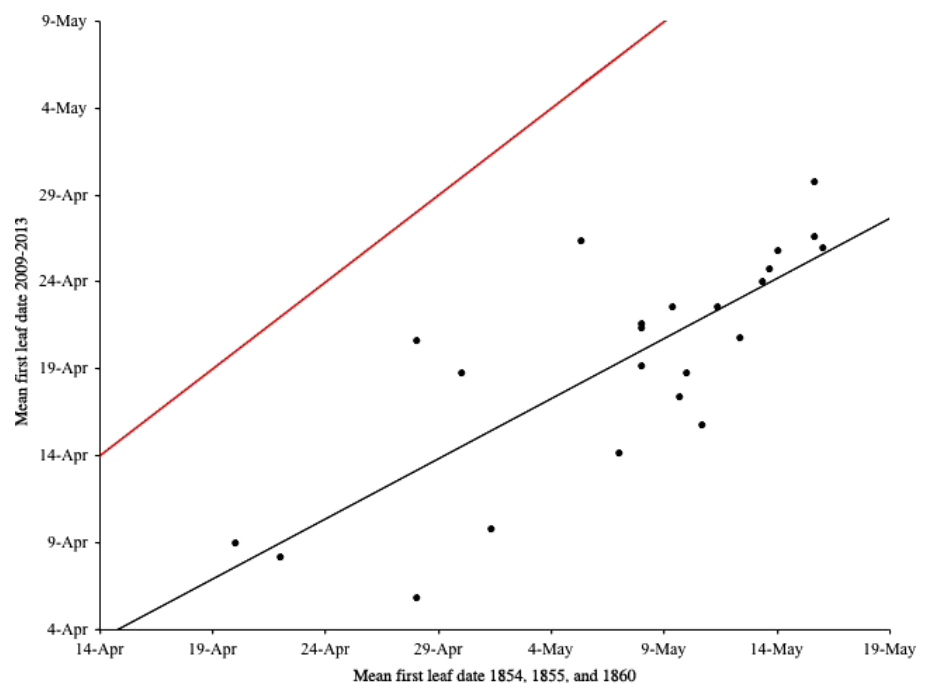

Fig. 1 Mean first leaf dates (FLDs) of individual species for recent years regressed against the mean FLDs for Thoreau's years. The data included in this figure are those for which we have complete data for the years 1854, 1855, 1860 and 2009–2013. The relationship between the two variables is highly significant (slope = 0.69; $R^2 = 0.62$; $P < 0.001$). All of the study species are leafing earlier in our years than they did in Thoreau's years. The red line shows the line along which the points would fall if first leaf dates had not changed; the black line is the regression line through the points.

(Fig. S1). The mean March and April temperature for 2009–2013 was 5.1°C (Fig. S1). There was a significant negative relationship between mean March and April temperature and FLD for all study species (Table S3). Among the study species, the sensitivity of FLD to temperature ranged from $-2.8 \pm 7.3 \, d \, °C^{-1}$ for silver maple (*Acer saccharinum*) to $-7.1 \pm 15.2 \, d \, °C^{-1}$ for late lowbush blueberry (*Vaccinium angustifolium*). The mean response to temperature was $-5.0 \pm 1.0 \, d \, °C^{-1}$; that is, for each 1°C warming, plants leafed out *c.* 5 d earlier on average. We also found a significant relationship between sensitivity to temperature and mean FLD; species that were most sensitive to spring temperatures tended to leaf out earlier in the spring ($P = 0.038$).

Laboratory chilling study

In the pilot study, 16 of the 17 species took the same number of days to leaf out in each of the two day length treatments, indicating a lack of photoperiod response. For the American beech, twigs collected in November, January and February and placed in the ambient day length treatment never leafed out in the laboratory, presumably because this species had strong winter chilling and photoperiod requirements. However, beech twigs collected at the same time and kept under extended day conditions did leaf out after more than a month. For samples collected on 9 March 2012, the last date of collection in the pilot study, twigs leafed out after 28 d in the extended day length treatment and after 32 d in the ambient day length treatment.

There was a significant difference among species and among collection periods in the number of days it took for leaf out to occur for both the pilot study in 2011–2012 (Table S2) and the main study (Table 1, Fig. 2), as determined by ANOVA ($P < 0.001$ in all cases). In each case, the number of days required to leaf out declined with successive collection dates (Fig. 2).

Overall, winter chilling requirement categories in the main study were minimal for 11 species, mild for 15 species, moderate for 17 species and strong for seven species (Table 1). A Spearman rank correlation showed a significant correlation between the winter chilling requirement categories as determined in the pilot study and the main study ($r = 0.75$; $P = 0.001$) despite changes in methodology between the 2 yr. For example, in both studies, multiflora rose (*Rosa multiflora*) and privet (*Ligustrum compactum*) were in the minimal chilling requirement category, whereas American beech and spicebush were in the strong chilling requirement category. Although the results of the pilot and main study were correlated, there was some variation that may be attributed to the change in definitions of the chilling requirement categories.

Functional groups (e.g. invasive shrubs, native shrubs and native trees) largely fell into different chilling categories, with invasive shrubs requiring mostly minimal winter chilling and native trees having moderate or strong winter chilling requirements (Fig. 3; Table 1). Native shrubs largely had mild and moderate chilling requirements. Species with minimal chilling requirements included eight invasive shrubs, one non-native tree (apple), one invasive vine (bittersweet) and one native shrub (American elder, *Sambucus canadensis*). Species with mild chilling requirements included primarily native shrubs. Species with moderate chilling

requirements included a mix of trees and native shrubs, although the group also included one invasive shrub (buckthorn, *Rhamnus frangula*). Species with strong chilling requirements were all native trees, except for one native shrub (spicebush) (Table 1).

Because a majority of species had met their winter chilling requirements by March, we used the number of days required to leaf out following March collection as a metric for species responsiveness to spring warming as measured in the laboratory. Using one-way ANOVA, functional groups had significantly different times to leaf out in March ($P < 0.001$), with native trees leafing out significantly later (27 d) than invasive shrubs (11 d) and native shrubs (17 d) (Table 3; Fig. 2). Two-way ANOVA showed a significant difference among chilling requirement categories in the number of days to leaf out ($P < 0.001$), but no significant difference among functional groups ($P = 0.34$), indicating that invasive species leaf out quickly largely as a result of their minimal and mild chilling requirements. Species with minimal chilling requirements were the fastest to leaf out in March (mean $= 11 \pm 4$ d), whereas species with moderate chilling requirements were the slowest (mean $= 19 \pm 6$ d), with species with mild chilling requirements in between (mean $= 15 \pm 6$ d). Species with strong chilling requirements that had not met their winter chilling requirements by March (as indicated by requiring > 40 d to leaf out in the laboratory) were excluded from this analysis; these species included American beech, silver maple, sugar maple (*Acer saccharum*), spicebush, black gum (*Nyssa sylvatica*), sassafras (*Sassafras alba*) and greenbriar (*Smilax rotundifolia*).

Connecting field observations and chilling results

One-way ANOVA indicated that functional groups differed significantly ($P < 0.001$) in their FLDs in the field – invasive shrubs leafed out significantly earlier (mean = April 15) than native shrubs (26 April) and native trees (28 April; Table 3). Two-way ANOVA indicated a significant association between FLD in the field in 2013 and chilling requirement category as determined in the laboratory ($P = 0.005$), but not with functional group ($P = 0.87$). The species with minimal chilling requirements, as determined in the laboratory, were the first to leaf out in the field, whereas species with moderate and strong chilling requirements leafed out later (Fig. 4). The lack of a significant effect of functional group in the two-way ANOVA suggests that the association of functional groups with FLDs is through their correlation with chilling categories.

Spearman rank correlation indicated that the order in which the 50 species leafed out in March in the laboratory was strongly related to the order in which they leafed out in the field in 2013 ($r = 0.64$; $P < 0.001$; Table 1), indicating that clipped twigs serve as a valid proxy for leaf-out phenology of wild plants. Apple, Japanese barberry (*Berberis thunbergii*) and multiflora rose all took 8 d – the fewest days of any species – to leaf out in the laboratory after the March collection, and shared the earliest leaf-out date in the field on 9 April. Sassafras, greenbriar and black gum, however, took at least 40 d to leaf out in the laboratory after the March collection and leafed out in the field between 6 and 9 May in 2013, among the latest dates of any species in our study (Table 1).

The sequence of leaf out across species was largely the same in the field in 2013 as it was in the laboratory after January collection
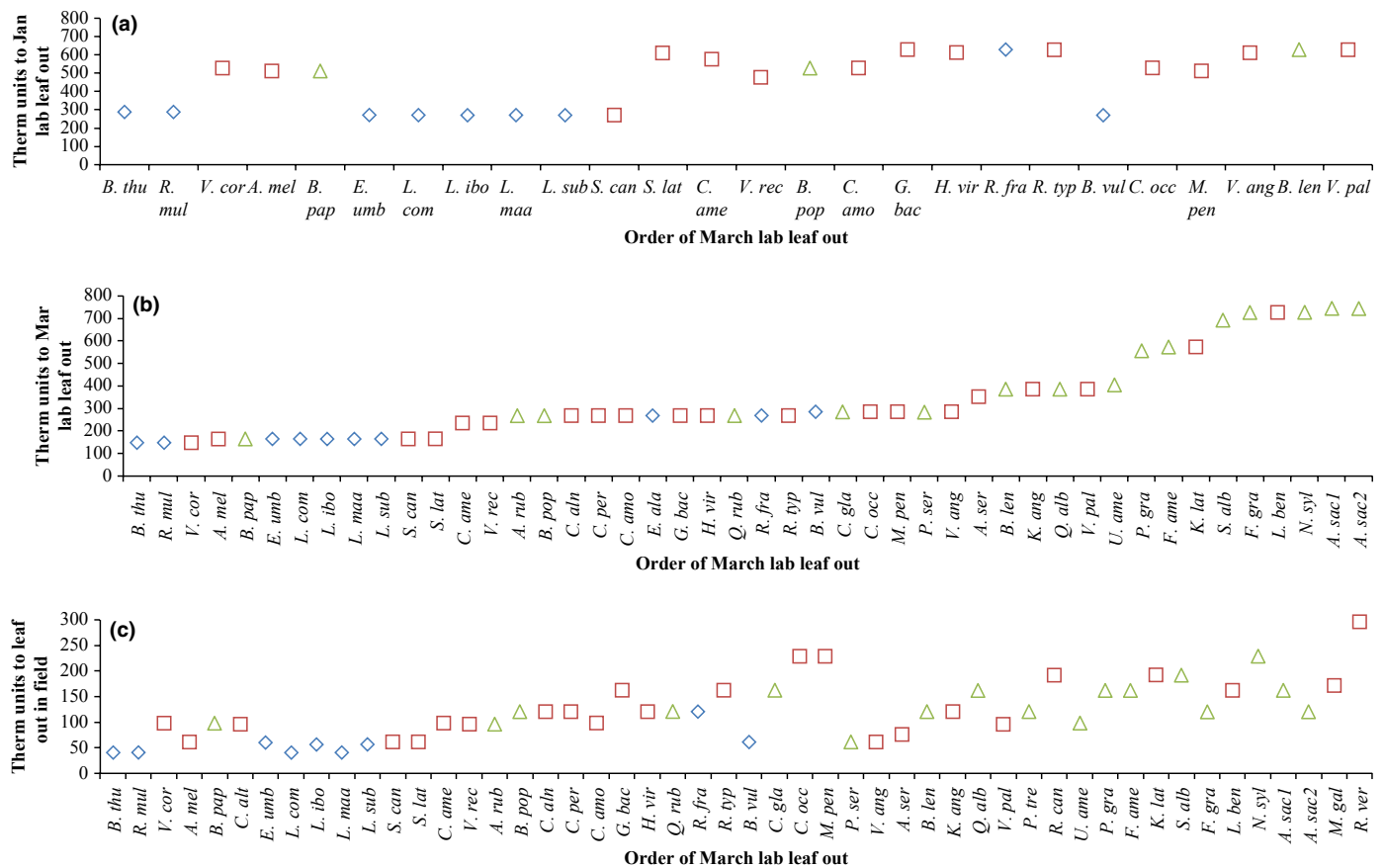
Fig. 2 The number of thermal units of warming to which the twigs of a species had been exposed on the day on which they leafed out in 2013, for (a) the twigs collected in January of the laboratory experiment, (b) the twigs collected in March of the laboratory experiment and (c) the plants growing outside (see Table 1). Along the x-axis, species are listed in the order in which the twigs collected in March leafed out in the laboratory for the first time. For species that leafed out for the first time on the same day, the rankings were assigned alphabetically. The abbreviations stand for the first letter of the genus and first three letters of the species; *A. sac1* is *Acer saccharinum* and *A. sac2* is *Acer saccharum*. For (a), thermal units are calculated as 22°C times the number of days in the experimental conditions. For (b), the thermal units are 22°C times the number of days in the experimental conditions plus 13, the thermal units experienced outside before collection. For (c), thermal units are based on weather records collected at the Blue Hills Meteorological Observatory (data from ncdc.noaa.gov). It should be noted that the y-axis ranges from 0 to 800 for (a) and (b), and from 0 to 300 for (c). Thermal units were calculated in order to put the field and experimental data on comparable scales; however, it is important to note that thermal units were calculated differently for the field and laboratory study, and the plants in each study probably experienced thermal units differently (e.g. because day and night temperatures did not differ in the experimental set-up, plants in the laboratory probably experienced more thermal units than they could developmentally use). Functional group: blue diamonds, invasive shrub; red squares, native shrub; green triangles, native tree.

(Spearman rank correlation *r* = 0.66; *P* < 0.001). This suggests that an extremely warm winter and early spring would not substantially affect the sequence in which species leaf out. The exceptions were black cherry (*Prunus serotina*) and wild alder (*Alnus serrulata*), which took > 40 d to leaf out in the laboratory after being collected in January, but which leafed out relatively early in the field (16 and 18 April, respectively). Bayberry (*Myrica pensylvanica*) and buttonbush (*Cephalanthus occidentalis*) were also exceptions and leafed out in 30 and 31 d after January collection, but did not leaf out in the field until relatively late, both on 10 May.

For the subset of 43 species monitored by Henry David Thoreau and for which we have sensitivity to temperature in the field as calculated using field observations from the 1850s and the present, neither chilling requirement category nor functional group explained the sensitivity to temperature (two-way ANOVA; *P* = 0.61; Table S3). However, this dataset excludes all of the invasive species, with the exception of European barberry (*Berberis*

vulgaris), because Thoreau did not monitor the leafing out times of these species.

Discussion

Changes in timing of leaf out

Our study is unusual in exploring links between changing leaf-out times in the field and winter chilling requirements using laboratory studies. The most significant results are as follows: all of the species examined are leafing out earlier now than they did 160 yr ago because of warmer spring temperatures; non-native invasive species leaf out earlier in the spring and have weaker winter chilling requirements than native trees and shrubs; and the leaf-out dates of invasive shrubs will probably continue to advance faster than those of native trees and shrubs as winter chilling is reduced and spring temperatures continue to warm.
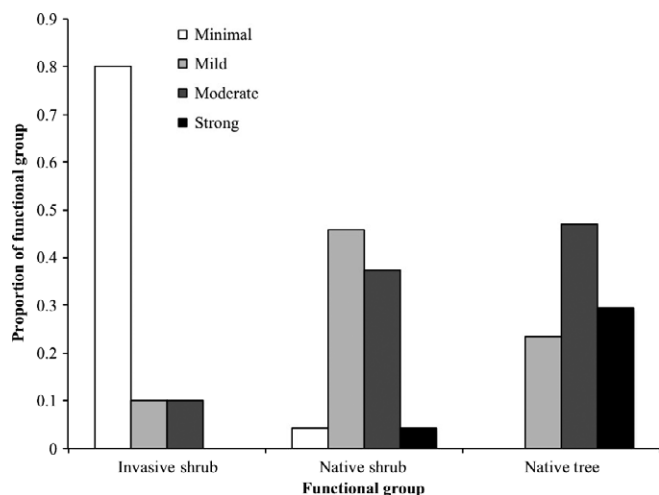
Fig. 3 Proportion of total species in each functional group with minimal, mild, moderate and strong winter chilling requirements. Functional groups are invasive shrubs ($n = 10$), native shrubs ($n = 20$) and native trees ($n = 16$).

Table 3 Mean days to leaf out in March and 2013 first leaf-out date (FLD) for each functional group

Functional group	Days to leaf out ($\pm$ SD)	2013 FLD ($\pm$ SD)
Invasive shrubs	10.7 ± 3.2 (b)	April 15 $\pm$ 6.4 d (a)
Native shrubs	16.9 ± 8.2 (b)	April 27 $\pm$ 8.8 d (b)
Native trees	26.8 ± 12.3 (a)	April 28 $\pm$ 8.2 d (b)

Values given are the number of days taken by species in each functional group to leaf out in the laboratory, and the mean first leaf date in the field in 2013. Functional groups include invasive shrubs ($n = 10$), native shrubs ($n = 20$) and native trees ($n = 16$). Within columns, groups that share a letter (a or b) are not significantly different ($P > 0.05$) as determined by Tukey's honestly significant difference test.
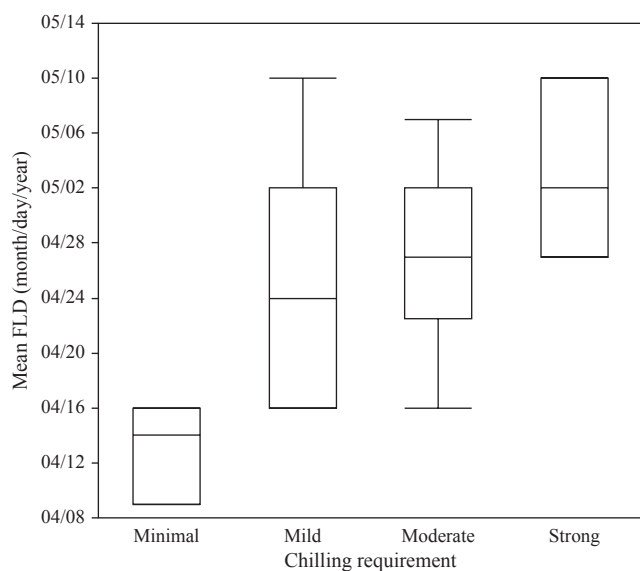

Fig. 4 First leaf-out dates (FLDs) of species in each chilling requirement category. Chilling requirements include minimal (11), mild (15), moderate (17) and strong (7). Central lines are mean FLDs, box boundaries are quartiles and boundaries beyond boxes indicate the range of FLDs. The outlier in the minimal chilling category is the invasive vine bittersweet (*Celastrus orbiculatus*).

In particular, we found that 23 woody species in Concord, MA are now leafing out an average of 18 d earlier than they did in Henry David Thoreau's time (*c.* 1850s) and that these changes are related to warmer spring temperatures. On average, these species leafed out 5 d earlier for each 1°C warming in spring (March–April) temperatures. This response is very similar to the sensitivity of -4.6 d°C^{-1} (annual temperature) found in a meta-analysis of temperate plants around the world (Wolkovich *et al.*, 2012). It appears that the temperature sensitivity of leaf-out phenology is somewhat greater than the sensitivity of flowering phenology in Concord, MA, which was -3.3 d°C^{-1} (Miller-Rushing & Primack, 2008). Similar to flowering phenology, however, we found that species that leaf early in the season show a stronger response to temperature than those that leaf later in the growing season. In the future, this difference in responsiveness may contribute to a larger difference in leaf-out timing between early and late leafing species.

Interspecific variation and implications for future forests

The speed of leaf out in the laboratory seemed to depend on two factors: whether the chilling requirement had been met before clipping; and the time needed for the plant to put out leaves in response to warm conditions. In both years of the winter chilling experiment, invasive shrubs showed the weakest chilling requirements, whereas native trees showed the strongest chilling requirements, with native shrubs intermediate (Fig. 3). The time needed for plants to put out leaves after they had met their chilling requirements in March varied, with invasive shrubs leafing out fastest and native shrubs and trees taking longer. In both of these capacities – chilling requirements and speed of putting out leaves once the chilling requirement is met (calculated as both days and heat sums) – invasive shrubs, which already tend to leaf out early in the season relative to natives, are poised to respond to warming temperatures and shortening of the chilling season in the wild faster than native shrubs and trees. They are well positioned to take advantage of newly emerging temporal niches early in the growing season before native canopy trees leaf out (Richardson & Rejmanek, 2011; Fridley, 2012). Trees and shrubs from more southern locations, which might have lower chilling requirements, may also be able to enter this new early-season niche.

The role of chilling

Although the results of our experiments revealed that most of the species examined have winter chilling requirements which, if not met, can delay the timing of leaf out, our field observations suggest that chilling (or lack thereof) has not yet affected leaf-out dates in wild-growing plants in Concord, MA. The lack of effect in the field is evidence that, in the current climate, all or most of these species are still meeting their chilling requirement in Massachusetts, and the effects of winter chilling will only be seen with additional winter warming. Even during the record warm winter of 2011–2012, when there were only half as many chilling days as 2013, plants apparently still received enough chilling to respond to the warm

spring and leaf out early. Abundant early season warmth may have compensated for species that did not meet their full chilling requirements. Observations in the southeastern USA have shown delays in leaf out linked to insufficient winter chilling (Zhang *et al.*, 2007). Our evidence suggests eventual delays will probably occur further north with more warming.

Photoperiod had little or no effect on leaf-out phenology. American beech was the only species in which we detected a weak photoperiod requirement on leafing out during the pilot study, as shown in earlier studies (Heide, 1993b). Laube *et al.* (2013) also found that photoperiod has a minor effect on leaf out compared with chilling time. Our results suggest that photoperiod requirements will play only a minor role in plant leaf-out responses to a warming climate in southern New England; however; further experimental work is needed to determine the precise relationship between photoperiod and leaf out in North America.

Methodological considerations

There may be some concern that the method of cutting twigs from plants growing outside and exposing them to warm laboratory conditions does not result in realistic experimental conditions. However, this method is increasingly being used to investigate leafing out requirements (Heide, 1993a; Ghelardini *et al.*, 2010; Laube *et al.*, 2013). The best indication that cut twigs in the laboratory serve as an accurate proxy for trees leafing out in the field is that the order of leaf out following March collection was strongly correlated with the order of leaf out in the spring of 2013. The order of leaf out in January was also strongly correlated with the order of leaf out in the field, indicating that the order of leaf out remains largely the same regardless of the length of chilling. It is also true that not all twigs and species responded well to the twig cutting process. For example, red maple (*Acer rubrum*), grape and the two *Lonicera* species used in the main study did initially leaf out, but the leaves either wilted or did not develop further. However, the great majority of twigs in our experiment leafed out normally and showed no signs of ill health. Researchers using this method need to take precautions to ensure the health and responsiveness of the twigs.

Future work

The combination of using observational and historical data from the field and experimental data represents an unusual, but useful way to examine some of the complex factors affecting the leaf-out response of plants to temperature, how the timing of leaf out has changed over time and the physiological factors behind these responses. Our study also demonstrates that the experimental method of using cut twigs can be employed to effectively examine the leaf-out requirements of entire communities of woody species. We suggest that comparable surveys of plant communities from other locations and surveys of widespread species across latitudinal gradients could provide further ecological and evolutionary insights into the leaf-out process during this period of climate change.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Mean combined spring (March and April) temperatures in Massachusetts for the years 1852–2013.

Table S1 Collection dates for twigs in the 2011–2012 pilot study and the 2013 main study, together with chilling units and thermal units that had occurred up to the day of collection, and the day length

Table S2 Days to first leaf out for study species for each collection period under the natural day treatment and chilling categories for the first year pilot laboratory study

Table S3 Results of simple linear regressions measuring the response of leaf-out timing to mean March and April temperature for the field study in Concord, MA, USA

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