

Vulnerability of wild American ginseng to an extreme early spring temperature fluctuation

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Abstract Frost events in natural plant populations can have dramatic demographic consequences. For many plant species, spring emergence occurs when probability of damaging frost is low. Climate change, however, may alter weather patterns such that the environmental cues signaling spring emergence no longer coincide with periods of low frost risk, rendering plant populations susceptible to damaging frost events more frequently than in the past. In 2007, a spring freeze occurred in the eastern United States after a period of unusually warm temperatures. We took advantage of a long-term demographic dataset for American ginseng (*Panax quinquefolius* L.) to examine among and within population patterns of frost damage, as well as the effects of the frost on ginseng demography. Higher temperatures prior to the frost event increased the probability and extent of frost damage at the population level. Within populations, large plants tended to be damaged more frequently than smaller plants. Survival, growth, and reproduction were reduced in frost-damaged plants compared to undamaged plants in the year of the frost event, and negative effects on growth and reproduction persisted the following year. For plants such as ginseng, increases in frost frequency will negatively impact population growth, and likely have serious ramifications for long-term population viability.

Keywords Climate change · Demography · Frost · *Panax* · Winter warming

Introduction

Historic records of the timing of natural and agricultural events have made it possible to track the effects of increasing temperatures on the phenophases of a diversity of taxa across a broad regional spectrum. While the overall signal is clear that phenological events are occurring earlier in response to warming, the magnitude of response varies substantially among species (Bradley et al. 1999; Parmesan and Yohe 2003; Badeck et al. 2004; Schwartz et al. 2006; Parmesan 2007; Miller-Rushing and Primack 2008). There have been ecological surprises as well; a number of species have not responded to warming at all, and a few species have responded in the opposite direction to that predicted (Parmesan 2007). Phenological response of species to climate change is important, because the timing of life history events can have drastic effects on fitness (Hall and Willis 2006; Hall et al. 2007; Jentsch et al. 2009). Indeed, adaptive differentiation has been demonstrated with respect to a variety of phenological traits (Ducousso et al. 1990, 1996; Myking and Heide 1995; Leinonen 1996; Aitken and Adams 1997; Li et al. 1997; Leinonen and Hänninen 2002; Savolainen et al. 2004; Ghelardini et al. 2006; Hall and Willis 2006; Kudo and Hirao 2006; Green 2007; Hall et al. 2007; Sjøgaard et al. 2008; Vitasse et al. 2009a). In the northern hemisphere, timing of the initiation of spring growth is critical for success, especially in the case of plant species. Numerous studies have found that early spring emergence is positively related to biomass accumulation and fecundity (Ross and Harper 1972; Kalisz 1986; Miller 1987; Stratton 1992; Verdú and Traveset 2005). Selection for earlier emergence times increases with plant density, indicating that plants that emerge early gain a competitive advantage over neighbors (Miller et al. 1994) primarily by accessing and usurping light resources (van der Toorn and

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Pons 1988). However, early emergence is risky. In a dynamic climate, frosts are possible in early spring, and can have severe negative repercussions for plant growth and reproduction.

A clear connection has been made between rising global temperatures and increased frost risk in montane, arctic, and boreal ecosystems due to decreased snow accumulation. Decline in winter snow cover, which acts as insulation against harsh climatic conditions, may expose plants to low temperature extremes, resulting in winter freeze damage (Ögren 2001; Bélanger et al. 2002; Bannister et al. 2005). Because snow cover also acts as a buffer against exposure to unusually warm temperatures, decreasing snow accumulation, in conjunction with elevated winter temperatures, may cause premature dehardening of cold-tolerant species (Ögren 2001; Bélanger et al. 2002; Bannister et al. 2005). When dehardening is initiated, carbohydrate concentrations within plant material decrease, leaving the plant vulnerable to freeze damage (Ögren 1997). For perennial wildflower species in alpine systems, snowmelt is often the primary cue to break winter dormancy (Inouye 2008). Decreasing winter snow accumulation, and the resultant advancement in spring snowmelt date, has triggered premature emergence of plant populations even when the probability of frost remains high (Inouye 2000, 2008; Inouye et al. 2002). In this case, the environmental cue that prompts emergence is no longer meaningful, because snowmelt is no longer correlated with low frost risk.

Novel climatic conditions projected by climate models may interact directly with plant phenology to increase frost risk in the future, even in ecosystems where snow accumulation plays little to no role in plant dynamics. In these environments, increased frost risk as a result of elevated temperatures is seemingly counterintuitive. In fact, climate models project a lengthening of the frost-free period and a decrease in the number of freeze events as a result of rising global temperatures (Solomon et al. 2007). These projections are consistent with the observations of climatologists over the last several decades (Easterling et al. 1997). However, these climatological studies refer generally to the occurrence of below freezing temperatures, and do not distinguish frost events that cause damage to plant or crop species. For most plant species, particularly in temperate environments, spring emergence dates are not immutable, and often depend on temperature cues (Rathcke and Lacey 1985). For this reason, vegetation-damaging spring frost events may, in fact, increase in frequency due to two phenomena predicted by global climate models: (1) increased climatic variability, and (2) disproportionate warming of winter temperatures relative to summer temperatures. With respect to the first phenomenon, frost risk may increase with greater climatic

variability, because of increased frequency of unusually warm, growth-stimulating temperatures in late winter or early spring, or conversely, unusually low temperatures following warm periods (Gu et al. 2008; Rigby and Porporato 2008). In terms of the second factor, milder winters mean that the period of transition from winter to summer, when temperatures may both stimulate growth and also drop below freezing, is longer, leaving populations susceptible to frost damage for a greater portion of the year.

The effect of increasing mean temperatures on frost risk has been extensively examined using phenology-based models. The majority of models find an increase in frost risk associated with climatic warming as a result of ‘premature’ spring development during periods when frosts are still possible (Cannell and Smith 1984, 1986; Hänninen 1991; Kellomäki et al. 1995; Pepin 1997; Linkosalo et al. 2000; Jönsson et al. 2004). Several models contradict these projections, however, finding no increase in the probability of damaging frosts (Eccel et al. 2009), or even a decrease in future frost risk (Kramer 1994; Scheffinger et al. 2003). The disparity in model outcomes is partially explained by species-level differences in phenological response to temperature. Future frost risks are diminished when models use species that require low winter temperatures in order to develop at a maximum rate during spring (Hänninen 1991; Murray et al. 1994). In these cases, winter chilling requirements are not fully met due to increased mean winter temperatures, and therefore these plants do not develop ‘early’ in response to elevated spring temperatures. Additionally, models that use environmental cues, such as photoperiod, to trigger spring bud-burst generally find a decrease in climate-mediated frost risk (Linkosalo et al. 2000). When the date of budburst is constrained, trees develop after the threat of sub-zero temperatures has receded, because the date of the last freezing event advances with increasing mean temperatures (Easterling et al. 1997). Methods of modeling climate change also affect model outcome. One study found that models that simulated climatic warming by uniformly increasing mean temperature across the entire year showed a much greater decline in frost frequency compared to models that incorporated disproportionate warming of winter relative to summer temperatures (Kramer 1994).

There is no clear consensus as to whether mean increases in global temperature will increase frost risk via direct effects on plant phenology, though the majority of models suggest this is the case. Changes in frost risk due to climatic warming are likely to differ among species depending on latitude, responsiveness of spring development to temperature cues, and the mechanisms by which species regulate spring growth and

development (e.g., winter-cold requirements, photoperiod response) (Hänninen 1991; Linkosalo et al. 2000; Murray et al. 1994). While discrepancies exist among phenology-based models of frost risk, increased climatic variability projected by global climate models will almost certainly contribute to higher occurrence of damaging frosts in future seasonal climates (Rigby and Porporato 2008).

Frosts may cause physical damage to plant tissue as water within or among plant cells freezes and expands (Pearce 2001; Inouye 2008). Freezing also causes cellular dehydration as developing ice crystals draw water from plant cells, the detrimental effects of which are actually more common than the damage caused by ice crystal formation (Pearce 2001). Significant secondary damage due to frost can occur, because cellular lesions act as portals for plant disease (Pearce 2001). The net effect of freezing temperatures on plant tissue is extensive, or total, loss of leaves, buds, and shoots. For example, in a long-term study of *Helianthella quinquenervis* in the American Rockies, late spring freezes resulted in losses of between 65 and 100% of flower buds in 7 of the 8 study years (Inouye 2008). It is clear from such studies that frost events are important in the long-term demography of species in seasonal climates (Inouye 2000, 2008). The direct effect of a frost event on plant populations is overwhelmingly negative. However, occasionally, frosts may play a beneficial role in reducing the abundance of herbivores, seed predators, and other pests and pathogens that negatively impact plant vigor (Inouye 2000).

Plant-damaging frost events are stochastic and usually infrequent, making patterns and effects of frost in natural ecosystems difficult to study. However, the impacts of warming periods followed by frosts are disproportionately high compared with their frequency, and deserve greater attention in ecological research. In 2007, one such extreme warming–freezing cycle caused extensive damage across a large west to east swath in the eastern deciduous forest. This particular event was captured and analyzed using remote sensing, and significant losses to agriculture were also well documented (Gu et al. 2008). Our study took advantage of a long-term demographic dataset for American ginseng (*Panax quinquefolius* L.) to examine patterns and demographic ramifications of the frost for a widespread native forest species with economic and cultural significance. Specifically, we asked: (1) can temperatures preceding and/or during the frost event predict observed patterns of frost damage among populations; (2) do size characteristics of plants affect the probability of a plant being damaged by frost; and (3) what are the effects of the frost on demographically important parameters, including survival, growth, and reproduction?

Materials and methods

Study species

American ginseng is a widespread herbaceous understory perennial found in deciduous forests of the eastern United States and southern Canada (Anderson et al. 1993; McGraw et al. 2003). Ginseng is an important harvested commodity in the Appalachian region, generating millions of dollars annually in supplemental revenue (Robbins 2000). The aboveground portion of the plant consists of an aerial sympodium, between 1 and 5 leaves, and an umbelliferous inflorescence. An inflorescence contains ca. 1–100 flowers, and flower number depends greatly on size (Schlessman 1985). In a study by Schlessman (1985), mean flower production across 88 reproductive plants was 12.3, with 2-leaved individuals producing an average of 7.5 flowers per inflorescence and 3-leaved plants producing 17 flowers per inflorescence. Ginseng flowers are hermaphroditic, 5-merous, and contain an inferior ovary with 1–2 (rarely 3) styles. Flowers are protandrous, and mature centripetally during mid-summer (Schlessman 1985). Ginseng has a mixed mating system. Known pollinators of ginseng include syrphid flies and halictid bees, both generalist pollinators (Lewis and Zenger 1983). Ginseng plants overwinter as a subterranean taproot and rhizome, and the root is the plant's primary storage organ. In the spring, the aboveground plant develops from a bud that forms on the rhizome in the previous growing season. If the aboveground portion of the plant is damaged, it cannot regenerate during that growing season, but may re-emerge the following year. While the perennating tissue may survive periodic damage to aboveground parts, repeated damage increases mortality. Ginseng plants are long-lived, with old plants attaining ages of 50 years or more (Mooney and McGraw 2009).

Ginseng demography has been modeled using a stage-based population projection approach (Charron and Gagnon 1991; Van der Voort et al. 2003). New seedlings are always 1-leaf plants, though plants may remain 1-leaved for several years, and as such, do not reproduce. Once a plant has attained two leaves, it is considered juvenile, and reproduction is generally low and intermittent. Reproduction in 3-, 4-, and 5-leaf adult plants increases linearly as a function of leaf area. Large adult plants are capable of producing over 100 seeds, but this is unusual and mean seed production per adult plant is typically low. Ginseng plants do not reproduce clonally, except on the rare occasion that physical injury separates a portion of the root or rhizome, which may produce another plant.

Ginseng census and data collection

Data on ginseng survival, growth, recruitment, and seed production were collected as part of a long-term censusing project. In total, 30 populations distributed across 7 states (IN, KY, NY, PA, MD, VA, WV) were censused, containing a grand total of 4,227 plants. Exact locations of these populations are withheld for conservation purposes. In order to avoid attracting the attention of harvesters, plants were marked with a subterranean tag. Plants were relocated using a ‘phototrail’ method, in which photographs and accompanying directions, and/or maps indicate ginseng locations. During the first of two annual censuses, leaf area and sympodium height were measured, and a search was conducted for new seedlings, which were then tagged and measured. While collecting these data, we noticed widespread leaf necrosis and deformity in several ginseng populations. Foliar deformities were ascribed to damage caused by a freezing event based on several lines of evidence. Principally, the visual appearance of leaf damage matched confirmed cases of frost damage in agricultural species. In an examination of a 2003 spring frost event on cultivated ginseng root yield and seed production in Ontario, Canada, Schooley and Proctor (2003) described the morphological symptoms of freeze damage on ginseng foliage and sympodia. Symptoms included: deformed, twisted sympodia, shriveled inflorescences, wrinkled, creased, or necrotic leaves, and/or complete loss of foliar material. The visual appearance of plants affected by frost in our census populations was consistent with descriptions by Schooley and Proctor (2003). Secondly, the damage was widespread geographically, and not species-specific; many trees and other plants in the vicinity were also affected. Temperature data loggers at the sites confirmed freezing temperatures following an extended warm period in April. Notably, the warming, followed by the frost, was widely observed across the eastern United States (Gu et al. 2008). Frost-damaged plants were simply noted as frost damaged or not during the first census. For plants that survived to the end of the growing season, frost damage was confirmed, typically by a second observer. During the second census, ginseng seed production was also measured. Temperature data used in the analyses were collected from HOBO pendant dataloggers (HOBO temperature/light pendant datalogger 64; Onset Computer, Bourne, MA, USA) that recorded temperature and light data every hour.

Analyses

To address our first question regarding predictors of plant-damaging frost on a regional scale, we explored several possibilities. Especially for mild frost events, small differences in freeze severity could greatly affect the

probability of a population being frost damaged if temperatures vary around the frost tolerance threshold of the plant. We used the minimum temperature during the frost period as a metric of frost severity. The period of frost was defined by contiguous days in which temperatures fell below 0°C, which on average was around 7 days for any given population. A population was considered to have sustained frost damage if at least one plant within the population had been recorded as damaged by frost. The independent variable, the minimum temperature during the freezing event, was related to frost damage using logistic regression.

Regional differences in temperature preceding the frost event could also affect the likelihood of a population being damaged. Specifically, warmer temperatures, or longer periods of high temperature, could accelerate development of plants in some populations, leaving them more vulnerable to frost. Mean average, minimum, and maximum temperatures were calculated for five time periods corresponding to 1, 5, 10, 15, and 20 days prior to frost. This 20-day time period was bracketed by below zero temperatures. The same criterion as above was used to label a population as ‘frost-damaged’. Logistic regression was used to determine whether the probability of a population incurring frost damage depended on each climatic variable. Among populations that were damaged by frost, we then tested whether these same climatic factors, as well as the minimum temperature during the frost, could explain differences in damage extent, defined as the percentage of plants damaged by frost in a population. For this analysis, we selected all populations that were considered ‘frost-damaged’, again using the criterion that at least one plant in the population had sustained frost damage, and calculated the percent of the total population that had incurred damage due to frost. Percentages were log-transformed and regressed on mean average, minimum, and maximum temperatures for 1-, 5-, 10-, 15-, and 20-day periods, as well as the minimum temperature during the frost. The residuals of the regression were tested for deviations from normality.

To address our second question regarding differential effects of frost within populations, we selected the largest ginseng population ($n = 153$) that had sustained a high level of frost damage. For that population, we then tested whether physical attributes of the ginseng plant, specifically plant height and leaf area, influenced the likelihood that a plant was damaged by frost using logistic regression. For these two analyses, as well as all subsequent analyses, three outliers suspected of being cultivated genotypes were excluded on the grounds that they likely originated from a different climatic zone and therefore may have differed from native plants in terms of their response to frost. These three plants were considerably larger than the other plants, were located in an area suspected to contain cultivated

ginseng, and differed from other plants in terms of morphology and phenology.

To address our third question, we investigated the effect of the frost on demographically important parameters within the previously selected ginseng population. Browsing by white tail deer negatively impacts ginseng population viability (McGraw and Furedi 2005), and loss of leaf material due to deer browsing is in some ways analogous to the loss of leaf material due to frost. To avoid confounding these two factors, all deer-browsed plants were excluded from subsequent analyses. In order to determine whether removal of deer-browsed plants biased our analyses, we also tested whether the likelihood of being deer-browsed differed as a function of being frost-damaged or not, using a log-likelihood analysis. We then examined whether being frost-damaged affected the probability of survival: (1) to the end of the growing season, and (2) to the spring of the following year using logistic regression. In plants, survival often increases as a function of size. For this reason, leaf area was used as a covariate in the analyses. Leaf area was calculated from field measurements of leaf length and width using a regression equation, which related these measurements to leaf area ($r^2 = 0.9327$). The model was parameterized with leaf areas calculated from digital images of 100 adult ginseng plants. Digital images were processed using the free image processing software, NIH Image J. The effects in the statistical model therefore included: frost damage (FD, yes or no), leaf area (LA, cm^2), and their interaction (FD \times LA).

The effect of frost damage on plant growth was also analyzed. Because the frost caused foliar deformities, leaf area growth rate was calculated for years bracketing the frost event. For this reason, only plants that were present all three seasons were used in the analysis. Relative growth rate (RGR) on a leaf area basis was calculated for the period of 2006–2008, using the following equation (McGraw and Garbutt 1990):

$$\text{RGR}_{\text{LA}} = \frac{\ln \text{LA}_2 - \ln \text{LA}_1}{t_2 - t_1}$$

The data were tested for normality, and analyzed using a one-way analysis of variance, with frost damage as the main effect in the model.

Reproductive responses to frost damage were partitioned into three components. First, logistic regression was used to determine whether the probability of forming a reproductive structure depended on being damaged by frost. Next, among plants that formed reproductive buds, logistic regression was used to test whether the probability that a plant would produce seeds differed as a function of being frost damaged or not. Finally, in order to determine whether the frost event affected the number of seeds produced per seed producing plant, an analysis of covariance

(ANCOVA) was performed. All analyses were conducted for both 2007, the year of the frost occurrence, and 2008, 1 year later. Leaf area was used as a covariate, as in prior analyses, using the full factorial model.

Results

Of the 30 populations of ginseng that we censused, 14 were affected by frost. Frost extent, measured as the proportion of damaged plants in the population, differed substantially among populations. In the population most severely affected by frost, 36.8% of ginseng plants showed symptoms of frost damage, whereas in the population least affected by frost, less than 1% of plants incurred frost damage. The mean percent of the population affected by frost was 10.9%.

Temperatures before and during the frost event did explain among-population patterns of frost damage. The minimum temperature during the frost event influenced the likelihood of a population being frost damaged ($\chi^2 = 5.71$, $P = 0.0169$); however, contrary to expectation, warmer temperatures during the frost increased the likelihood of a population being damaged. All temperature summaries for the time periods prior to the frost, except the 1-day period, strongly affected the probability of a population being frost damaged ($P < 0.01$ in all cases; Table 1; Fig. 1). Greater pre-frost temperatures increased the probability that a population would incur frost damage (Fig. 1 shows one such relationship). The lowest temperature during the frost did not affect the proportion of frost-damaged plants within a population ($\beta = 0.0005$, $P = 0.9875$). However, minimum temperatures 5, 10, 15, and 20 days prior to the frost affected the extent of frost damage, with minimum temperatures explaining the largest amount of variation in y (Table 1). Warmer minimum temperatures prior to the freeze increased the percent of the population damaged, and minimum temperatures averaged over a 15-day period explained the most variation in extent of frost damage ($\beta = 0.2735$, $P = 0.0008$; Table 1; Fig. 2). In frost-damaged populations, maximum temperatures did not predict extent of frost damage (Table 1; $P > 0.05$).

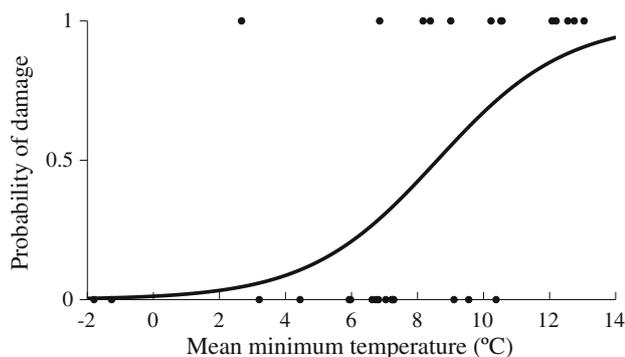
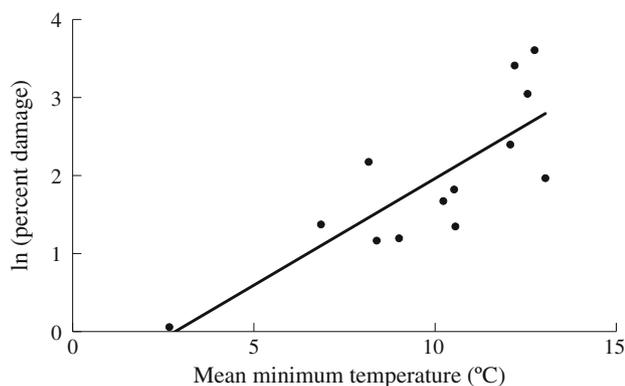
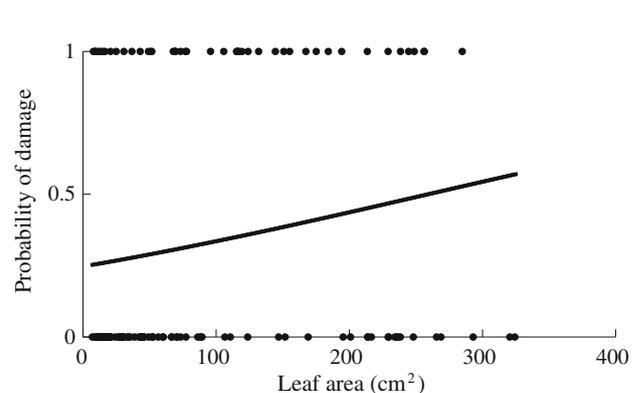
Size-related traits of ginseng plants influenced the likelihood of sustaining frost damage. Plants with larger leaf area were more likely to be damaged by frost ($\chi^2 = 4.37$, $P = 0.0365$; Fig. 3). However, there was no effect of height on likelihood of incurring frost damage ($\chi^2 = 0.79$, $P = 0.3734$).

Overall, the frost event negatively impacted ginseng demography. The effect of leaf area on persistence to the end of the growing season in 2007 depended on the effect of frost damage ($\chi^2 = 3.18$, $P = 0.0298$; Fig. 4a, b). For frost-damaged plants, the likelihood of being absent at the

Table 1 Summary of two statistical analyses examining among population patterns of frost damage of American ginseng (*Panax quinquefolius* L.) in terms of temperature prior to the frost event

Method of summarizing temperature data	Time period (days) over which mean temperatures were calculated	χ^2 for logistic regression	P values ₁ for logistic regression	β values for linear regression	r^2 for linear regression	P values ₂ for linear regression
Minimum	1	0.043	0.8359	0.088	0.023	0.6175
Maximum	1	0.819	0.3655	-0.098	0.113	0.2611
Minimum	5	12.566	0.0004	0.316	0.534	0.0046
Maximum	5	7.217	0.0072	0.055	0.028	0.588
Minimum	10	11.678	0.0006	0.261	0.636	0.0011
Maximum	10	7.239	0.0071	0.078	0.041	0.5063
Minimum	15	11.182	0.0008	0.273	0.652	0.0008
Maximum	15	8.162	0.0043	0.123	0.156	0.1819
Minimum	20	11.154	0.0008	0.283	0.631	0.0012
Maximum	20	8.604	0.0034	0.124	0.200	0.1257

Mean temperatures have been calculated from daily minimum and maximum temperatures over time periods 1, 5, 10, 15, and 20 days before the freeze. χ^2 and P values₁ correspond to logistic regressions of populations incurring frost damage or not as a function of temperature preceding the frost event. β , r^2 , and P values₂ are derived from regressions of the percent of the population damaged by frost on the temperature prior to freeze

**Fig. 1** The probability of a population of American ginseng (*Panax quinquefolius* L.) being frost-damaged as a function of mean minimum temperature (°C) for the 15-day period prior to the frost event. Closed circles are data points**Fig. 2** Regression of the percentage of the population damaged by the frost on mean minimum temperature (°C) for the 15-day period prior to the frost event. The fit of the regression was improved by taking the natural log of the percentage of the population damaged by frost**Fig. 3** The probability that an individual plant will incur frost-damage as a function of leaf area. Closed circles are data points

end of the growing season increased with greater leaf area. There was also a tendency for frost-damaged plants to be absent the following growing season more frequently than expected ($\chi^2 = 2.65$, $P = 0.1037$), but this effect did not depend on leaf area ($\chi^2 = 1.13$, $P = 0.2884$).

Frost damage reduced relative leaf area growth rate of ginseng from 2006 to 2008 ($F = 7.2644$, $P = 0.0089$; Fig. 5). In 2007, neither frost ($\chi^2 = 1.71$, $P = 0.4656$), nor leaf area ($\chi^2 = 2.14$, $P = 0.1437$), nor the interaction between these factors ($\chi^2 = 1.13$, $P = 0.5675$), affected the presence of an inflorescence. In 2008, leaf area alone affected the likelihood of a plant forming a reproductive structure ($\chi^2 = 30.56$, $P < 0.0001$), but frost damage, and the interactive term, did not ($\chi^2 = 0.09$, $P = 0.7622$; $\chi^2 = 0.21$, $P = 0.6465$, respectively). Of the plants that produced an inflorescence in 2007, only leaf area influenced the probability of a plant producing seeds

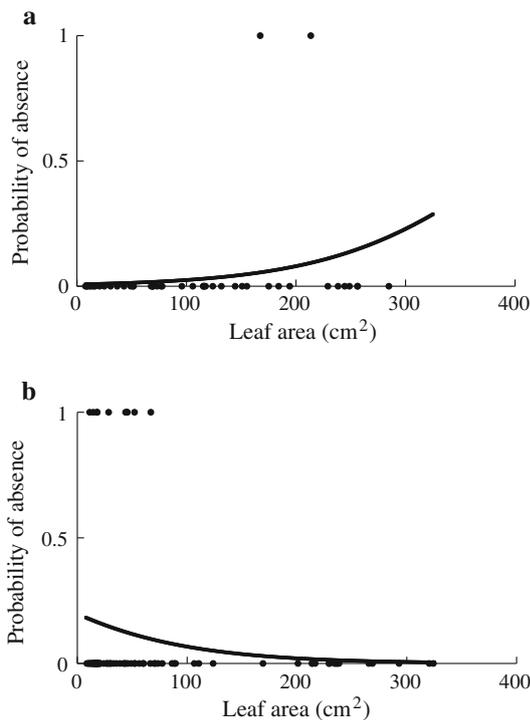


Fig. 4 The probability of a plant being absent as a function of leaf area for **a** frost-damaged plants and **b** undamaged plants. Closed circles are data points

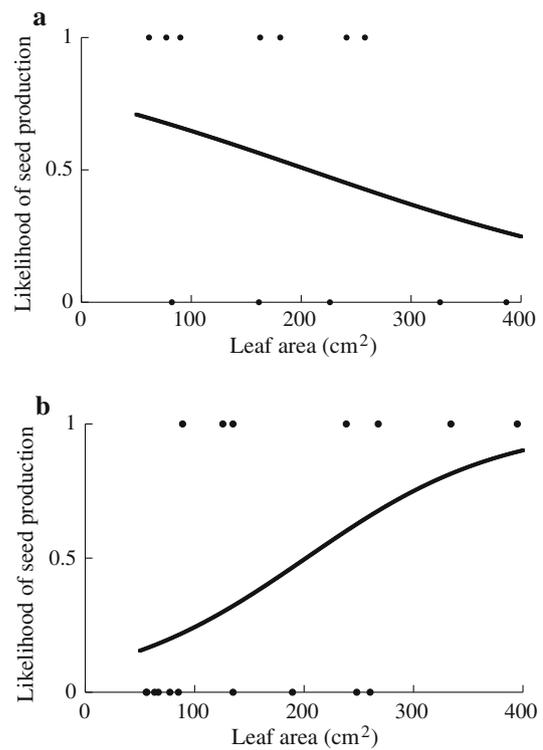


Fig. 6 The probability of a plant producing seeds in 2008 as a function of leaf area for **a** frost-damaged plants and **b** undamaged plants. Closed circles are data points

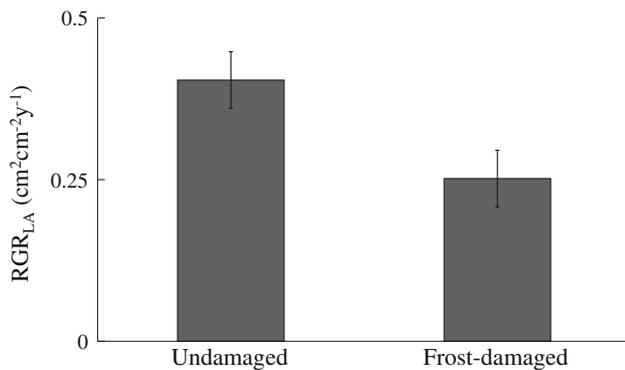


Fig. 5 Comparison of the relative growth rate of leaf area from 2006 to 2008 for frost-damaged and undamaged plants (± 1 standard error)

($\chi^2 = 4.51$, $P = 0.0336$), while frost damage and the interaction of frost damage and leaf area had no effect ($\chi^2 = 0.19$, $P = 0.6641$; $\chi^2 = 0.11$, $P = 0.7459$, respectively). As expected, leaf area increased the likelihood of producing seeds. Interestingly, in 2008, frost damage and leaf area alone did not affect the probability of producing seeds ($\chi^2 = 0.87$, $P = 0.3514$; $\chi^2 = 0.03$, $P = 0.8565$, respectively), but there was a statistically significant interactive effect between these two factors ($\chi^2 = 6.05$, $P = 0.0139$; Fig. 6a, b). As leaf area increased, those

plants that were frost damaged in the prior year showed reduced probability of seed production, while the expected increase in seed production was observed for undamaged plants. Among plants that produced seeds, neither frost damage, nor leaf area, nor the interactive effect influenced the number of seeds produced in 2007 ($F = 2.6703$, $P = 0.1568$) or in 2008 ($F = 1.5426$, $P = 0.3652$).

Discussion

Relatively little is known concerning the role of damaging frosts in the long-term demography of natural populations. Extreme early warming events followed by hard frost occur seldom and at random, making them hard to study in the absence of long-term censusing projects. In 2007, just such a dramatic temperature fluctuation (Gu et al. 2008) occurred in the concomitant censusing of ginseng populations, providing a rare opportunity to examine among- and within-population patterns of frost damage, and to quantify demographic ramifications of such an infrequent, but important, event. We found the likelihood that a population would be damaged by frost, and the percentage of frost-damaged individuals within a population, clearly depended on temperatures prior to the freeze. The most parsimonious

explanation is that plant emergence increased as a function of temperature prior to the freeze, thus increasing the likelihood that plants would be exposed when the frost occurred, as well as increasing the number of plants that had emerged at the time of the frost event. Several lines of evidence support this conclusion. In comparison to spring ephemerals, ginseng emerges relatively late in the spring, indicating a frost avoidance, rather than tolerance strategy. Additionally, the ability of temperature preceding the frost to explain differences in frost damage extent was temporally dependent, suggesting an ontogenetic mechanism.

We expected lower minimum temperatures during the frost event to increase the likelihood that a population would be damaged by frost. The fact that the opposite pattern was observed suggests that ginseng was not frost tolerant at any freezing temperatures, so severity of freezing did not matter. Instead, minimum temperatures prior to and during the frost event are likely correlated, and therefore, it was those populations with accelerated emergence that experienced the highest amount of damage.

Failure for temperature to explain all of the among-population variation in frost damage extent may be due to genetic differentiation with respect to phenological traits. In tree species, for instance, spring bud burst is highly heritable (reviewed in Howe et al. 2003). Ecotypic variation of bud burst has been observed in many tree species, even when high levels of gene flow oppose local adaptation (Ducousso et al. 1996; Hall et al. 2007; Vitasse et al. 2009a, b). Regional differences in frost-free days explain large amounts of variation among temperature cues that stimulate bud break in these studies (Myking and Heide 1995).

Ginseng populations were censused approximately 1 month after the frost occurred. Because the most severely damaged plants may have senesced prior to our arrival, it is possible that we are underestimating both the number of plants damaged by the frost as well as frost effects on demography. In the same vein, the tendency for larger plants to be frost-damaged more frequently than smaller plants may be a result of size-dependent senescence rather than reflecting any real difference in likelihood of damage. Specifically, smaller plants damaged by frost may have senesced earlier than larger plants due, in part, to differences in carbon stores. An alternative explanation for size-dependent damage is that larger plants emerged earlier than small plants. Rates of emergence and development in perennials have been shown to vary as a function of carbon storage; larger ginseng with greater photosynthate stores may have developed at a faster rate compared to smaller plants (Pillar and Meekings 1997; Bustamante and Búrquez 2008). Whatever the mechanism by which the frost injured larger plants, the demographic consequences were enhanced because these primary seed producers were more frequently impacted.

Indeed, when examining demographic parameters, we found that the frost event had an overall negative impact on ginseng demography. The likelihood of a plant being absent by our second demographic census increased as a function of leaf area in frost-damaged plants, representing a complete reversal from the usual positive association of size and survival. Early senescence of frost-damaged plants reduced growing season length, and eliminated their reproductive contribution in 2007. To put ginseng seed production in perspective, reproductive plants from this study population produced an average 1.08 seeds per year during the 8-year study period. With such low rates of seed production, even seemingly small effects on reproduction may have profound effects on long-term population growth for ginseng. The negative effects of the frost persisted in the subsequent year by both decreasing RGR of frost-damaged ginseng compared to undamaged ginseng, and by decreasing the likelihood of reproduction in frost-damaged plants. For many plant species, there is an apparent trade-off between high reproductive effort and future growth and survival (Galen 1993; Bañuelos and Obeso 2004). The observed reduction in probability of seed production in frost-damaged plants may be an adaptive response to loss of carbon gain resulting from damaged photosynthetic machinery after the frost event, thus ensuring long-term survival and reproductive success of these individuals.

Stochastic events can have dramatic effects on long-term population demography (Menges 1990). These effects are often greater than might be expected based on their low frequency. The 2007 spring frost negatively impacted ginseng growth, reproduction, and survival. Notably, the effects of the frost on ginseng growth and reproduction were detected even though the physical damage caused by the frost was mild in the population that we examined in detail for this study. In several other populations, frost damage was so severe that the plants were completely denuded by the time we censused them. Because these populations were small, it was not statistically feasible to analyze the effects of the frost on demography. However, for these small populations, such frost events could be one of many such perturbations leading down an extinction vortex (Morris and Doak 2002).

Frosts are clearly a strong selective force in natural populations, levying severe demographic penalties against 'early riser' genotypes of susceptible species. Evidence from provenance and transplant studies suggests that spring emergence within species is aligned with regional frost patterns (Beuker 1994; Myking and Heide 1995; Ducousso et al. 1996; Myking 1999; Savolainen et al. 2004; Ghe-lardini et al. 2006; Hall et al. 2007). If the climate is altered such that the historical environmental cues that signal plant emergence no longer coincide with low frost probability, then plant survival, growth, and reproduction may be

jeopardized. Outside arctic, alpine, and boreal ecosystems, however, there is a paucity of ecological research that considers the demographic effects of frost on long-term population growth, and the potential for climate change to modify frost frequency. Models from silvicultural and agricultural arenas suggest that climate change may interact with plant phenology, increasing the frequency of damaging frost events in the future (Hänninen 1991, 1996, 2006; Kellomäki et al. 1995; Kramer et al. 1996; Linkosalo et al. 2000; Jönsson et al. 2004). This study provides empirical evidence for the kind of effects that would be expected with increasing frequency if such models are correct. Observed patterns of frost damage among populations illustrate how even small increases in temperature greatly increased the probability of being affected by the frost. This study further suggests that species like ginseng, which appear to have low frost tolerance, yet whose emergence is highly sensitive to changes in temperature (Farnsworth et al. 1995), are susceptible to frost damage, with serious negative consequences for population growth.

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