Experimental climate warming alters aspen and birch phytochemistry and performance traits for an outbreak insect herbivore

MARY A. JAMIESON[1], EZRA G. SCHWARTZBERG[1,2], KENNETH F. RAFFA[1], PETER B. REICH[3,4] and RICHARD L. LINDROTH[1]

[1]*Department of Entomology, University of Wisconsin-Madison, Madison, WI 53706, USA,* [2]*Adirondack Research, Saranac Lake, NY 12983, USA,* [3]*Department of Forest Resources, University of Minnesota, St. Paul, MN 55108, USA,* [4]*Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW 2751, Australia*

Abstract

Climate change and insect outbreaks are key factors contributing to regional and global patterns of increased tree mortality. While links between these environmental stressors have been established, our understanding of the mechanisms by which elevated temperature may affect tree–insect interactions is limited. Using a forest warming mesocosm, we investigated the influence of elevated temperature on phytochemistry, tree resistance traits, and insect performance. Specifically, we examined warming effects on forest tent caterpillar (*Malacosoma disstria*) and host trees aspen (*Populus tremuloides*) and birch (*Betula papyrifera*). Trees were grown under one of three temperature treatments (ambient, +1.7 °C, +3.4 °C) in a multiyear open-air warming experiment. In the third and fourth years of warming (2011, 2012), we assessed foliar nutrients and defense chemistry. Elevated temperatures altered foliar nitrogen, carbohydrates, lignin, and condensed tannins, with differences in responses between species and years. In 2012, we performed bioassays using a common environment approach to evaluate plant-mediated indirect warming effects on larval performance. Warming resulted in decreased food conversion efficiency and increased consumption, ultimately with minimal effect on larval development and biomass. These changes suggest that insects exhibited compensatory feeding due to reduced host quality. Within the context of observed phytochemical variation, primary metabolites were stronger predictors of insect performance than secondary metabolites. Between-year differences in phytochemical shifts corresponded with substantially different weather conditions during these two years. By sampling across years within an ecologically realistic and environmentally open setting, our study demonstrates that plant and insect responses to warming can be temporally variable and context dependent. Results indicate that elevated temperatures can alter phytochemistry, tree resistance traits, and herbivore feeding, but that annual weather variability may modulate warming effects leading to uncertain consequences for plant–insect interactions with projected climate change.

Keywords: aspen (*Populus tremuloides*), birch (*Betula papyrifera*), climate change, forest tent caterpillar (*Malacosoma disstria*), herbivore resistance, herbivory, plant defenses, plant–insect interactions

Introduction

In recent decades, the combined effects of climate change and insect outbreaks have contributed to increased tree mortality and large-scale forest dieback (Raffa *et al.*, 2008; McDowell *et al.*, 2011; Jamieson *et al.*, 2012; Ryan & Vose, 2012; Weed *et al.*, 2013). Phytophagous insects play a key role in regulating forest primary production, and insect outbreaks are major drivers of natural disturbance in forests (Mattson & Addy, 1975; Logan *et al.*, 2003; Kirilenko & Sedjo, 2007). Moreover, the frequency and severity of outbreaks by some forest insects is predicted to intensify with climate change

(Volney & Fleming, 2000; Logan *et al.*, 2003; Tobin *et al.*, 2014). Global mean temperatures are projected to increase 1–4 °C in the next 50–100 years (IPCC, 2013). Because forests provide ecological services with great socioeconomic value, understanding how climate warming could interact with insect pests to affect trees is a key research priority for forest ecology and management.

While a number of studies have addressed the direct effects of warming on plant and insect phenology, physiology, and distribution, less attention has been given to understanding the potential consequences of elevated temperature on trophic interactions and indirect effects (Bale *et al.*, 2002; Walther, 2010; Jamieson *et al.*, 2012). Interactions between herbivores and host trees are influenced by various phytochemicals,

Correspondence: Mary A. Jamieson, tel. (303) 827 4932, fax (608) 262 3322, e-mail: maryajamieson@gmail.com

including nutrients, such as proteins and carbohydrates, and secondary metabolites, such as lignin, condensed tannins, phenolics, and terpenes (Scriber & Slansky, 1981; Mattson & Scriber, 1987; Awmack & Leather, 2002; Haukioja, 2005; Behmer, 2009). Together, these metabolites affect food quality for herbivores by influencing the nutrition, palatability, digestibility, and/or toxicity of foliage. Temperature plays a central role in tree physiology and may influence foliar quality for herbivores via changes in primary and secondary metabolism or development (Zvereva & Kozlov, 2006; Bidart-Bouzat & Imeh-Nathaniel, 2008; Chung *et al.*, 2013). In general, elevated temperature decreases foliar sugar and starch concentrations, potentially related to developmental shifts, while foliar nitrogen (an index of protein) shows no consistent response (Zvereva & Kozlov, 2006). In temperate and boreal trees, foliar nitrogen, carbohydrate, and water concentrations tend to decline with leaf age, while condensed tannin, terpene, and lignin concentrations typically increase (Mattson & Scriber, 1987; Hunter & Lechowicz, 1992). Climate warming may alter phytochemicals by advancing plant development or by altering plant physiology and resource allocation.

Compared with primary metabolites, less is known about how elevated temperatures may influence secondary metabolites (Bidart-Bouzat & Imeh-Nathaniel, 2008). The growth-differentiation balance hypothesis proposes that warming-accelerated photosynthesis should contribute to growth rather than defense, when resources, such as soil moisture and nutrients, are not limited (Herms & Mattson, 1992). Based on this hypothesis, secondary metabolites are expected to decline with elevated temperature, independent of developmental shifts related to warming. Evidence to date, however, suggests warming has variable effects on different groups of chemical defenses (Zvereva & Kozlov, 2006). For example, phenolic constituents, such as flavonoids and tannins, tend to decrease with warming, while terpenes increase (Zvereva & Kozlov, 2006; Bidart-Bouzat & Imeh-Nathaniel, 2008).

In general, research addressing the effects of elevated temperature on phytochemistry and insect performance is limited (Zvereva & Kozlov, 2006; Bidart-Bouzat & Imeh-Nathaniel, 2008; Chung *et al.*, 2013). Moreover, few experimental studies have examined the influence of warming on host quality and tree–insect interactions in an ecologically realistic setting (Zvereva & Kozlov, 2006; Chung *et al.*, 2013). While greenhouse and environmental chamber studies are valuable for investigating mechanisms underlying plant and insect responses to warming, incorporation of greater ecological realism into climate change experiments will enhance our understanding of potential climate change effects. For example, variation in abiotic and biotic factors (e.g. nutrient, water, light availability, plant competition) in a field setting may shape plant response to warming, altering the magnitude and/or direction of response. In this study, we used a multi-year open-air forest warming experiment to investigate
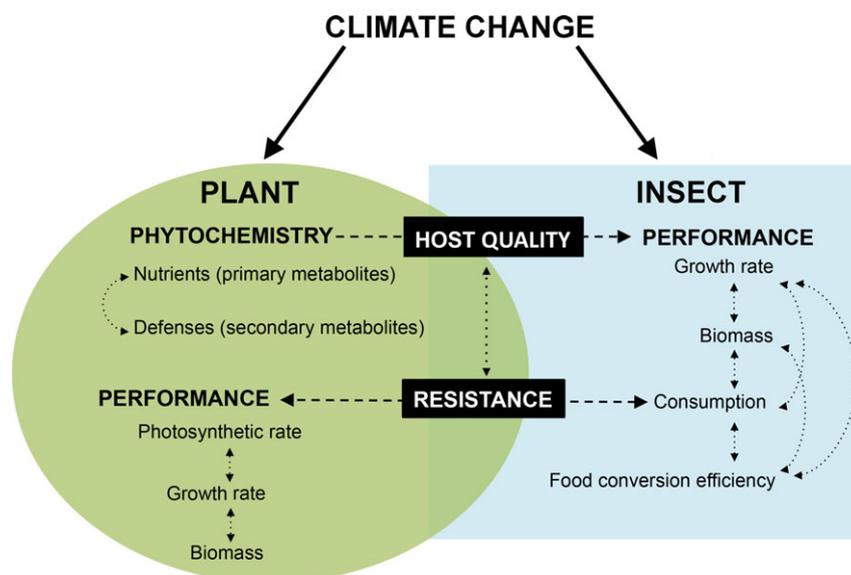

Fig. 1 Conceptual framework illustrating potential direct (solid lines) and indirect (dashed lines) effects of climate change on plants and insects. Climate change, including elevated temperatures, may directly influence plant primary and secondary metabolism. Corresponding changes in phytochemistry, in turn, could alter host quality and resistance via plant-mediated indirect effects on insect performance.

the influence of elevated temperature on forest tent caterpillar (*Malacosoma disstria*) and two host tree species – trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*).

The goal of our study was to examine warming effects on phytochemistry, host quality for herbivores, and tree resistance to herbivory (Fig. 1). Additionally, we aimed to determine whether response patterns were consistent across host tree species. Tree species have different heat tolerance mechanisms and physiological sensitivities to temperature (e.g. Darbah *et al.*, 2010). Thus, warming may lead to species-specific changes in primary and secondary metabolism. We expected that climate warming would alter foliar nutrients and defense chemicals due to temperature-induced changes in tree physiology and carbon allocation to growth and defense. Further, we hypothesized that such modifications in phytochemistry would influence host quality and resistance, which would be demonstrated by changes in insect feeding and performance. To investigate hypothesized direct and indirect effects of climate change on plant–insect interactions (Fig. 1), we conducted a field study to evaluate warming effects on foliar nutrients (nitrogen, sugars, starch) and defense chemicals (lignin, condensed tannins, phenolic glycosides). We also assessed warming effects on tree resistance and insect performance traits in laboratory bioassays. Finally, we used field and bioassay data to examine the relationship between phytochemistry and insect performance.

Materials and methods

Study organisms

Forest tent caterpillar (*M. disstria* Hbn.) is an early-season univoltine lepidopteran folivore that exhibits eruptive population dynamics, with cyclic outbreaks occurring every 10–12 years, on average, and lasting approximately 2–4 years (Roland, 1993; Fitzgerald, 1995; references therein). This generalist herbivore is one of the most damaging native defoliators in North American forests (Mattson *et al.*, 1991; Fitzgerald, 1995). Larvae feed gregariously during early instars on a variety of host trees. The primary host species for forest tent caterpillar is trembling (also known as quaking) aspen (*P. tremuloides* Michx.). Trembling aspen is the most widely distributed tree species in North America. Successive years of defoliation by forest tent caterpillars, in combination with other environmental stressors, such as warming and drought, have been linked to stand-level mortality and regional declines of aspen in North America (Worrall *et al.*, 2013). Paper birch (*B. papyrifera* Marshall) is another favored host species for forest tent caterpillar, especially in boreal forests in the northern U.S. and Canada. Other host species, which were not evaluated in this study,

include sugar maple (*Acer saccharum* Marshall), red oak (*Quercus rubra* L.), and basswood (*Tilia americana* L.)

Experimental design

Our field study was conducted using an open-air climate change experiment (the 'B4WarmED' project) in northern Minnesota, USA. Study plots were located at two sites in the temperate–boreal forest transition zone: Cloquet Forestry Center in Cloquet, MN (46°40′45″N, 92°31′10″W) and Hubachek Wilderness Research Center near Ely, MN (47°57′2″N, 91°44′55″W). The experimental setup consisted of six replicate plots per temperature treatment (ambient, +1.7 °C, +3.4 °C) at each of the two sites for a total of 36 circular forest plots (3 m in diameter). Plots were arranged in a randomized block design with three blocks per site. Plots were cleared of mature trees in 2007, and the residual shrub and herbaceous vegetation was in-planted with juveniles of 11 species (11 individuals per species per plot) in 2008. Warming treatments were initiated in spring 2009. The study presented here was conducted in 2011 and 2012. Aspen trees were in their fifth and sixth growing season during the study, and birch trees were in their fourth and fifth years.

In recent decades, mean annual temperature has risen ~1.5 °C in our study area and is expected to increase an additional 3–9 °C in the next 75–100 years (Kling *et al.*, 2003; Wuebbles & Hayhoe, 2004). Thus, warming treatments simulated temperatures well within the projected range of climate change in coming decades. In warming plots, temperatures were elevated using belowground heating cables (Danfoss GX, Devi A/B, Denmark) and aboveground ceramic heating elements (Salamander Model FTE-1000; Mor Electric Heating Assoc., Inc., Comstock Park, MI, USA). Above- and belowground temperatures were elevated via plot-level feedback controls that maintained temperature differentials 24 h per day for 8 months per year, during a mostly snow-free period (methods in Rich *et al.*, 2015). At the beginning of our study, we randomly selected two trees per plot for each species to use for phytochemical analyses (2011 and 2012) and insect bioassays (2012). Dates for temperature treatments, budbreak phenology, foliar sample collections, and bioassays are presented in Table S1.

Foliar sample collection, preparation, and chemical analyses

In 2011, collection dates for foliar samples were based on degree days (dd), which allowed phytochemical traits to be compared among treatments at the same degree day accumulation (~450 dd) and roughly equivalent tree developmental stage, rather than calendar date (see Table S1 for degree day calculation). In 2012, foliar samples were collected based on the development of *M. disstria* larvae reared in field plots (~350 dd), so phytochemical analyses would correspond with foliage fed to larvae in laboratory bioassays (Table S1). In both years, leaves (~15–25) were haphazardly selected from an individual tree, clipped at the petiole, placed in paper coin

envelopes, kept on ice (~1 h), vacuum dried (~48 h), and then stored at -20 °C until further preparation for chemical analyses.

Leaves were ground using a Wiley mini-mill (20-mesh sieve) and samples were vacuum dried again prior to analyses. We quantified concentrations (% dry weight) of foliar primary and secondary metabolites. Foliar nitrogen was measured using a Thermo Finnigan Flash 1112 elemental analyzer. Soluble sugars and starch were measured spectrophotometrically using a dinitrosalicylic acid method to assay total nonstructural carbohydrates (Lindroth *et al.*, 2002). Soluble condensed tannins were extracted in 70% acetone (containing 10 mM ascorbic acid as an antioxidant) with sonication at ~4 °C for 30 min. Condensed tannins were quantified using a spectrophotometric acid butanol assay, with purified aspen and birch condensed tannins as reference standards (Lindroth *et al.*, 2002). Lignin concentrations were determined gravimetrically by sequential acid-detergent extraction using an ANKOM 200 fiber analyzer (ANKOM Technology Macedon, NY, USA).

For aspen foliage, we quantified phenolic glycosides using a Waters ACQUITY ultra high performance liquid chromatography system coupled with an electrospray ionization single quadrupole mass spectrometer (UPLC-MS) operated in negative mode (methods adapted from Abreu *et al.*, 2011 and Rubert-Nason *et al.*, 2014). Phenolic glycosides were extracted in methanol containing 10 mg mL^{-1} β-resorcylic acid as a control standard with sonication at ~4 °C for 15 min. After initial extraction, samples were diluted 1 : 1 with methanol amended with salicylic acid-d$_6$ as an internal standard (100 mg L^{-1}). In this study, phenolic glycosides refer to the sum concentration of salicortin and tremulacin. We focus on these compounds because they are the primary phenolic glycosides (also known as 'salicinoids' and 'salicylates'; Lindroth *et al.*, 1987a; Boeckler *et al.*, 2011) found in aspen (>85% of total). Additionally, these compounds are the two aspen phenolic glycosides most biologically active against lepidopteran herbivores (Lindroth *et al.*, 1987b, 1988).

Laboratory bioassay

In 2012, we performed feeding trials with penultimate (fourth) instar forest tent caterpillars to investigate the influence of elevated temperatures on tree resistance to herbivory and insect performance. We examined the feeding behavior and performance of fourth instars because food consumption and growth during the penultimate and final instars comprise more than 75% of the total value of these measures over the course of larval development (Slansky & Scriber, 1985). Additionally, evaluation of a single instar reduces variation in performance measures related to allometric scaling. Moreover, several earlier studies (e.g. Hwang & Lindroth, 1997) indicate that forest tent caterpillar responses to food quality, including quality of aspen foliage, are similar for early and late instars. For the bioassay, we used a common environment approach (i.e. environmental chambers) to control for direct temperature effects on insect physiology, development, and feeding. Thus,

bioassays focused on plant-mediated indirect warming effects on insects.

Larvae used in bioassays were reared on site from eggs, enclosed in mesh sleeves on aspen and birch trees adjacent to field plots. Egg bands were collected from naturally occurring *M. disstria* populations in the local area (46 °8′28″N, 93°27′35″ W) in late October 2011. They were partitioned into two sets of approximately 50 egg bands and were placed outside at field sites to overwinter. Multiple cohorts of larvae were established at each field site by exposing egg bands to different temperature regimes in ambient and warmed plots. Elevated temperatures advanced egg hatch by 4–5 days at the first level of warming and 8–10 days at the second level (Schwartzberg *et al.*, 2014). We initially reared phenologically advanced larvae in warmed plots for ~10–15 days, until ambient trees broke bud. Then, we moved these larvae onto ambient trees to prevent direct effects of warming on insects. Larvae grew on ambient foliage for approximately 2–3 weeks prior to feeding trials. All larvae used in the bioassay developed under ambient field conditions on aspen or birch trees, enclosed in mesh bags, until they were 3rd instars. The aim of this approach was to provide larvae with a common food source and growth conditions prior to the bioassay.

When larvae reached the end of the third stadium, they were moved into the laboratory and placed in labeled petri dishes with aspen or birch foliage. We surveyed these larvae four times daily (08:00, 12:00, 16:00, 20:00), and newly molted fourth instars were selected for bioassays (larvae that molted overnight were not used). For bioassay feeding trials, larvae were reared individually in petri dishes on foliage collected from field plots (three larvae per tree species per plot). Feeding trials were staggered to reflect warming-induced shifts in insect phenology (Schwartzberg *et al.*, 2014). Prior to initiating a feeding trial, we recorded the initial weight and time of molt for each larva. Individual larvae were placed on individual leaves in separate petri dishes and then into a Sherer environmental chamber with a 16 : 8 h light : dark cycle and day : night temperature regimes roughly corresponding to conditions at field sites (23 : 18 °C for Cloquet and 21 : 16 °C for Ely).

Bioassay leaves were removed from trees by clipping at the base of the petiole. Individual leaves were placed separately into labeled plastic bags in a cooler and taken to the laboratory. Each leaf was weighed and the petiole was placed in a water pick. A single leaf was presented to a larva in a petri dish. In addition to bioassay leaves, we collected and weighed three replicate foliar samples (~3 leaves per sample) from each tree species by treatment by site combination. These leaves and a subset of 4th instar larvae ($N = 3$ per species × treatment × site combination) were weighed fresh, freeze-dried, and then weighed again to calculate fresh to dry weight conversion factors. Additionally, we estimated foliar water content using these leaf samples. We used conversion factors to calculate dry weights for initial larval and initial leaf weights (at onset of feeding trials). Larvae fed freely in petri dishes, and individual leaves were changed when more than 50% of the leaf tissue was consumed, or as needed. A feeding trial was complete when the larva molted into a fifth instar. The

time of molt was recorded, and then newly molted fifth instars were weighed, frozen, freeze-dried, and then weighed again. For individual bioassays, we collected, freeze-dried, and weighed all unconsumed leaf material.

We calculated measures of insect performance using dry weights (larval and plant dry weights before and after feeding in bioassay trials). We evaluated larval development time (days), final larval weight (newly molted 5th instar dry weight in mg), total leaf consumption (dry weight in mg), and food conversion efficiency (%) for larvae during their penultimate stadium (from 4th to 5th instar). Food conversion efficiency was calculated as biomass gained/total consumption $\times$ 100. Insect response variables were averaged across two to three larvae for each of 12 trees per species per plot.

Statistical analyses

Statistical analyses were performed in JMP$^{\circledR}$ Pro v. 11 (SAS Institute Inc., Cary, NC, USA). Data were transformed to meet assumptions of statistical models. Total consumption data were log-transformed and phytochemical concentrations (proportion dry weights) were arcsine square root transformed. For field and bioassay data, we first examined the effects of warming treatment, host tree species, and their interaction on tree and insect response variables using linear mixed models (fit model platform with standard least squares and restricted maximum-likelihood methods). Then, we performed univariate analyses to evaluate within-species warming effects. Finally, we followed up with Tukey-HSD *post hoc* tests to identify significant mean differences among treatment groups.

For linear mixed models, statistical models included warming treatment, species, and their interaction as fixed effects, and site, block [site], and plot [block, site] as random effects, with brackets denoting nesting. Within-plot tree response variables were averaged by species (~2 trees per species). Thus, plot was the experimental unit of replication ($N = 12$ plots per temperature treatment). We performed separate analyses for foliar chemistry in each study year due to differences in sample collections approaches.

For bioassay data, we averaged insect performance measures across replicate insects reared on foliage from an individual tree (~2–3 insects per tree). Statistical models included warming treatment, species, and their interaction as fixed effects, and initial larval weight as a covariate. Site was included as a random effect to account for variation in larval rearing conditions. For analyses of insect performance data, tree/plot was the experimental unit of replication ($N \approx 12$ trees/plots per species per temperature treatment).

To examine the influence of phytochemistry on insect performance, we used partial least squares regression (PLSR), which is preferred over ordinary least squares regression when predictor variables are correlated or nonindependent (Wold *et al.*, 1984, 2001; Carrascal *et al.*, 2009; Couture & Lindroth, 2012). See Table S2 for correlation matrices relating foliar nutrients and defense chemicals. Additionally, PLSR can be more reliable for determining and assessing relevant explanatory variables compared with multiple regression techniques, including multiple regression alone and when combined with principal components analysis, especially when sample sizes are small (Carrascal *et al.*, 2009). The PLSR method extracts linear combinations of predictors, or latent factors, to develop a model that optimally explains variation in response and predictor variables. Data were centered and scaled, the number of latent factors was selected by minimizing predictive residual sum of squares (PRESS) scores using *K*-fold crossvalidation and nonlinear iterative partial least squares (NIPALS) methods, and explanatory variables for final models were selected using variable of importance for projection (VIP) threshold scores.

We also conducted correlation analyses to examine relationships among insect performance traits, given that these response variables are physiologically related. Because the calculation for food conversion efficiency involves total consumption, a correlation was expected for these variables. In this case, correlation analyses were used to evaluate the strength of the relationship between these two metrics of insect performance.

Results

Effects of warming on phytochemistry

Warming treatments altered foliar nutrients and defense compounds, with differing phytochemical responses observed across species and years (Table 1; Figs 2–3). In 2011, warming influenced foliar sugar, starch, condensed tannins, and lignin. In aspen, sugar concentrations showed a decreasing but nonsignificant trend, and starch responded nonlinearly to elevated temperature (Table 1b; Fig. 2a). In birch, sugar concentrations decreased by 14%, and starch increased by 25%. Condensed tannin and lignin concentrations were ~20% lower in warmed plots compared with ambient plots, with similar patterns across species. Elevated temperatures had no effect on aspen phenolic glycosides (Table 1b; Fig. 3a).

In 2012, elevated temperatures influenced foliar nitrogen, sugars, and starch (Fig. 2b), but not defense chemistry (Fig. 3b). Foliar nitrogen was significantly lower in +1.7 °C plots compared with ambient and plots for both tree species (Table 1; Fig. 2b). For aspen, warming decreased sugar concentrations by 13% (Table 1b; Fig. 2b), but did not affect starch. For birch, warming had no effect on foliar sugars, but decreased starch by ~30% (Fig. 2b). Elevated temperature did not affect condensed tannins, lignin, or phenolic glycosides (Table 1b; Fig. 3b). Foliar samples from the bioassay showed that elevated temperatures reduced foliar water concentrations for both tree species ($F_{2,29} = 12.77$; $P < 0.001$), with an approximate 6% reduction for aspen and 10% for birch.

Table 1 Aspen and birch phytochemical response to experimental warming. Data are F_{df} and P values for fixed effects from linear mixed models examining warming treatments (a) across host tree species and (b) within host species. Significant ($P \leq 0.05$) results are shown in bold ($N = 12$ plots per temperature treatment). See Fig. 1 for corresponding data illustrating mean responses

(a)

	Warming		Species		Warming × species	
	$F_{2,28}$	P	$F_{1,33}$	P	$F_{2,33}$	P
2011						
Nitrogen	0.63	0.541	3.40	0.074	1.92	0.163
Sugar	3.47	**0.045**	152.98	**<0.001**	0.27	0.763
Starch	14.78	**<0.001**	168.49	**<0.001**	1.37	0.268
Lignin	18.28	**<0.001**	199.54	**<0.001**	1.44	0.251
Condensed tannins	6.45	**0.005**	19.80	**<0.001**	1.91	0.165
2012						
Nitrogen	10.15	**0.0005**	5.05	**0.032**	0.93	0.403
Sugar	6.93	**0.004**	779.09	**<0.0001**	2.94	0.067
Starch	7.65	**0.002**	61.91	**<0.0001**	6.37	**0.005**
Lignin	1.05	0.363	58.70	**<0.0001**	1.68	0.202
Condensed tannins	0.09	0.916	43.58	**<0.0001**	0.99	0.383

(b)

	Aspen		Birch	
	$F_{2,28}$	P	$F_{2,28}$	P
2011				
Nitrogen	1.84	0.177	0.11	0.896
Sugar	1.04	0.366	3.71	**0.038**
Starch	19.61	**<0.001**	3.60	**0.041**
Lignin	7.61	**0.002**	17.66	**<0.001**
Condensed tannins	5.00	**0.014**	6.56	**0.005**
Phenolic glycosides	0.25	0.782	na	na
2012				
Nitrogen	5.42	**0.011**	10.13	**<0.001**
Sugar	10.16	**<0.001**	1.40	0.263
Starch	0.51	0.608	14.50	**<0.001**
Lignin	0.71	0.501	2.42	0.107
Condensed tannins	0.29	0.747	0.72	0.494
Phenolic glycosides	0.99	0.383	na	na

na, not applicable.

Plant-mediated effects of warming on insect performance

Bioassay results revealed plant-mediated warming effects on insect performance (Table 2; Fig. 4). Moreover, we found significant species and warming by species interaction effects, indicating host-specific insect responses to elevated temperatures. Indirect warming effects significantly, but modestly, increased larval development time and final weight (by ≈10%) in birch, but not aspen (Table 2b; Fig. 4). In contrast, warming had strong effects on foliar consumption and food conversion efficiency for larvae reared on both tree species. Total consumption was 50–100% higher and food conversion efficiency was 30–40% lower for larvae reared on aspen and birch foliage collected from trees in

warmed plots compared with ambient plots (Table 2b; Fig. 4). These results suggest that warming reduced host quality, leading to compensatory feeding, which in turn resulted in minimal to no change in larval growth and biomass.

Insect performance traits were significantly correlated with each other, with both similarities and differences between larvae reared on aspen and birch (Table 3). Except in one case (a nonsignificant response), the direction of the correlations for larvae fed on birch matched those for larvae fed on aspen. For aspen, larval weight was negatively related to development time and positively correlated with consumption. Some performance traits, however, were decoupled for birch-fed larvae, as demonstrated by nonsignificant
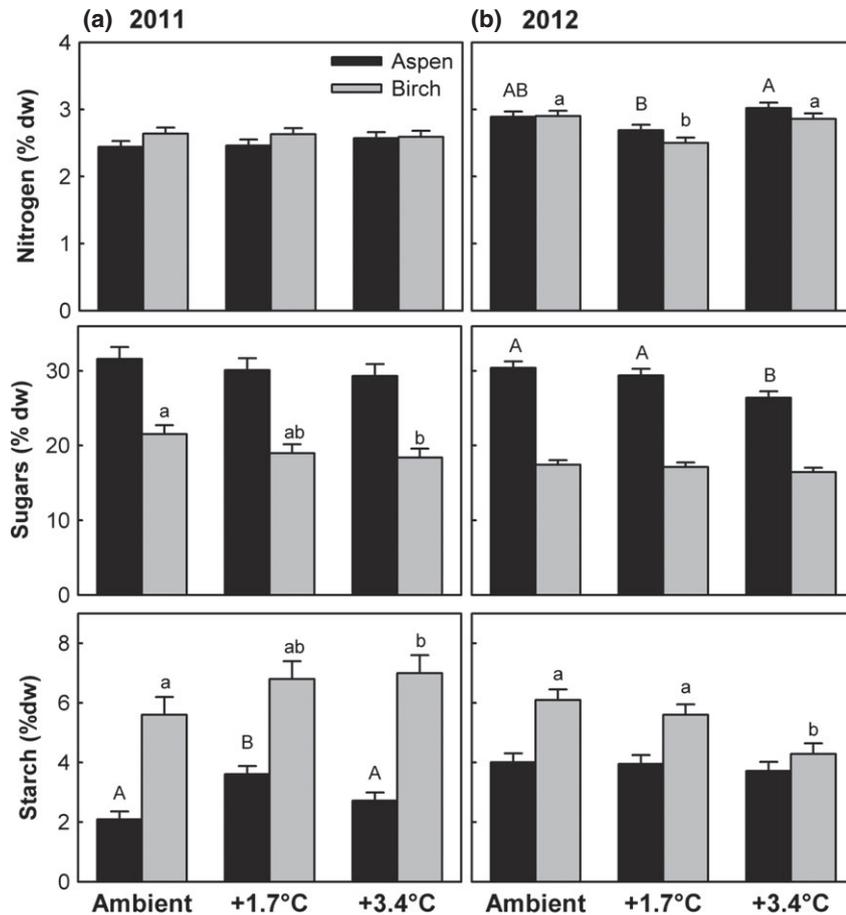
Fig. 2 Effects of warming on aspen and birch foliar nutrients. Bars are means (+1 SE) for untransformed percent dry weights. Letters show within-species pairwise differences ($P \leq 0.05$), with capital letters for aspen (black bars) and lower case letters for birch (gray bars). Data for (a) 2011 are displayed on the left and for (b) 2012 on the right. $N \approx 12$ trees per species per treatment per year.

correlations. For example, in contrast to aspen-fed larvae, development time was not significantly related to total consumption or food conversion efficiency for birch-fed larvae.

Influence of phytochemistry on insect performance

We used approximately half of the trees sampled for phytochemistry in the bioassay. Temperature-related phytochemical responses (Table 4) were mostly similar in bioassay trees compared with overall patterns described for the field study (Figs 1b and 2b). For example, birch foliar nitrogen was lowest in +1.7 °C warming plots in both field and bioassay samples in 2012. Additionally, elevated temperatures decreased foliar sugars and starch in bioassay foliage, roughly mirroring patterns from field study results described above. There were differences, however, that likely reflect variation due to random sampling and smaller sample sizes. For example, +1.7 °C warming did not significantly affect foliar nitrogen in aspen bioassay

trees, as it did in the overall field study. Additionally, we found significant differences in sugar and lignin concentrations for birch bioassay trees, which were not observed in the overall study.

Both foliar nutrients and defense chemicals influenced insect performance, with variation across host tree species (Table 5). Statistical models including phytochemical traits and initial larval weight explained 20–47% of the variation in performance traits. For both tree species, foliar nitrogen was a key explanatory variable for development time, final larval weight, total consumption, and food conversion efficiency. Greater foliar nitrogen concentrations were associated with reduced development time and increased food conversion efficiency for both host species. Surprisingly, however, higher nitrogen concentrations were related to increased consumption for aspen, but reduced consumption for birch. Statistical analyses indicated that foliar carbohydrates also influenced insect performance, and the relative importance of these nutrients varied by host species.
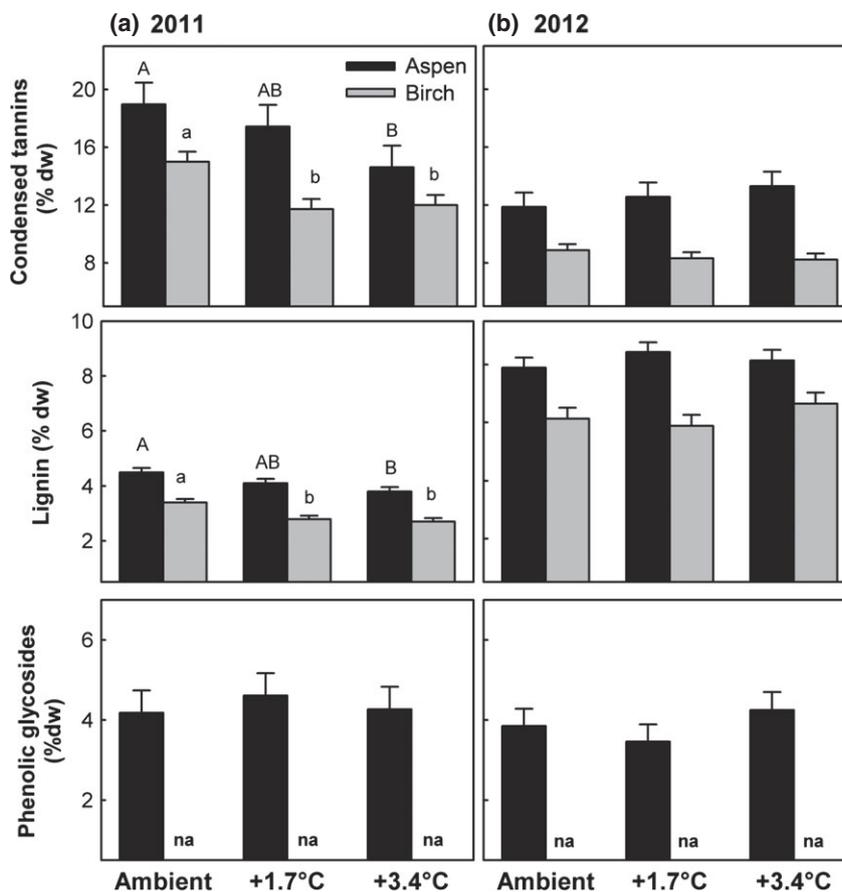
Fig. 3 Effects of warming on aspen and birch foliar defenses. Bars are means (+1 SE) for untransformed percent dry weights. Letters show within-species pairwise differences ($P \leq 0.05$), with capital letters for aspen (black bars) and lower case letters for birch (gray bars). Data for (a) 2011 are displayed on the left and for (b) 2012 on the right. $N \approx 12$ trees per species per treatment per year.

Total consumption was enhanced by higher sugar concentrations in aspen and higher starch concentrations in birch. Food conversion efficiency was negatively affected by aspen sugars and positively affected by birch sugars and starch.

Condensed tannins and phenolic glycosides were important predictors of insect performance, and results showed that these phytochemicals were negatively associated with host quality. Specifically, higher condensed tannin concentrations were associated with increased larval development times for aspen and decreased larval weight and food conversion efficiency for both host species. For aspen, higher phenolic glycoside concentrations also increased larval development time and reduced food conversion efficiency. Foliar lignin was not an important explanatory variable for insect performance.

Discussion

Climate change projections indicate forests will be exposed to more extreme warming events, including heat waves, than imposed in our study. Even with a conservative approach, we found that elevated temperatures resulted in changes in foliar nutrients and defenses, with consequences for insect performance. Temperature effects on phytochemistry varied, however, between host species and across years. Many studies examining the effects of global change drivers, such as elevated temperature, carbon dioxide, or ozone, on phytochemistry and tree–insect interactions also reveal complex patterns of plant and insect response (e.g. Zvereva & Kozlov, 2006; Tylianakis et al., 2008; Lindroth, 2010; Couture & Lindroth, 2012; Couture et al., 2012; Robinson et al., 2012). Elevated temperatures may interact with other climatic or environmental factors to influence phytochemistry (e.g. Veteli et al., 2007; Moreira et al., 2014; Virjamo et al., 2014) and plant–herbivore interactions (e.g. De Sassi et al., 2012; Rasmann et al., 2014). Such interactive effects complicate interpretations and predictions of plant and insect responses to climate change. Nonetheless, experimental warming studies, such as this one, improve our understanding of the potential range of responses with future

Table 2 Effects of experimental warming on insect performance traits. Data are F_{df} and P values for fixed effects from linear mixed models examining warming treatments (a) across host tree species and (b) within host species. Significant ($P \le 0.05$) results are shown in bold ($N \approx 12$ trees per species per treatment). See Fig. 2 for corresponding data illustrating mean responses

(a)

	Warming		Species		Warming × Species		Initial larval weight	
	$F_{2,56}$	P	$F_{1,56}$	P	$F_{2,56}$	P	$F_{1,56}$	P
Development time	6.03	**0.004**	43.75	**<0.001**	1.76	0.182	4.77	**0.033**
Final larval weight	3.55	**0.035**	0.08	0.785	1.31	0.277	18.67	**<0.001**
Total consumption	17.81	**<0.001**	10.25	**0.002**	11.68	**<0.001**	7.63	**0.012**
Food conversion efficiency	10.54	**<0.001**	19.40	**<0.001**	4.34	**0.018**	0.46	0.499

(b)

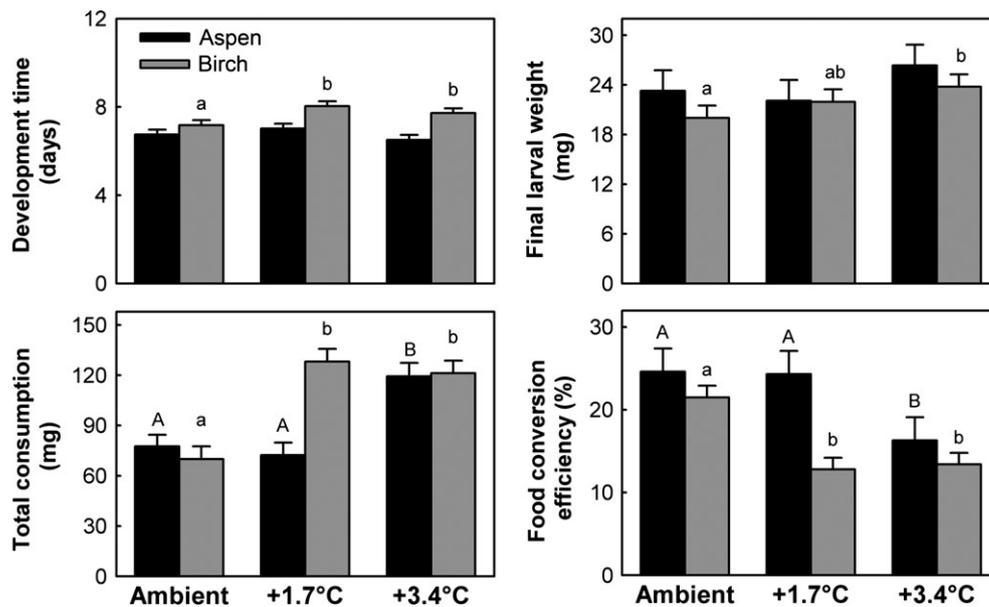|	Aspen				Birch			
	Warming		Initial larval weight		Warming		Initial larval weight	
	$F_{2,23}$	P	$F_{1,23}$	P	$F_{2,31}$	P	$F_{1,31}$	P
Development time	2.85	0.078	4.38	**0.048**	7.50	**0.002**	0.30	0.586
Final larval weight	1.44	0.258	0.39	0.537	3.34	**0.049**	23.82	**<0.001**
Total consumption	9.60	**0.001**	0.27	0.617	17.90	**<0.001**	9.29	**0.005**
Food conversion efficiency	5.23	**0.013**	0.47	0.502	10.42	**<0.003**	1.14	0.294

Fig. 4 Indirect effects of warming on insect performance traits. Bars are means (+1 SE) for untransformed data ($N = 28$ for aspen; $N = 36$ for birch). Letters show within-species pairwise differences ($P \le 0.05$), with capital letters for aspen (black bars) and lower case letters for birch (gray bars).

increases in temperature as well as which species may be especially sensitive to climate change.

In the first year of our study (2011), spring temperatures were average relative to mean temperatures over the last few decades (1981–2012 'climate normals'). In 2012, however, study sites experienced the warmest and earliest spring on record, to date (Midwest Regional Climate Center, http://mrcc.isws.illinois.edu). In

Table 3 Relationships between insect performance traits. Pearson's correlation coefficients (r) are shown with significant correlations in bold

	Aspen (N = 28)		Birch (N = 36)	
	r	P	r	P
Development time and final weight	−0.79	**<0.001**	−0.31	0.063
Total consumption and final weight	0.68	**<0.001**	0.57	**<0.001**
Development time and total consumption	−0.46	**0.015**	−0.19	0.397
Food conversion efficiency and development time	−0.40	**0.036**	−0.27	0.117
Food conversion efficiency and final weight	0.37	0.051	−0.31	0.274
Food conversion efficiency and total consumption	−0.41	**0.031**	**−0.83**	**<0.001**

Table 4 Phytochemical concentrations in 2012 (mean percent dry weight ± 1 SE) for trees used in bioassays

	Aspen (N = 28)			Birch (N = 36)		
	Ambient (n = 12)	+1.7 °C (n = 9)	+3.4 °C (n = 7)	Ambient (n = 12)	+1.7 °C (n = 12)	+3.4 °C (n = 12)
Nitrogen	2.8 ± 0.1	2.8 ± 0.1	3.2 ± 0.1	2.9 ± 0.1 a	2.5 ± 0.1 b	3.1 ± 0.1 a
Sugar	30.8 ± 1.3 a	29.0 ± 1.5 ab	25.4 ± 1.4 b	17.3 ± 0.3 ab	18.2 ± 1.1 a	15.6 ± 0.5 b
Starch	4.3 ± 0.3	3.8 ± 0.4	3.2 ± 0.4	6.0 ± 0.5 a	5.7 ± 0.4 ab	4.3 ± 0.5 b
Lignin	8.1 ± 0.6	8.1 ± 0.3	7.3 ± 0.4	5.9 ± 0.3 ab	5.6 ± 0.3 b	7.0 ± 0.4 a
Condensed tannins	11.5 ± 1.2	10 ± 1.2	11.5 ± 1.5	8.7 ± 0.6	7.8 ± 0.7	8.2 ± 0.7
Phenolic glycosides	2.9 ± 0.5	3.0 ± 0.8	4.2 ± 1.0	na	na	na

Letters show significant ($P \leq 0.05$) within-species differences.
na, not applicable.

Table 5 Partial least squares regression analyses examining the influence of phytochemistry on insect performance traits (N = 28 for aspen and N = 36 for birch). Values are overall model fit and standardized regression coefficients, which indicate the direction and strength of relationships between explanatory and response variables

	Development time		Final larval weight		Total consumption		Food conversion efficiency	
	Aspen	Birch	Aspen	Birch	Aspen	Birch	Aspen	Birch
Model fit (R^2)	0.47***	0.44***	0.35**	0.42***	0.20*	0.38**	0.21*	0.36**
Nitrogen	−0.21	−0.77	0.17	–	0.19	−0.20	0.10	0.57
Sugar	0.29	−0.05	−0.24	–	−0.22	–	−0.14	0.32
Starch	–	−0.12	–	–	–	−0.24	–	0.51
Lignin	–	–	–	–	–	–	–	–
Condensed tannins	0.13	–	−0.15	−0.23	–	–	−0.19	−0.06
Phenolic glycosides	0.13	na	–	na	–	na	−0.20	na
Initial larval weight	−0.21	–	0.21	0.56	0.13	0.57	–	−0.29

Explanatory variables included in models were selected based on variable of importance for projection (VIP) scores (VIP > 0.80), which provide a measure of the amount of variation explained by each independent variable. Dashes indicate explanatory variables did not meet VIP threshold scores and were not included in final models.
*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$.

the average climate year, elevated temperatures reduced condensed tannins and lignin for aspen and birch as well as aspen sugar concentrations. These response patterns are consistent with trends demonstrated in the meta-analysis by Zvereva & Kozlov (2006). In the atypically warm year, elevated temperatures did not affect defense chemicals, but did reduce aspen sugar and birch starch concentrations. Again, these changes in carbohydrates support trends shown in Zvereva & Kozlov (2006). Overall, warming-induced

phytochemical shifts were nominal and chemical profiles were relatively consistent across years. Condensed tannin and lignin concentrations, however, demonstrated considerable variation across years. While warming treatments had no effect on these defense compounds in 2012, there was a large shift in relative concentrations of condensed tannins and lignin in 2012 compared with levels in 2011. These differences may be related to variable weather conditions across years. In addition to potential effects on plant–insect interactions, temperature- or climate-related shifts in condensed tannins and lignin could have important implications for community and ecosystem level processes (e.g. Schweitzer *et al.*, 2008).

In 2012, bioassay results indicated that elevated temperatures increased larval consumption and decreased food conversion efficiency. Insects evidently exhibited compensatory feeding due to reductions in host quality, which resulted in minimal effects on larval growth and biomass. Overall, concentrations of primary metabolites demonstrated greater variation than did secondary metabolites in this study year. Within the context of these temperature-related phytochemical shifts, primary metabolites (i.e. nitrogen and carbohydrates) were stronger predictors of forest tent caterpillar performance traits than were secondary metabolites. For aspen and birch, bioassay results indicated that warming-induced reductions in carbohydrates were associated with increased consumption.

In general, secondary metabolite concentrations showed only modest variation among warming treatments in 2012. Moreover, weak relationships between those compounds and insect performance were likely due, in part, to their low levels. For example, aspen phenolic glycoside concentrations were low (~3–4% dry weight) compared with levels shown to significantly affect insect performance (e.g. up to 10–15% in Hemming & Lindroth, 1995; Donaldson & Lindroth, 2007; Couture *et al.*, 2014). Likewise, condensed tannin concentrations were low in 2012. In some cases, early instars may be more susceptible to low levels of defense compounds compared with late instars (e.g. Lahtinen *et al.*, 2004; Ruusila *et al.*, 2005). For forest tent caterpillar, however, previous research indicates that early and late instars respond similarly to aspen quality (e.g. Hwang & Lindroth, 1997). Ultimately, the influence of individual chemical constituents on insect performance depends on relative proportions of other metabolites, including nutrients (Mattson & Scriber, 1987; Awmack & Leather, 2002; Behmer, 2009), in addition to the interaction between direct and indirect defenses (Gatehouse, 2002; Haukioja, 2005). While chemical defenses, including phenolic compounds, can be induced in response to defoliation (e.g. Stevens & Lindroth, 2005), we found no evidence of induction in this study. Our experimental design, however, did not allow us to adequately evaluate constitutive vs. induced defenses.

Overall, we found that birch bioassay trees demonstrated greater phytochemical variation in response to warming than aspen. Consistent with this trend, larvae reared on birch were more responsive to plant-mediated warming effects. Our results show that elevated temperatures can differentially affect foliar nutrients and defense chemicals, leading to corresponding host species-specific changes in insect performance. Such warming-induced phytochemical shifts could influence insect preferences for and performance on different host tree species. Variation in phytochemistry, however, did not fully explain temperature effects on insect performance. In bioassays, the food source for phenologically advanced insects was shifted from ambient foliage prior to feeding trials to warmed foliage during bioassay feeding trials. It is possible that this shift contributed to unexplained variation in insect performance. Warming-induced changes in plant and insect phenological synchrony (e.g. Schwartz-berg *et al.*, 2014) may influence relationships among phytochemical, host quality, and insect performance traits. Additionally, elevated temperatures can reduce food conversion efficiency when a species experiences temperatures above its thermal optima (Angilletta, 2009; Bauerfeind & Fischer, 2013). Future climate warming will likely affect insect performance and herbivore damage to host plants via a number of mechanisms, including direct effects on plant and insect physiology as well as indirect effects mediated by organisms at higher trophic levels (Jamieson *et al.*, 2012).

Conclusions

While the consequences of climate warming on trophic interactions remain uncertain, our study demonstrates that direct and indirect warming effects can alter important tree resistance and insect performance traits. Additionally, results presented here suggest that annual weather conditions and interannual climate variability will likely influence the overall effects of warming trends on tree chemical profiles and host quality for herbivores. Given that many aspects of weather conditions, including temperature, moisture, and their interaction, vary from year to year, teasing apart the underlying mechanisms affecting phytochemistry will require longer time series to evaluate plant response. Moreover, further research is needed to determine

whether warming-induced shifts in phytochemistry and insect performance could influence insect population dynamics and herbivore damage to host trees.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Project timeline, including dates for temperature treatment, tree budbreak, leaf sample collection, and insect bioassay.
Table S2. Correlation matrices relating foliar nutrient and defense constituents.