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Temperature affects insect outbreak risk through tritrophic interactions mediated by plant secondary compounds

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Citation: Kollberg, I., H. Bylund, T. Jonsson, A. Schmidt, J. Gershenzon, and C. Björkman. 2015. Temperature affects insect outbreak risk through tritrophic interactions mediated by plant secondary compounds. Ecosphere 6(6):102. http://dx.doi.org/10.1890/ES15-000021.1

Abstract. Global warming may affect population dynamics of herbivorous insects since the relative impact of bottom-up and top-down processes on herbivore survival is likely to be influenced by temperature. However, little is known about the mechanisms by which warming could affect regulation of populations, particularly when indirect effects across trophic levels are involved. We quantified larval survival of the needle-feeding European pine sawfly, *Neodiprion sertifer*, either protected from (caged) or exposed to natural enemies at three geographically separated localities in Sweden. The study shows that larval survival is affected by temperature but the direction of the effect is influenced by plant secondary compounds (diterpenes). The results suggest that survival of exposed larvae feeding on needles with high diterpene concentrations will decrease with increasing temperature, while larval survival on low diterpene concentration is less predictable with either no change or an increase with temperature. This food quality dependent response to temperature is probably due to diterpenes having a double-sided effect on larvae; both a negative toxic effect and a positive anti-predator defense effect. Increased temperature had also consequences at the population level; an established population model parameterized using data from the study to evaluate the influence of temperature and plant secondary compounds on the regulation of the sawfly predict that, depending on food quality, outbreak risks could both decrease and increase in a warmer climate. If so, effects of plant secondary compounds will play an increasing role for larval survival in a future warmer climate and temperature will, via multitrophic effects on larval survival, strongly influence how sawfly and other insect populations are regulated.

Key words: climate change; diterpenes; European pine sawfly; natural enemies; *Neodiprion sertifer*; plant defense; population dynamics; predation; regulation; Sweden.

Received 12 January 2015; revised 20 February 2015; accepted 3 March 2015; final version received 27 April 2015; **published** 25 June 2015. Corresponding Editor: D. P. C. Peters.

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INTRODUCTION

The world's climate is changing (IPCC 2013) and ecological effects of global warming have been documented across many levels of biological organization and across many different ecosystems (Schröter et al. 2005, Parmesan 2006, Walther 2010, Bellard et al. 2012). As climate warms, it is likely that regulatory processes of populations will be affected. One reason for this is that the strength of interactions within and between trophic levels often depends on climatic conditions and that species at different trophic levels may respond differently to increasing temperature (Berggren et al. 2009, Gilman et al. 2010, Montoya and Raffaelli 2010). Insects are

particularly sensitive to changes in temperature, and increasing temperatures due to global warming have in general been predicted to lead to improved performance and more generations per year (Bale et al. 2002). For pest insects, improved performance, e.g., higher fecundity or increased survival, could lead to increased frequency, and/or magnitude of outbreaks, with negative economic consequences for society. Previous studies on insect herbivore performance in relation to climate change have mainly focused on direct temperature effects on host plants (Ayres and Lombardero 2000), herbivores (Bale et al. 2002) and natural enemies (Colinet et al. 2007). In some studies indirect effects of temperature on herbivore performance, through effects on another trophic level, have been considered (Virtanen and Neuvonen 1999, Joern et al. 2006), but studies including tritrophic interactions are rare. However, since herbivore response is a result of both bottom-up and top-down processes, such studies are needed to increase our understanding of how changes in temperature will affect species of interest.

Both insect herbivores and their invertebrate predators are likely to increase their activity with increasing temperature (Uvarov 1931). Whether the increased activity will result in more herbivore damage to plants depends on whether different trophic levels respond similarly or not to increasing temperature (Berggren et al. 2009). In some systems it has been shown that the temperature sensitivity of species increases with increasing trophic level. This suggests that top-down regulation of herbivorous insects by natural enemies could be higher at high temperatures (Virtanen and Neuvonen 1999, Voigt et al. 2003, Barton et al. 2009). How bottom-up effects, i.e., plant quality, interact with top-down effects and modify the response to temperature is largely unknown.

One aspect of plant quality is secondary compounds, which in many cases function as chemical protection against herbivores. The herbivore often respond to secondary compounds in a quantity dependent way and the compounds can affect the herbivore directly (by decreasing survival and/or growth rate) and indirectly by, e.g., attracting the herbivore's natural enemies (Feeny 1992). In some cases, the herbivore has overcome the plant's defense and uses the secondary compounds in its own anti-predator defense (Eisner 1970). If predators and parasitoids become more active at higher temperatures, herbivore anti-predator defenses, such as sequestered plant secondary metabolites, may become increasingly important for the survival of the herbivore and need to be considered when predicting the response of insect herbivores to warming.

The European pine sawfly, *Neodiprion sertifer*, is an example of a species where the defense capability of the larvae increases with the amount of secondary compounds (diterpenes) in the food (Björkman and Larsson 1991, Björkman et al. 1997). The food (pine needles) is detoxified and the diterpenes are stored in special pouches connected to the foregut of the larvae. When enemies approach the larvae, they regurgitate sticky resin droplets that repel the enemy. The cost of detoxifying their food is expressed as increased mortality during early larval stages and longer developmental times for larvae feeding on needles with high diterpene content (Larsson et al. 1986), while the gain is manifested as increased survival due to reduced mortality from arthropod predators (Björkman and Larsson 1991, Björkman et al. 1997). How increasing temperature affects the net outcome of this double-sided effect of diterpenes in the sawfly and how such tritrophic interactions may affect herbivore survival and population regulation in a warmer climate has not been studied.

The purpose of this study was to estimate *N. sertifer* larval survival in relation to temperature, evaluating the roles of food quality (concentration of diterpenes in needles) and predation mortality from natural enemies to increase our knowledge about the regulatory mechanisms for the population dynamics of this species in a warmer climate. To achieve this we studied the relative importance of bottom-up and top-down processes using geographically separated localities that differed in temperature regimes. We expected that higher temperatures should strengthen the top-down control of this species (due to increased activity of natural enemies), which could result in reduced forest damage in the future. More specifically, our hypotheses were that (1) larval mortality due to predatory arthropods increases with temperature, but (2) the mortality would be lower among larvae

feeding on needles with a high diterpene concentration, due to a better anti-predator defense. Furthermore, to investigate the potential net effect of temperature and diterpene concentration on larval survival, and thus, how increased temperature could alter the regulation of population growth and probability of outbreak, we used a population model originally developed by Larsson et al. (2000).

Material and Methods

Study species

The European pine sawfly is a herbivorous insect with irregular outbreaks, considered to be one of the major pest species in boreal forests in Eurasia and North America. The eggs are laid in the autumn: the females insert 60–100 eggs into pockets in pine needles (Kolomiets et al. 1979). The larvae hatch in the spring and then feed gregariously on pine (*Pinus* spp.) needles. The larvae normally stay on the branch as long as there is food available (Griffiths 1959). The average size of a late instar larval group is about 20–60 individuals (I. Kollberg, *personal observation*). The main natural enemies in the larval stage are ants, spiders, predatory beetles and parasitoids, while birds seem to be of less importance (Kolomiets et al. 1979). Male larvae pass through four instars and females five before dropping to the ground to pupate (Kolomiets et al. 1979). Predation on the pupae by small mammals has shown to be density dependent and suggested to regulate sawfly populations at low densities (Holling 1959, Hanski and Parviainen 1985).

Study area

The study was conducted in 2010 at three localities in Sweden; Asa (57°11′ N, 14°47′ E), Uppsala (60°00′ N, 18°18′ E) and Vindeln (64°12′ N, 19°50′ E) (Appendix A). At each locality, two replicate sites (A and B) were chosen 500–1000 m apart. A data logger recorded the temperature every hour at each site. The logger was placed on the northern side of a spruce stem (to minimize impact from direct sunlight) at a height of approximately 1.5 m. The sites consisted of naturally generated *Pinus sylvestris* dominated forests, approximately 15 years old. Other common tree species were birch and spruce. At each site 12–15 pines of a manageable working size, i.e., approximately 2–3 m, were haphazardly selected (Appendix A).

Larval performance

On 3 May 2010, eggs of *N. sertifer* were collected in an outbreak area close to Ramnäs (59°46′ N, 16°16′ E). It would have been preferable to have collected eggs at several areas, but we were restricted to locations where sawflies could be found in sufficient numbers. Egg origin however, does not seem to influence the direct effect of temperature on larval survival (Kollberg 2013). Egg batches were collected by cutting off egg-bearing branches and placing a plastic bag around each twig with eggs to prevent the spread of potential virus infection between twigs. Branches were then stored in buckets of water at 5°C in the lab for later use. About one week before it was time to start the experiment the eggs were placed at room temperature to hatch. Needles with 15–27 newly hatched larvae, or eggs just about to hatch, were removed from the shoots, threaded onto insect pins and kept in small plastic containers. The larvae were then transferred to the experimental branches on the 12–15 selected pine trees at each field site by attaching one pin with egg-bearing needles per branch, allowing the larvae to crawl over to the fresh needles. This method for transferring larvae has been successfully used in previous studies (Larsson et al. 1986, Björkman 1997, Björkman et al. 1997).

The transfer of eggs/newly hatched larvae was timed to coincide approximately with the natural hatching of *N. sertifer* larvae at each locality and therefore occurred at different dates. The emergence of the larvae is mostly temperature dependent (Henson et al. 1970) and we thus started the experiment on 12 May in Asa, on 21 May in Uppsala and on 2 June in Vindeln. For each pine two similar sized experimental branches within the same whorl were selected, one branch to host larvae enclosed within a fine-meshed cage (to exclude predators) and one branch to host larvae without a cage (to allow exposure to predators) (see Appendix A for experimental setup). Weekly survival and development of the larvae were monitored by four weekly visits, until the larvae were five weeks old and in their later instars. The uncaged

(exposed) larvae was easy to keep track of thanks to their stationary and gregarious behavior (Griffiths 1959). Moreover, natural disturbances like heavy rain and high winds have been shown to not easily dislodge the larvae from the branch (Schedl 1937) and if knocked down most larvae soon return to the same tree (Teräs 1982). However, no larvae were seen on neighboring branches, indicating negligible loss due to migration. Furthermore, the natural densities of *N. sertifer* at the experimental sites were low and the likelihood for ''natural'' larvae to join the experimental groups was considered to be close to zero. We thus consider the differences in weekly survival between the open and caged treatments to mainly indicate predation rate on the larvae. This method of measuring predation, using sleeve cages, is well established and has been used many times before for this system (Larsson et al. 1986, Björkman 1997, Björkman et al. 1997).

To assess to what degree the cages affected the microclimate the temperature was monitored within and outside a cage during 24 hours. This showed that the daily mean temperature was about 0.5°C lower in the cages.

Food quality

Needles collected from each tree used in the field experiment were analyzed for diterpenes. The needles were collected in 2012 (in June/July) from the branch closest above the branch that had hosted the exposed larvae. Previous studies have shown that diterpene concentration is similar among branches from the same and adjacent whorls of pines growing in young stands (Gref and Tenow 1987). Since the branches had been marked they were easy to find again two years after the experiment. Age and shading are two factors that may have affected absolute diterpene concentrations between years (Gref and Tenow 1987). Regarding age, there are no significant differences in the relative diterpene concentration between years of mature needles (that the larvae are feeding on) (Gref 1982) and shading is not likely to have caused changes in diterpene concentrations between years since the stands were all young with homogenous light conditions. We thus consider the data on diterpene concentrations in 2012 as representative of the conditions in 2010. Unfortunately, the stand at one of the sites in Asa had been thinned in 2012 which might have affected diterpene concentrations. Therefore, this site was not included. Immediately after removal from the trees the needles were put on dry ice and transported to the laboratory. The analyses were conducted following the procedure described in Kollberg et al. (2013). The concentration of diterpenes in a tree was expressed as the amount relative to the tree with the lowest amount (diterpene $\text{content}_{\text{tree}X}$/diterpene $\text{content}_{\text{tree}MIN}$).

Statistical analysis

To analyze whether larval survival differed among localities due to temperature, experimental treatment (caged/exposed larvae) and food quality (diterpene concentration of needles) we used a mixed effects logistic regression model (as implemented by the glmer-function in R package lme4, Bates et al. 2011, R version 2.15.1, R Development Core Team 2012). Repeated measurements of weekly survival were used as the response variable. The survival was formulated as a binomial variable which consisted of the number of larvae that had survived from one week to the other (successes) and the number of larvae that had disappeared (failures). Since both eggs and larvae were present at the start of the experiment, the first count, after one week when all larvae had hatched, was used as the initial number of larvae in the model. The assumption of independence was violated by repeated measurements on the same tree and larval group, but this was dealt with by introducing tree identity as a random effect in the model. In the full model, locality (the two sites at each latitude pooled) and treatment (caged/exposed larvae) were used as categorical explanatory factors. Weekly mean temperature, the amount of diterpenes in the needles, larval development stage, larval development rate (the difference in the proportion of larvae in a certain larval stage between two weeks), group size (number of surviving larvae at the beginning of the week), the abundance of surrounding trees and pine diameter were used as continuous covariables. The latter two, abundance of surrounding trees (measured as the vertical projection of trees within a 3 m radius of the experimental pines) and pine diameter at breast height, were included as potential proxies for predator abundance

(based on the hypotheses that abundance of natural enemies in general is larger in diverse habitats [Root 1973, Russell 1989] and increases with the size of pines [Jactel et al. 2005]) because we could not estimate the abundances of natural enemies around trees directly without interfering with the experiment.

The focal interaction term in the model was the locality × temperature × diterpene concentration × experimental treatment interaction. This term tests for differences in larval survival among localities as a function of temperature, food quality and exposure to natural enemies. Significant interactions with the treatment (caged/exposed larvae) would indicate a different response in the survival of the exposed larvae to that of the caged larvae. Interaction terms between treatment (caged/exposed larvae) and each of the continuous variables were also included in the full model.

Model reduction was conducted by first removing non-significant interactions and then non-significant variables (not included in significant interactions) one by one from the initial model until it contained only significant explanatory variables as well as significant interactions and their associated variables. Prior to analysis, all continuous variables were centered to their mean values to avoid parameterizations at unrealistic values, e.g., at a group size of zero.

The reduced model was then used to examine quantitatively the effect of temperature on larval survival. Diterpene concentration was used as a continuous variable in the statistical model but in order to facilitate visualization and interpretation of the results we chose to reduce this variable to either "low" or "high", corresponding to the mean ± 1 SD of the concentration of diterpenes in pine needles at each locality. That is, the slope of the relationship between larval survival and temperature was obtained for every combination of (1) caged and exposed larvae, feeding on needles with (2) low or high diterpene concentration, at (3) each locality.

Population model

To analyze how the combined effect of temperature and food quality on larval survival, as documented by the field study, might scale up to population level characteristics (such as population growth rate and subsequently probability of outbreak) we modified a discrete population model of the European pine sawfly, originally developed by Larsson et al. (2000), to incorporate effects of temperature (T) and diterpene concentration of pine needles (D) on larval survival. The model assumes that (1) larval survival is density independent but affected by temperature and food quality, and (2) pupal survival is density dependent due to predation by small mammals (Holling 1959, Larsson and Tenow 1984, Larsson et al. 2000). Survival rates in all other life stages are assumed to be density independent and constant so that they can be aggregated into one single background survival (s_b). We choose to model the number of pupae, since this figure has been used to characterize the extent of damage from pine sawfly populations (Hanski 1987). For details on the model see Appendix B.

Results

Weekly mean temperatures at each locality fluctuated, albeit with an overall increasing trend over time (Fig. 1; Appendix C). The relative concentration of diterpenes in needles varied among pines at each site, but were on average highest in Asa A, intermediate in Uppsala B and Vindeln B and lowest in Uppsala A and Vindeln A (Appendix C). The total survival (over the four week study period; Appendix C), as well as weekly survival (Fig. 1), was at all sites higher for the caged than for the exposed larvae.

The statistical analysis revealed an overall increase in survival with increasing larval development rate, larval developmental stage and larval group size (Appendix D), but no significant effect of abundance of surrounding trees or pine diameter. More importantly, the analysis revealed a significant four-way interaction between locality, temperature, diterpene concentration and treatment (caged/exposed larvae) ($p < 0.01$; Appendix D). This result means that larval weekly survival was affected by temperature and food quality (diterpene concentration of needles) but that the relationship (between survival, temperature and food quality) differed among localities and experimental treatment (caged/exposed larvae). The complex interaction was further explored by studying the relationship between larval survival and temperature sepa-

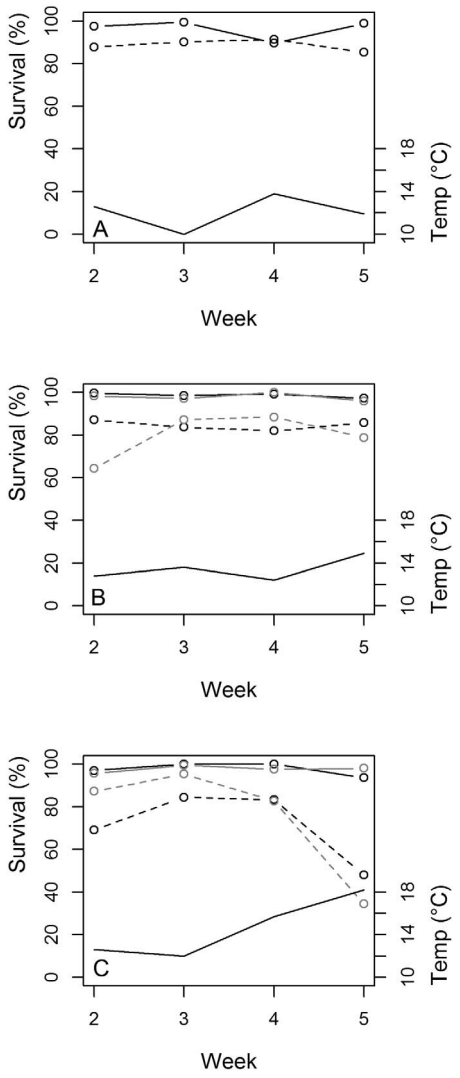

Fig. 1. Weekly larval survival (left y-axis) of *Neodiprion sertifer* during the study period (four weeks) at three different localities in 2010; Asa (A), Uppsala (B), and Vindeln (C). At each locality there were two sites (black and grey lines). The survival is shown for larvae protected against natural enemies (solid lines) and exposed to natural enemies (dashed lines). For the same period, weekly mean temperatures (right y-axis) pooled over the two sites at each locality is also shown. Differences in mean temperatures between the sites within localities were small (Appendix C).

rately for caged and exposed larvae, feeding on needles with either low or high diterpene concentrations at each locality (Table 1, Fig. 2). For caged larvae (Fig. 2A–C), survival tended to decrease with increasing temperature at all localities except for larvae feeding on low diterpene needles in Asa (which were unaffected by temperature). For exposed larvae (Fig. 2D–F), however, the effect of temperature depended on the diterpene concentration. The temperature × diterpene interaction was most apparent in Vindeln, which also showed the greatest temperature range (Fig. 2F). Here the weekly survival of exposed larvae feeding on high diterpene needles decreased significantly with increasing temperature, while survival among larvae feeding on low diterpene needles tended to increase with increasing temperature. Although both mean and range in temperature was lower at Asa (Appendix C), qualitatively, the same effects were seen here (Fig. 2D), i.e., with larval survival tending to decrease with temperature on high diterpene needles and increase with temperature on low diterpene needles. In Uppsala, which showed the smallest temperature range, weekly survival of exposed larvae on low diterpene needles decreased with increasing temperature but was unaffected on high diterpene needles (Fig. 2E).

When comparing the survival of exposed larvae in relation to temperatures to that of caged larvae, the slopes differed significantly for larvae feeding on high concentrations of diterpenes in Uppsala and low concentrations of diterpenes in Vindeln, and similar (but nonsignificant) trends were also seen among larvae on low diterpene needles in Uppsala and on high diterpene needles in Vindeln (Table 1).

Discussion

We here report interesting effects of temperature on tritrophic interactions in a plant–insect system, driven by the interplay among plant quality, herbivore anti-predator defense and natural enemies. More specifically, larval survival of an herbivorous pest species, the European pine sawfly, was found to be affected by temperature, but the direction of the effect, depended on the concentration level of chemical defense compounds (diterpenes) in the host plant (Fig. 2). In the locality with the largest temperature range, survival tended to increase with temperature on low diterpene concentrations and decreased on high diterpene concentrations.

The difference in slope between exposed and

Table 1. Model parameter estimates of the effect of temperature on larval (*Neodiprion sertifer*) survival at three different localities in Sweden depending on the concentration of diterpenes in the needles and whether the larvae had been protected (caged) or exposed to natural enemies.

Treatment	Slope	SE	z	p
Asa (temperature 10.1–13.8°C)				
Low concentration of diterpenes (5.0)				
Caged larvae	0.007	0.47	−0.014	0.99
Exposed larvae	0.22	0.18	1.20	0.23
Exposed larvae-caged larvae†	0.22	0.50	0.45	0.65
High concentration of diterpenes (16.3)				
Caged larvae	−1.52	0.94	−1.62	0.11
Exposed larvae	−0.32	0.19	−1.67	0.10
Exposed larvae-caged larvae†	1.20	0.95	1.27	0.21
Uppsala (temperature 12.3–14.9°C)				
Low concentration of diterpenes (2.8)				
Caged larvae	−1.09	0.34	−3.17	0.002
Exposed larvae	−0.50	0.22	−2.29	0.02
Exposed larvae-caged larvae†	0.59	0.38	1.54	0.12
High concentration of diterpenes (8.8)				
Caged larvae	−0.92	0.36	−2.59	0.01
Exposed larvae	−0.09	0.20	−0.46	0.65
Exposed larvae-caged larvae†	0.83	0.39	2.13	0.03
Vindeln (temperature 11.8–18.2°C)				
Low concentration of diterpenes (2.8)				
Caged larvae	−0.64	0.19	−3.40	<0.001
Exposed larvae	0.18	0.12	1.53	0.12
Exposed larvae-caged larvae†	0.83	0.22	3.69	<0.001
High concentration of diterpenes (7.2)				
Caged larvae	−0.23	0.12	−1.97	0.05
Exposed larvae	−0.44	0.08	−5.28	<0.001
Exposed larvae-caged larvae†	−0.21	0.14	−1.51	0.13

Note: The diterpene concentrations in the needles are set to be either low (the mean value at each locality − 1 SD) or high (the mean value at each locality + 1 SD), all other variables in the model are set to their mean.

† The difference between the slopes of the caged and exposed larvae as temperature increases indicates the relative importance of bottom-up and top-down effects. A positive difference suggests an indirect bottom-up effect on the survival through a stronger anti-predator defense and a negative difference suggests a relative stronger top-down pressure from natural enemies.

caged larvae (Table 1) indicates how the relative importance of bottom-up and top-down effects change with increasing temperature at each locality. From this it can be seen that both the temperature range at each locality and the amount of diterpenes in the needles influenced the relative role of bottom-up and top-down effects, and hence larval survival. Comparably low temperatures (as in Asa) resulted in little change in the survival of exposed relative to caged larvae on low diterpene needles, but for larvae feeding on needles with very high diterpene concentration, even a small increase in temperature tended to reduce survival due to bottom-up effects (as indicated by a positive difference in slope between exposed and caged larvae; Table 1). Larvae exposed to higher temperatures (as in Vindeln) seemed to make particular use of the diterpenes in their own anti-predator defense when feeding on low diterpene needles, since the survival of exposed larvae was less reduced than that of the caged. However, high temperatures in combination with high concentrations of diterpenes, seemed to induce a stronger top-down effect. Thus, our experimental data suggest that the response of pine sawfly larvae to a changing climate is determined by the combined effect of plant quality on two higher trophic levels, as manifested through (1) the direct toxic effect of diterpenes on larval performance and (2) the indirect effect of diterpenes on larval mortality, via effects on the anti-predator defense of the larvae. This tritrophic interaction in combination with how temperature shifts the balance between the direct and indirect effect is likely to have population level consequences, affecting population growth rate, with not easily foreseen implications for the probability of

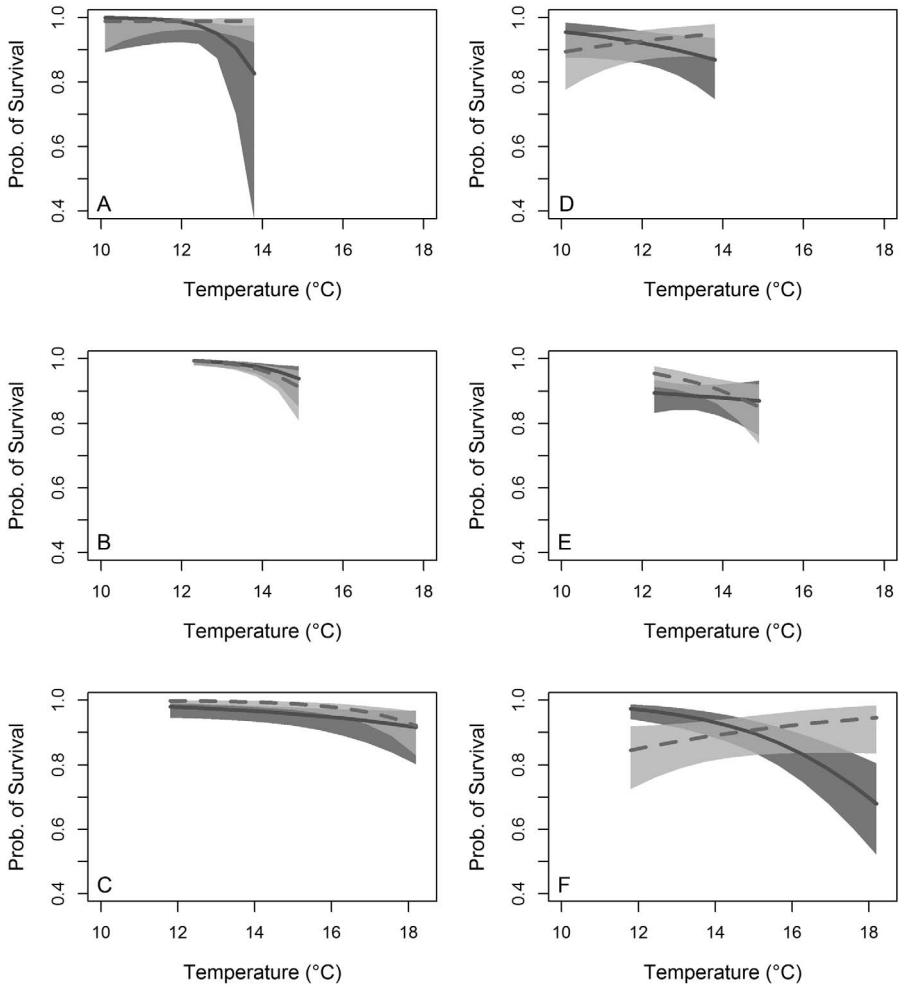

Fig. 2. Weekly survival of sawfly (*Neodiprion sertifer*) larvae relative to temperature and needle diterpene concentration at three localities in Sweden; Asa (A and D), Uppsala (B and E), and Vindeln (C and F). Figures are derived from the model presented in Appendix D. The probability of survival is shown for larvae being either protected (A–C) or exposed to natural enemies (D–F). The diterpene concentrations in the needles are set to be either low (the mean value at each locality − 1 SD) or high (the mean value at each locality + 1 SD), all other variables are set to their overall mean values. The temperature range differed for each locality, hence the different location of the slopes on the x-axes. The dashed lines and light grey areas represent the probability of survival ± CI of larvae feeding on needles with low diterpene content and the solid lines and dark grey areas represent the survival of larvae feeding on high diterpene needles.

outbreak of the pine sawfly in a changing climate. To assess the potential consequences of increasing temperature on the pine sawfly dynamics, a population model (Appendix B: Eqs. 1–3) was parameterized using survival data from the locality with the largest observed mean and range in temperature (Vindeln). That is, from the statistical model (Appendix D) we extracted quantitative predictions of the strength (slope) of the effect of temperature on larval survival (Appendix B: Eq. 3) for exposed larvae feeding on needles with low as well as high concentrations of diterpenes (LD and HD, respectively), yielding $\alpha_{LD} = 0.662$, $\alpha_{HD} = 1.515$, $\beta_{LD} = 0.016$ and $\beta_{HD} = -0.046$, where α is the intercept and β is the slope. Applied to the population model, these values result in increasing population growth rate (λ) with temperature at low diterpene

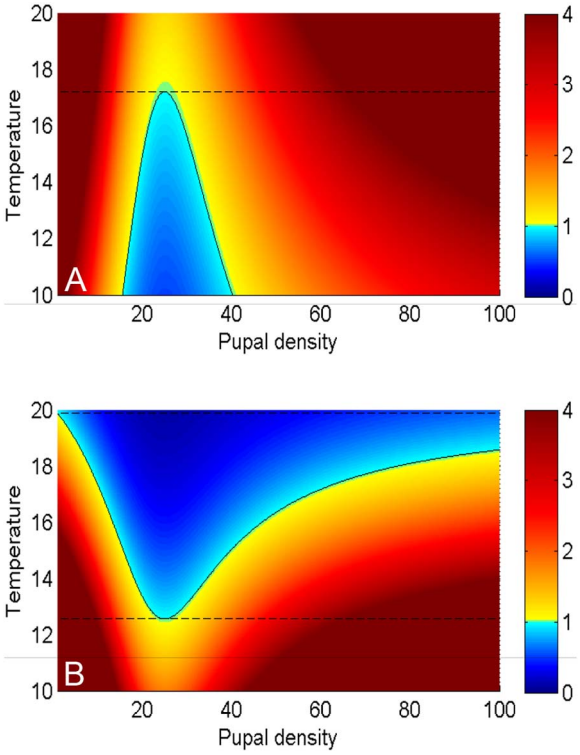

Fig. 3. Effect of temperature and pupal density on population growth rate (λ) of sawfly (*Neodiprion sertifer*) populations feeding on needles either low (A) or high in diterpenes (B). Colors denote regions with stabilizing regulation (low outbreak risk, $\lambda < 1$, different shades of blue) and destabilizing dynamics (producing deterministic outbreaks, $\lambda > 1$, different shades of red) respectively (see colorbar for conversion to value of λ).

concentrations, but decreasing growth rate at high diterpene concentrations (Fig. 3).

More specifically, our analysis shows that the population model, with these parameters, has a region of density dependent control (where $\lambda < 1$ and from which the population will return to low densities) for both low and high diterpene concentrations, at low to intermediately high temperatures (Fig. 3). For low diterpene concentrations, this region is narrow and shrinking with increasing temperature (meaning that increasing temperature is predicted to be destabilizing), and above a threshold (here ≈17°C) the model is unstable (deterministically produces outbreaks), no matter what the starting density of pupae is. For high diterpene concentrations instead, the model is unstable at low temperatures (<12°C), but for an intermediate temperature range (≈12–18°C) temperature is stabilizing (the region of density dependent control increases rapidly with increasing temperature). However, above a threshold temperature (≈20°C), the model enters a new region of instability where the population always crashes (deterministic extinction). This double-sided effect of temperature on population dynamics of the pine sawfly (destabilizing for low, and stabilizing for high diterpene concentrations) is further illustrated by the recruitment function of the population model (Appendix E). For low to intermediate temperatures it is evident that predation mortality on pupae can maintain a regulating function on sawfly populations at low population densities on low diterpene concentrations. Thus, as long as temperature remains below a critical threshold in this scenario (here ≈15°C), population densities cannot reach outbreak densities from small starting densities. However, if average temperature increases or temperature fluctuates and occasionally exceeds the critical threshold, the population may escape control and reach outbreak densities. On high diterpene concentrations instead, this scenario is reversed (with predation mortality maintaining a regulating function at low population densities for intermediate to high temperatures). Thus, the nature of temperature fluctuations, both within and among years, will according to the model be crucial to determine the frequency of outbreaks under various climatic scenarios. This is furthermore illustrated by our analysis of how the outbreak risk can be expected to change due to warming when temperature also fluctuates (Fig. 4). With a non-fluctuating weekly temperature, corresponding to the mean of that observed at most localities in the field experiment (13.5–15°C; Appendix C), sawfly populations can, according to the model, not reach outbreak densities (>100 individuals/m^2) from endemic densities, neither on low nor on high diterpene needles. When weekly temperatures fluctuate however, outbreak risk in this temperature region is, although small (<25%), not zero (Fig. 4). Furthermore, on low diterpene needles the outbreak risk increases rapidly with increasing temperature, but decreases to zero on high diterpene needles. We conclude that effects of plant secondary com-

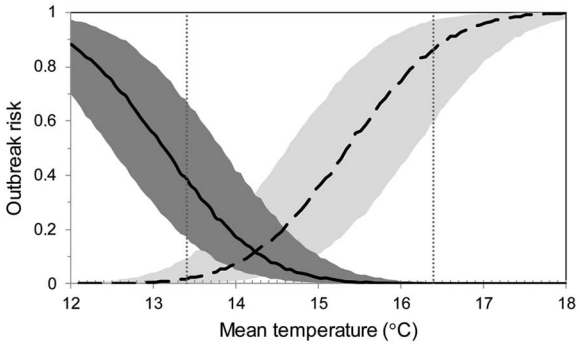

Fig. 4. Outbreak risk in a population model of the pine sawfly (*Neodiprion sertifer*) with larvae feeding on needles either low (dashed line) or high in diterpenes (solid line) and where weekly mean temperature varies. Outbreak risk is the probability of reaching a threshold pupal density of 100 individuals/m^2 within five years, from a starting density of 1 individual/m^2. Shaded areas show the robustness of predicted outbreak risk to uncertainty in the sensitivity of larval survival to temperature (by a 5% increase and decrease respectively of the estimated slope of the effect of temperature on weekly larval survival; Appendix B, Eq. 3). Horizontal dotted lines indicate the mean weekly temperature in the field experiment (13.4°C) and a predicted increase in temperature during the 21st century (to 16.4°C). For details on model simulation see Appendix B.

pounds will play an increasing role for larval survival in a future warmer climate where temperature will strongly influence how sawfly populations are regulated via multitrophic effects on larval survival.

Why does the balance between bottom-up and top-down effects change with temperature, and why is the response fundamentally different for larvae feeding on low and high diterpene needles? We suggest that this is caused by a metabolic response of larvae to temperature. It is known from previous studies that increased diterpene concentrations increase the chemical defense of larvae (Björkman and Larsson 1991), but probably only up to a limit, higher concentrations of diterpenes instead become lethal (Björkman 1997). However, since temperature affect insect metabolic rate (Brown et al. 2004), it is likely that the food intake by larvae increases with temperature and hence also their intake of terpenoids. A consequence is that even though diterpene concentration in the food is relatively constant, temperature can strongly influence the effect on larval survival. The direct toxic effect of diterpenes is shown by the caged larvae for which the survival tended to decrease with increasing temperature; here the higher mortality may be explained by the intake of diterpenes becoming too high, and causing mortality. Among exposed larvae on the other hand, the increased intake of diterpenes with increasing temperatures may be beneficial for survival, but only for larvae feeding on low diterpene needles. A possible explanation is that the cost of detoxifying smaller amounts of diterpenes here was matched or even superseded by a gain in natural enemy defense. This positive effect though, was not seen among larvae feeding on needles high in diterpenes. Here there seems to be an increasingly negative effect of temperature on larval survival, presumably due to rapidly increasing costs of detoxifying high amounts of diterpenes as higher temperatures cause consumption rate of the larvae to increase.

Since temperature affects insect metabolism, it is likely that also insect natural enemies become more active and efficient in warmer conditions (Vucic-Pestic et al. 2011) which could result in increased predation on herbivores, unless predator defense mechanisms can provide more effective protection. The slope of the relationships between survival and temperature on high diterpene needles in Vindeln was steeper (more negative) for exposed compared to caged larvae (Fig. 2, Table 1) suggesting that this could be the case. However, the study design does not give opportunity to disentangle if the reduced survival was due to more active (and thus, per unit time, more efficient) predators or if it was an indirect effect on predation via the diterpenes in the needles. The latter effect could be possible if an increased detoxification of defense chemicals prolonged the development of the larvae. It is also possible that the sawfly anti-predator chemical defense became less efficient at higher temperatures (e.g., if the sawfly larvae cannot produce enough resin droplets when it is warm to counter an increased predation pressure from the more active natural enemies, or if higher temperatures interfere with the process of sequestering terpenoids from the food). To summarize, although the idea that warmer

temperatures strengthen top-down control is supported by our study, several interpretations of the mechanisms behind our results are possible. Thus, there is a great need for more studies on how species at the third trophic level respond to climate warming in order to disentangle mechanisms behind observed patterns.

Another concern is the lack of data on natural enemies. Mortality caused by natural enemies is likely to be associated with their abundance. Since it was difficult to obtain a reliable estimate of the abundances of natural enemies and at the same time not interfere with the experiment, we chose instead to measure the abundance of trees surrounding the experimental pines and rely on the hypothesis that natural enemies in general are more numerous in diverse habitats (Root 1973, Russell 1989). Predators of sawfly larvae are generalists and the abundance of trees surrounding an experimental pine would give a measure of available microhabitats for other prey species. Lots of potential prey would attract many natural enemies and we therefore assumed a correlation between tree abundance and the number of potential natural enemies. Neither tree abundance, nor pine diameter had any effect on the survival in the statistical model, but we cannot rule out that qualitatively and quantitatively differences in the local natural enemy pools could have affected the results, especially if some predators are more sensitive to temperature than other.

In this study system it seems likely that both bottom-up and top-down effects will become more important in a warmer climate, affecting larval survival directly as well as indirectly. This is in line with observed results in a recent meta-analysis (Rodriguez-Castaneda 2013); where both the effect of predation and of plant defenses on herbivores increased with temperature. However, none of the papers included in the meta-analysis examined the combined effects of both bottom-up and top-down processes on herbivore survival at the same time, as have been done here.

Conclusions

Climate change effects on ecological processes have recently been documented across many levels of biological organization and different types of ecosystems (Parmesan 2006, Walther 2010, Woodward et al. 2010). Many of these are comparably simple, involving direct (physiological) responses of species. However, warming also affects interactions between species (Emmerson et al. 2004, van der Putten et al. 2004). Such indirect effects (Walther 2010) may affect regulation and population dynamics of many species and has the potential to cascade through entire communities (Traill et al. 2010), but our understanding of effects of warming involving responses on several trophic levels is yet limited (Woodward et al. 2010). Because of the possible consequences for ecosystem functioning, such as pest control, it is important that we decrease our ignorance of such effects. To address this deficit, we have here studied the larval response of an insect herbivore to temperature and analysed the tritrophic mechanisms involved and their consequences for population regulation and thus outbreak risk of this forest pest. We show that the response is complex, but that considering interactions among three trophic levels aided in disentangling the chain of mechanisms responsible for the population level response of the focus species. In conclusion, our study illustrates that the ecological effects of warming need not be straightforward, but may, due to indirect effects, be context dependent (here depending on both plant quality and predation pressure). We anticipate that many more similar tritrophic, or even higher order effects present in other systems, are waiting to be unraveled, so that our understanding of warming across multiple scales and levels of organization can be increased. In order to make progress we need more studies where responses to climate variation at all relevant trophic levels are quantified simultaneously, preferably in systems where there are long-term population data that can be used to 'test' predictions of population models including climate effects on trophic interactions.

Acknowledgments

We thank the helpful staff at Asa and Vindeln research field stations and the forest companies Sveaskog and Korsnäs for letting us undertake fieldwork in their forests. We are also grateful to Karin Eklund, Lisa Fors, Carin Eriksson, Ling Shen and Marion Staeger for assistance in the lab and field and to Martin Schroeder, Tea Ammunét, Jonas Knape, Elizabeth Crone and two anonymous reviewers for

comments and discussion on earlier drafts of the manuscripts. Statistical consultancy was provided by Mikael Franko Andersson at the department of Economics, SLU. The study was funded by Formas, the Mistra-program "Future Forests" and the EU-project BACCARA.

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Supplemental Material

Appendix A

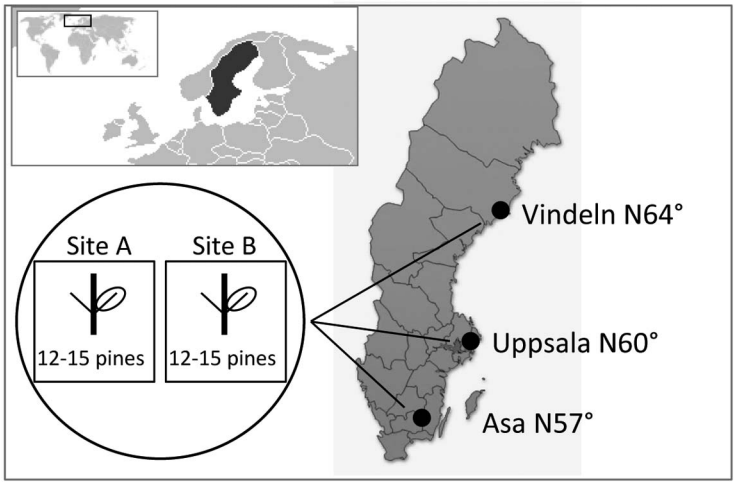

Fig. A1. Map showing the study areas and an indication of the experimental design used in the study of *Neodiprion sertifer* larval survival in Sweden. In each of the three localities (Asa, Uppsala and Vindeln), 12–15 pines at each of two sites (A and B) hosted larvae and their survival was estimated for four weeks. The larvae was either protected against natural enemies (caged) to estimate host quality (diterpene content) effects on larval survival or exposed to natural enemies to estimate predation effects. Caged and exposed larvae were feeding on the same whorl of a pine which has similar food qualities.

Appendix B

The population model projects the density of pupae (n_t) as a function of pupal survival ($s_p = s_p(n_t)$), female fecundity (f), sex ratio (r), larval survival ($s_l = s_l(T, D)$), and background survival (s_b), where T is temperature and D concentration of diterpenes, and takes the following simple form:

$$n_{t+1} = n_t \times s_p(n_t) \times f \times r \times s_l(T, D) \times s_b. \quad (1)$$

Based on Kolomiets et al. (1979), female fecundity (f: number of laid eggs) is set to 80, the sex ratio (r: proportion of female eggs) is assumed to be 0.5 and background survival (affected by e.g., viruses, parasitism, bird predation, egg mortality, etc.) is set to 0.35.

It has been proposed (Holling 1959) that predation mortality of sawfly pupae is density dependent due to a Type III functional response of generalist small mammal predators. Thus, we modelled pupal survival as (see Larsson et al. 2000 for details):

$$s_p = 1 - \frac{a \times n_t}{b^2 + n_t^2}. \quad (2)$$

Here, a (=45) is the asymptotic (maximum) number of pupae being predated (when pupal density gets large) and b (=25 cocoons/m^2) the inflexion point (i.e., the abundance where the density dependence of the functional response switches from being positive to negative) of vole predators (Larsson and Tenow 1984, Larsson et al. 2000). Mortality at the threshold b equals $a/2b$ and is here estimated to be 90%.

The combined effect of food quality and climate on larval survival is probably a continuous nonlinear function of both diterpene concentration of needles and temperature, but reliable parameterization of such a function would need more data than is available here. Thus, (pending more data) we chose to approximate the full

larval response as a linear effect of temperature, T, on weekly larval survival, σ_l, as modeled in the statistical analysis for Vindeln where we measured the largest temperature range. Sawfly population growth was then contrasted under low and high needle diterpene concentrations (LD and HD, respectively). Thus:

$$\sigma_l(T, D) = \begin{cases} \alpha_{\text{LD}} + \beta_{\text{LD}} \times T(\text{low diterpene concentration}) \\ \alpha_{\text{HD}} + \beta_{\text{HD}} \times T(\text{high diterpene concentration}) \end{cases}. \quad (3)$$

The two categories of diterpene concentrations were set at the mean value in Vindeln ± 1 SD (i.e., LD = 2.6 and HD = 7.6), and represented the majority (60%) of the observed data on diterpene concentrations at all sites. Hence, the difference in food quality, and resulting larval survival responses, can be expected to cover most of the variation occurring among pine trees in naturally regenerated pine stands. Since survival rates were empirically determined on a weekly basis, and larval development normally takes about five weeks, we assume for simplicity: $s_l = \sigma_l^5$. Parameterized in this way the population growth rate ($\lambda = s_p \times f \times r \times s_1 \times s_b$) and recruitment curve (n_{t+1} as a function of n_t) of the model (1) was analyzed. Furthermore, to analyze population growth of the pine sawfly in a climate change perspective we modelled population growth under a variable and increasing temperature scenario. More specifically, for a large number of model populations, five weekly temperatures (T) were drawn at random from a normal distribution with a fixed mean (x) and a standard deviation corresponding to that observed in the field experiment (i.e., $T = x \pm 2.1$). The effect of warming on the outbreak risk of the sawfly was analyzed by increasing the mean temperature (x) from 12° to 18°C, encompassing a rise from the observed mean, 13.4°C, during the field experiment, to 16.4°C, due to a predicted 3°C increase in temperature in Sweden during the 21st century (Lind and Kjellström 2008). For every temperature x, 10,000 replicates, of a starting population of 1 pupa/m^2 (corresponding to estimates of endemic sawfly population densities; Hanski 1987), were projected 5 years into the future and the probability of outbreak determined, as the proportion of replicates exceeding a threshold density of 100 pupae/m^2 (corresponding to lower estimates of when forest defoliation will be noticeable; Hanski 1987). The sensitivity of predicted outbreak risk to uncertainty in the strength of the effect of temperature on weekly larval survival was analyzed by increasing and decreasing the estimated slope (α) of Eq. 3 by 5%.

Appendix C

Table C1. Temperature (°C), needle diterpene concentration and larval survival (%) during four weeks of *Neodiprion sertifer* larval development. Mean values ± SD are given for each site in a latitudinal gradient in Sweden in 2010.

			Larvae survival	
Location	Temperature	Needle diterpene	Caged	Exposed
Asa A	12.1 ± 1.6	10.7 ± 5.6	87.7 ± 27.2	72.2 ± 18.5
Asa B	...	...	...	...
Uppsala A	13.5 ± 1.0	4.7 ± 3.3	97.4 ± 4.5	61.5 ± 32.9
Uppsala B	13.4 ± 1.2	6.7 ± 2.5	95.5 ± 7.5	49.7 ± 40.2
Vindeln A	14.8 ± 2.8	4.3 ± 1.7	97.0 ± 5.6	48.1 ± 31.4
Vindeln B	14.5 ± 2.9	6.5 ± 2.4	92.8 ± 6.3	68.7 ± 22.1

APPENDIX D

Table D1. Model parameter estimates, SEs, z-values and P-values from a reduced generalized linear mixed model of the survival of sawfly larvae (*Neodiprion sertifer*) either protected or exposed to natural enemies, based on data from a field experiment along a latitudinal gradient in Sweden in 2010. The protected larvae in Asa are used as the reference level and interaction terms describe a different effect of the explanatory variables from that seen among the protected larvae in Asa. The continuous explanatory variables are centered (i.e., the sample mean was subtracted from all values of the input variable) to facilitate interpretation of the estimates. The number of replicated trees was 42; each tree hosted a caged and an exposed larval group. Weekly records of larval survival were collected over four weeks.

Fixed effects	Estimate	SE	z	p
(Intercept)	4.15	0.81	5.2	<0.001
Uppsala	−0.28	0.84	−0.3	0.73
Vindeln	−0.29	0.87	−0.3	0.74
Exposed larvae	−1.36	0.85	−1.6	0.11
Temperature	−0.18	0.40	−0.5	0.65
Needle diterpene content	−0.23	0.13	−1.8	0.07
Larval growth rate	0.64	0.22	2.9	<0.01
Larval developmental stage	0.66	0.13	5.1	<0.001
Larval group size	0.05	0.02	2.1	0.03
Uppsala : Exposed larvae	−0.33	0.89	−0.4	0.71
Vindeln : Exposed larvae	0.14	0.93	0.2	0.88
Uppsala : Temperature	−0.81	0.48	−1.7	0.09
Vindeln : Temperature	−0.14	0.41	−0.3	0.74
Exposed larvae : Temperature	0.34	0.42	0.8	0.42
Uppsala : Diterpene content	0.26	0.15	1.7	0.09
Vindeln : Diterpene content	−0.22	0.23	−0.9	0.35
Exposed larvae : Diterpene content	0.14	0.13	1.1	0.27
Temperature : Diterpene content	−0.13	0.10	−1.3	0.20
Uppsala : Exposed larvae : Temperature	0.39	0.51	0.8	0.44
Vindeln : Exposed larvae : Temperature	−0.34	0.44	−0.8	0.44
Uppsala : Exposed larvae : Diterpene content	−0.24	0.16	−1.5	0.13
Vindeln : Exposed larvae : Diterpene content	0.47	0.24	2.0	0.05
Uppsala : Temperature : Diterpene content	0.16	0.13	1.2	0.21
Vindeln : Temperature : Diterpene content	0.23	0.12	2.0	0.05
Exposed larvae : Temperature : Diterpene content	0.09	0.11	0.8	0.42
Uppsala : Exposed larvae : Temp : Diterpene content	−0.05	0.14	−0.3	0.74
Vindeln : Exposed larvae : Temp : Diterpene content	−0.32	0.12	−2.6	<0.01

Appendix E

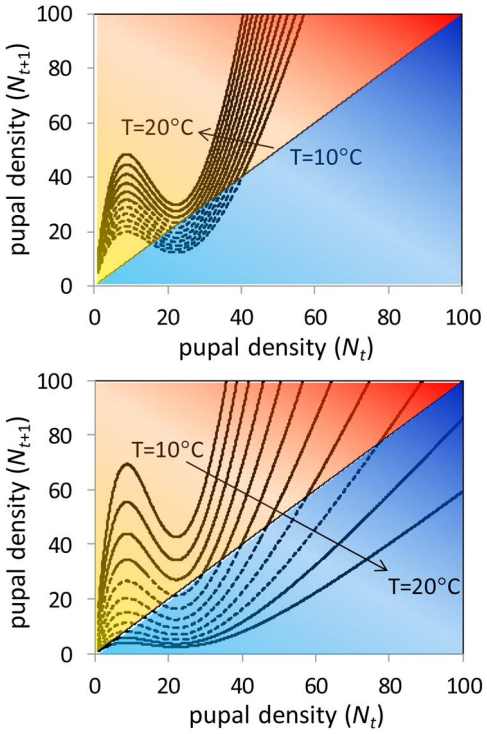

Fig. E1. Recruitment curves of sawfly (*Neodiprion sertifer*) populations feeding on needles either low (A) or high in diterpenes (B). Colors denote regions with stabilizing regulation (low outbreak risk, $\lambda < 1$, different shades of blue) and destabilizing dynamics (producing deterministic outbreaks, $\lambda > 1$, different shades of red), respectively. Solid parts of recruitment curves show pupal densities (N_t), for different temperatures from 10° to 20°C, from which the population will escape density dependent control (deterministically increase to outbreak densities or decrease to extinction), while dashed parts of recruitment curves show the region of pupal densities where the population will be maintained by density dependent control.