# Fungal infections and chemical quality of subarctic *Vaccinium myrtillus* plants under elevated temperature and carbon dioxide

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## Abstract

The environmental changes associated to the projected global climate change may alter the plant metabolism in a way that has consequences for plant resistance to natural enemies. Using open top chambers, we investigated the short-term effects of elevated temperature and carbon dioxide ($CO_2$) enrichment on the amino acids and phenolic secondary metabolites of subarctic *Vaccinium myrtillus* (L.) plants. The chemical data was correlated with severity of fungal infections on the plants, in order to find out whether the altered chemical quality could explain the abundance of fungal infections. The results demonstrated that the chemical quality of *V. myrtillus* leaves varies markedly during the growth season. Temperature elevation had the strongest capacity to alter the chemical quality and fungal infection patterns on *V. myrtillus*, whereas $CO_2$ enrichment had, at most, an additive effect. However, we did not find clear-cut and consistent relations between the measured plant metabolites and the severity of fungal infections. Thus, we conclude that the analyzed chemicals are not major determinants of the success of parasitic fungi on subarctic *V. myrtillus* plants under climatic perturbations.

## Introduction

According to the climate models, the global average temperature and atmospheric accumulation of human-made greenhouse gases, such as carbon dioxide ($CO_2$) will continue to rise during the 21st century (IPCC 2001, Novak *et al*. 2004). These changes are expected to cause alterations in the biogeochemical cycles of carbon (C) and nitrogen (N) (Lee 1998). Since C and N are essential elements in the biological processes, the climate change is expected to have substantial effects on the physiology and ecology of plants. Such effects may be especially pronounced in high-latitude and high-altitude areas where the plants have adapted to low temperatures and limited availability of nutrients (Tamm 1991). The projected ecological effects of climate change include alterations in abundance of plant natural enemies, i.e., pathogens and herbivores that may be directly affected by the environmental changes (Ayres & Lombardero 2000, Bale *et al*. 2002, Mitchell *et al*. 2003). However, since the levels of different C-based and N-based metabolites may strongly determine the plant quality to consumers (e.g., Harborne 1993, Biere *et al*. 2004 and refs. within), the ecological consequences of climate change may also derive from the environmentally induced changes in plant chemical quality. Due to the complex web of interactions between different external factors and feedbacks between plant C and N metabolism (Rustad *et al*. 2001, Norby & Luo 2004, Novak *et al*. 2004, Volder *et al*. 2004), it is difficult to forecast the outcome of plant-parasite/pest interactions during the climate change. To increase the precision of climatic models and predictions, more information about plant responses to environmental manipulations is needed.

Although climate change associated changes in the growth and chemical quality of northern plants have been actively studied (e.g., Laine & Henttonen 1987, Hartley 1999, Richardsson *et al*. 2002), only few studies have considered both the C-and N-based metabolites or tested the ecological importance of the possible changes in plant chemistry to pathogen infections. Here, we addressed the questions of whether elevated temperature and $CO_2$ may cause alterations in the chemical quality of subarctic *Vaccinium myrtillus* (L.) plants, and whether these alterations could explain the possible changes in abundance of fungal infections in the same treatments. The study was carried out as a short-term experiment with open top chamber (OTC) $CO_2$ treatments and soil/air warming in the subarctic woodland of northern Sweden. During one growth season, we studied the fungal infection status on *V. myrtillus* plants subjected to elevated $CO_2$ and temperature (administered individually and in combination). In order to detect whether the possible treatment-induced changes in fungal infection patterns could be explained by altered chemical quality of the plants, we quantified the easily digestible amino acids, as well as low molecular weight phenolic metabolites with potential antifungal properties. The chemical analyses were conducted at three different time points of the growth season in order to address the seasonal variations in plant chemistry.

## Material and methods

### *Study site*

The study site is located in Stordalen, northern Sweden near the Abisko Scientific Research Station (68º35´ N 18º82´ E, 380 m above sea level). The experiment was carried out in the dwarf shrub understorey of an open birch (*Betula pubescens* Ehrh. ssp. *tortuosa* (Lebed.) Nyman) woodland. The understorey is dominated by evergreen (*Empetrum hermaphroditum* Hagerup and *V. vitis-idaea* L.) and deciduous (*V. myrtillus* and *V. uliginosum* L.) dwarf shrubs (Sonesson & Lundberg 1974). The mean temperature of July (1961–1990) in the region is 11ºC. Hence the climate of the area is subarctic, when the 10ºC -isotherm is used to define arctic zones (Andersson 1996).

### *Experimental design*

The climate manipulation experiment was established in June 2000. The climate manipulation treatments were conducted on 0.5 $m^2$ plots that were surrounded by 30 cm high open-top chambers (OTC). The treatments were: 1. elevated temperature of the soil and air (control +5ºC; hereafter referred to as eTEMP), 2. elevated $CO_2$ (700 ppm; e $CO_2$) and 3. combination of these treatments (eTEMP + e $CO_2$). The soil warming was carried out with heated cables buried in the humic layer 5 cm below the soil surface (Hartley *et al*. 1999) and the air was simultaneously heated with infrared lamps. The $CO_2$ mixed with normal air was blown into the chambers to elevate the $CO_2$ level. Two types of controls were used: undisturbed control (control 1) and disturbance control (control 2) with unheated cables in the ground, OTC and circulating air. The experimental set up consisted of a total of 30 plots, which were randomly assigned to one of the five treatments (3 manipulations and 2 controls), which were repeated across 6 blocks, each of which contained each type of climate manipulation and controls.

### *Sampling and chemical analyses*

Current year shoots of *V. myrtillus* were collected at tree occasions during 2001, i.e., in the end of June, in the end of July and in the middle of September (hereafter referred to as June, July and September, respectively). At each sampling occasion, two shoots from each plot were randomly collected. One of the shoots was frozen on dry ice for amino acid analysis and the other shoot was air-dried in room temperature for phenolic analysis. Amino acids were extracted and analysed as their 9-fluorenylmethylchloroformate (FMOC) derivatives using HPLC with fluorescence detection (Nordin & Näsholm 1997). The extraction and HPLC-analysis of phenolics was carried out according to the method described by Witzell *et al*. (2003). The most abundant individual amino acids and phenolics were quantified. Here, were report the results for four individual amino acids and phenolic compounds.

### *Quantification of fungal infections*

In July 2001, the severity of fungal infections (i.e. presence of dark reddish or brownish spots or lesions) was visually estimated from shoots occurring along longitudinal transects on each plot. The number of shoots observed per plot varied from 18 to 21. In September 2001, leaves of 15 shoots were collected along longitudinal transects on each plot for a more detailed analysis of infection severity. The severity of fungal infestation on leaves was estimated by classifying the leaves to six groups according to the visual symptoms. The groups were as follows: no visible symptoms (group 0); infection symptoms covered less than 1 % of leaf area (group 0.5); estimated infected leaf area was about 1 % (group 1); 1–10 % (group 2); 10–30 % (group 3) or 30–80 % (group 4). The leaves on which the infections covered virtually the whole surface were classified to group 5.

To identify some of the potential causal agents of the symptoms, *V. myrtillus* leaves showing typical symptoms were collected from the immediate vicinity of the experiment, surface sterilized (4 % NaOCl for 1 min, 70 % EtOH 30 s, followed by rinsing with sterile water) and placed on potato dextrose agar (Sigma Chemicals Co, St Louis, MI, USA). On the basis of colony morphology, five of the most common fungi were selected for a more detailed identification at CBS (Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands).

### *Statistical analyses*

The MIXED -procedure of SAS (SAS Institute Inc., Cary, NC, USA, release 8.1) was used to study the treatment effects and within-seasonal (June, July and September 2001) fluctuations of the compound concentrations. The data was transformed to meet the criteria of normal distribution and homoscedasticity of variances. The main factors tested were block, time, eTEMP and $eCO_2$ using the repeated measurements option. The interaction between block, eTEMP and $eCO_2$ was used as a random factor. The control 2 (disturbance control) was chosen as the control-treatment to exclude disturbance effects from the results. The data on infection classes were analyzed with the same MIXED -model, which was used for the compound concentrations. The least squares means (LSM) of different factor combinations were compared with Tukey's post hoc test, and the slice-option of the MIXED -procedure was used to study the interactions between the factors. Disturbance by the experimental set-up, i.e. differences between controls 1 and 2, was tested with general linear model (GLM) -procedure at each sampling occasion with and without sample infection as covariate. The direct impact of infection frequency on the compound concentrations was tested with a parametric regression fit (SAS INSIGHT) between infection and concentrations of studied compounds in the controls.

## Results and discussion

### *Fungal infections of* V. myrtillus *leaves*

In July, only few symptoms were visible suggesting that the fungal infections were at the initiation phase. The proportion of the most severely infected leaves (group 3) was significantly increased in plants subjected to the combined eTEMP+e $CO_2$ treatment ($P_{eTEMP+e\,CO2} = 0.007$; Fig. 1a). In September, eTEMP significantly increased the proportion of healthy leaves ($P_{eTEMP} = 0.01$; Figure 1b) and reduced the proportion of leaves belonging to infection groups 2 and 3 ($P_{eTEMP} = 0.01$ and 0.009, respectively; Figure 1b). In addition, the proportion of leaves classified to the most severe infection group 5 tended to increase in eTEMP treatment ($P_{eTEMP} = 0.06$; Figure 1b). Significant main effects on fungal infections were not detected for $eCO_2$ (Figs. 1a, b) or for the combined eTEMP+$eCO_2$ treatment. The differences between controls were not consistent and significant, indicating that the OTC alone did

not systematically alter the infection patterns. Our results suggest that temperature elevation has a high potential to alter the fungal infection patterns on *V. myrtillus* leaves, whereas the effect of $CO_2$ enrichment on fungal infections appears to be negligible.

On basis of morphological features, at least ten different types of colonies could be separated among the fungi isolated on PDA medium. Of the isolates, *Hormonema prunorum* (C. Dennis and Buhagiar) and *Godronia cassandraea* Peck forma *vaccinii* (anamorph) could be identified to the species level, and *Melanconium* and *Isthmolongispora* to the genus level.

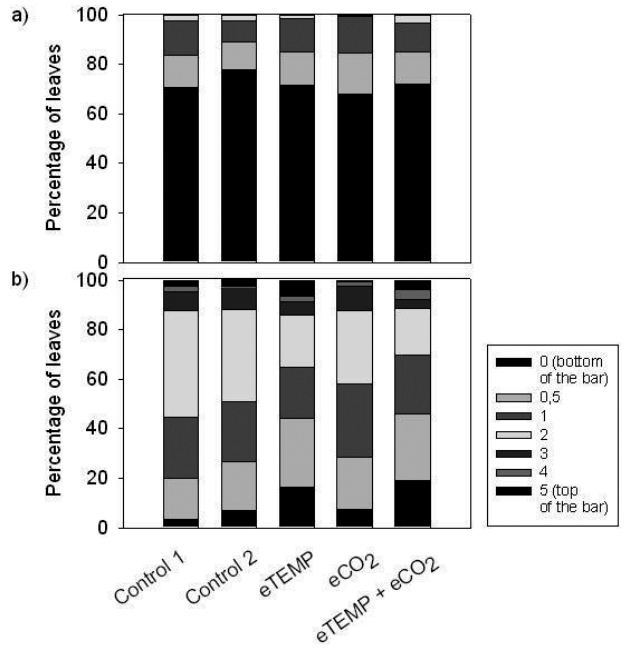


*Fig. 1. Severity of fungal infections on* V. myrtillus *leaves in June (a) and September (b) quantified as percentages of leaves (per shoot) classified to each infection group (0, 0.5, 1, 2, 3, 4 or 5). Shown are the mean values of 18–27 (June) and 15 (September) shoots. (n of treatments = 6).*

## *Within seasonal variation in plant chemistry*

The concentrations of the main amino acids in *V. myrtillus* leaves (aspartate, serine, glutamate and alanine, Fig. 2) showed significant temporal variation ($P_{TIME}$ =0.0001 for all amino acids). In addition, several of the analysed phenolic compounds showed individual seasonal kinetics ($P_{TIME}$ = 0.0001 for arbutin and *p*-coumaric acid, as well as for two minor quercetin glucosides for which data is not shown). These results emphasize the marked within-seasonal variation in the primary and secondary chemistry of *V. myrtillus* (see also Witzell & Shevtsova 2004), and show that parasitic fungi must cope with a highly variable chemical environment during their developmental phases on *V. myrtillus* leaves. Temporal variations in plant chemicals may reflect the various functions of individual compounds in plants. For instance, aspartate and glutamate are both assimilatory and transport amino acids (Buchanan *et al*. 2000). Within-seasonal fluctuations of phenolic compounds may reflect the temporally varying allocation of carbon to either growth or defence (cf. Bryant & Julkunen-Tiitto 1995).

## *Treatment effects on plant chemistry*

Elevated temperature, administered alone or in combination with $eCO_2$, decreased the concentration of glutamate especially in September ($P_{eTEMP}$ = 0.04; $P_{eTEMP \times CO2}$ = 0.003; Fig. 2). The concentrations of some phenolics (e.g., *p*-coumaric acid and flavonoids) increased in eTEMP-treated plants in June, but in July we found reduced levels of some phenolics in eTEMP-treated plants (Fig. 3, $P_{eTEMP}$ = 0.03 for *p*-coumaric acid; $P_{eTEMP \times TIME}$ = 0.01 and 0.002 for *p*-coumaric acid and the quercetin glycoside, respectively). We did not find significant main effects of $eCO_2$ on any of the analyzed amino acids or phenolics. Our results thus suggest that elevated temperature has the strongest capacity to affect the chemical quality of *V. myrtillus* leaves, whereas $eCO_2$ has no or only an additive effect. The lack of $eCO_2$ effect on amino acids suggest that there was no dilution of N concentration in *V. myrtillus* plants, although it is commonly reported in plants under elevated $CO_2$ (e.g. McGuire 1995). The carbon metabolism of *V. myrtillus* seemed to be generally unaffected by $eCO_2$, or rapidly acclimated to it, as indicated by the rather stable levels of phenolic metabolites under $eCO_2$.

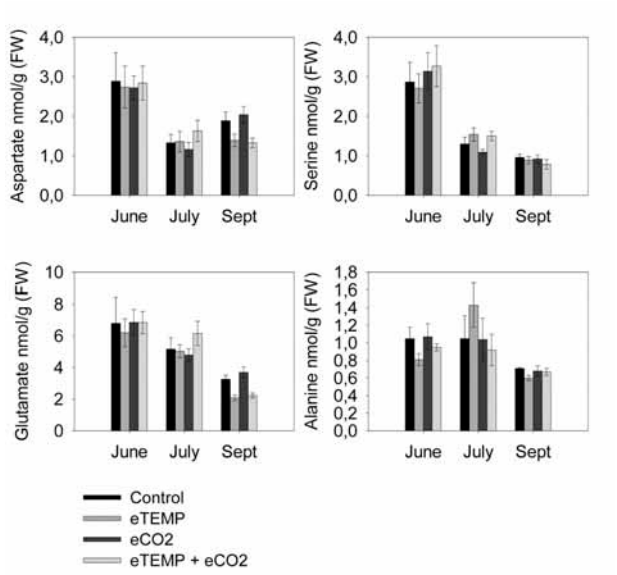


*Fig. 2. Concentrations of four amino acids (nmol $g^{-1}$ FW) in* V. myrtillus *plants at different climate manipulation treatments during one growth season. Shown are the means of 6 replicates. Vertical bars represent standard error of the mean.*

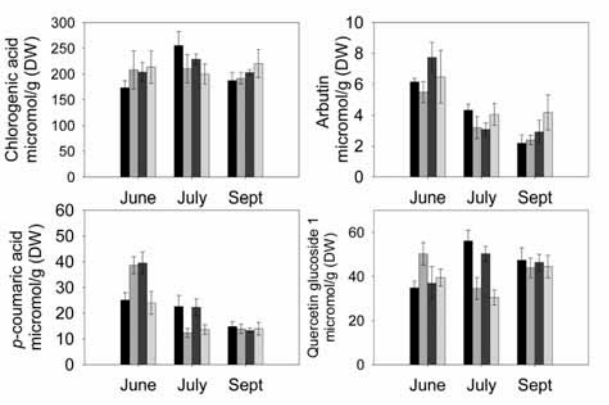


*Fig. 3. Concentrations of four phenolic compounds (µmol $g^{-1}$ DW) in* V. myrtillus *plants at different climate manipulation treatments during the growth season. Shown are the means of 6 replicates. Vertical bars represent standard error of the mean. See figure 2 for the treatment legend.*

### *Associations between plant chemistry and fungal infections*

At the study area, the outbreak of fungal infections occurred around mid of July and it is possible that the concurring eTEMP-associated decrease in phenolics (Fig. 3) rendered the plants to a better (less toxic) substrate for the parasites, allowing them to initiate leaf colonization. However, changes in amino acids and phenolics did not seem to explain the treatment-induced patterns in infections, such as the increased proportion of healthy leaves in plants treated with eTEMP (alone or in combination with $eCO_2$) in September. Rather, this response may have been associated with temperature-induced alteration in plant growth patterns (e.g., increased leaf biomass and area; data not shown) or to direct, microclimatic factors on the fungi. The lack of clear-cut and temporally consistent associations between the measured plant metabolites and severity of fungal infections suggests that the studied chemicals may not be major determinants of fungal success on *V. myrtillus* leaves. Thus, we conclude that the infection patterns on *V. myrtillus* plant under climate change conditions are likely to be more strongly dictated by other plant chemical characters, or by the direct effects of elevated temperature on the fungi.

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