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Source: Arctic, Antarctic, and Alpine Research, 40(3) : 561-567

Published By: Institute of Arctic and Alpine Research (INSTAAR), University of Colorado

URL: https://doi.org/10.1657/1523-0430(07-041)[PEHKONEN]2.0.CO;2

*Arctic, Antarctic, and Alpine Research, Vol. 40, No. 3, 2008, pp. 561–567*

# Relationships between *Vaccinium vitis-idaea* and the Frequency of Its Fungal Pathogen *Exobasidium splendidum*, and the Environment

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## Abstract

We investigated relationships between an evergreen dwarf shrub *Vaccinium vitis-idaea* and infection frequency of its annual specific pathogenic fungus *Exobasidium splendidum*, and environmental conditions in six boreal and subarctic populations in Finland during 1998–2002. The aim was to explore how environmental factors and plant characteristics influence the infection frequency of the rare pathogen in nature. Climate data, soil organic matter, and plant characteristics were recorded along with annual demographic observations on *V. vitis-idaea* ramet dormancy, growth, flowering and fungal infection. Infection frequency of *E. splendidum* in *V. vitis-idaea* populations varied between 0 and 4.8% and increased with decreasing temperatures. Along with low air temperature, high concentration of $NH_4^+$, low concentration of P, high cover of bare ground, and high density of *V. vitis-idaea* were positively associated with the frequency of *E. splendidum* in *V. vitis-idaea* populations. Diseased ramets were at greatest risk of being reinfected by the fungus. The results indicate that both environmental factors and plant characteristics can constrain the distribution of the rare pathogen. Although an observational study of this kind does not allow us unequivocally to identify causality underpinning complex plant-pathogen-environment relationships, we can speculate that a further decrease in the frequency of *E. splendidum* infections will occur under climate warming.

DOI: 10.1657/1523-0430(07-041)[PEHKONEN]2.0.CO;2

## Introduction

The susceptibility of plants to pathogen infections is affected by several factors, such as environment, plant life-history characteristics, and genetic factors. Climate may contribute to the plant-pathogen system by affecting the growth of the host plant populations or by affecting the growth, reproduction, and transmission of pathogens (e.g., Sundström, 1964; Ranta and Neuvonen, 1994; Green and Bayley, 2000; Roy et al., 2004). Availability of nutrients can affect the interaction between hosts and pathogens by altering the concentrations of amino acids in plant tissues (Strengbom et al., 2002). Disturbances in soil and vegetation may have an indirect impact on pathogen infections in plants, for example, by triggering active growth, which can be a susceptible stage to pathogen attacks (Pehkonen et al., 2002), or a direct impact e.g. due to increasing nutrient mineralization, which may affect the pathogen growth and reproduction (Wennström and Ericson, 1992).

The life-history characteristics of the host plant have an impact on the susceptibility to pathogen infections. Some clonal plants can avoid the pathogens by increasing lateral growth (D'Hertefeldt and van der Putten, 1998; Pan and Clay, 2002), while others rely on induced resistance (e.g., Parker, 1988; Schmid, 1994; Ranta et al., 2000). In some plant species, the need to defend against pathogens is minor due to greater palatability of diseased plant parts to herbivores, which decreases the pathogen frequency in the population (Ericson and Wennström, 1997). The responses may differ depending on the age or developmental stage of the host plant (Carlsson et al., 1990; Burdon et al., 1995; Dudycha and Roach, 2003). For the present, the demographic structure of the host plant population is a relatively unexplored facet of host-pathogen ecology (Dudycha and Roach, 2003).

An evergreen clonal dwarf shrub *Vaccinium vitis-idaea* L. is a dominant field layer species in boreal heath forests and timberline habitats in northern Fennoscandia. It is occasionally infected by the host-specific pathogenic fungus *Exobasidium splendidum* Nannf., which is restricted to northern latitudes and high elevations. There is little information on the dynamics of *E. splendidum* and on factors that influence its distribution (Nannfeldt, 1981). Disturbances in the soil and vegetation have been found to increase the infection frequencies of *E. splendidum* in *V. vitis-idaea* stands (Pehkonen et al., 2002), but factors influencing the relationship between *E. splendidum* and *V. vitis-idaea* are largely unknown. Pathogens restricted to cold regions may decrease in abundance as a consequence of warming temperatures (Kurkela, 1969; Nissinen, 1996; Wu et al., 1998). By investigating the relationship between environment, host plant characteristics, and infection frequencies of *E. splendidum* in nature, the present research provides basic information that could underpin an evaluation of whether the rare pathogen is able to survive under warming climate.

In this study we investigated, (1) whether temperature, precipitation and soil organic matter (SOM) nutrient concentrations have an influence on the frequency of *E. splendidum* infections in nature, (2) whether there is interaction between the frequency of *E. splendidum* infections and certain plant characteristics, such as vegetation cover and the density, growth, and demography of *V. vitis-idaea,* and (3) whether plant life-cycle stage (dormant, vegetative, flowering, diseased) has an impact on the risk of becoming infected by *E. splendidum*. We used field data from six *V. vitis-idaea* populations in northern boreal, subarctic, and subarctic-subalpine Finland during 1998–2002.


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**TABLE 1**

**Site and plant characteristics (mean ± SE) in three environments (Oulanka, Kevo, Kilpisjärvi). Variables were recorded in 1998 except for the ramet density, which was measured in 2000, and ramet growth during 1998–2002. Total study area refers to the combined area of sites 1 and 2 in each environment. Asterisks indicate differences between environments and sites significant at the 0.1% (***), 1% (**) and 5% (*) levels (Kruskal-Wallis and Mann-Whitney *U* tests ). If the difference was significant between environments, the comparison between sites was carried out separately for each environment. Different letters indicate significant differences between the sites within each environment at the 5% level (Mann-Whitney *U* test). Number of study plots: $n = 10$ at Oulanka and Kevo, $n = 6$ at Kilpisjärvi, total $n = 52$.**

| Site | Total study area ($km^2$) | Disturbance history | Bare ground (% ± SE) | Total vegetation cover (%) | Density of *V. vitis- idaea* (ramets $m^{-2}$) | Ramet growth (mm $yr^{-1}$) |
|---|---|---|---|---|---|---|
| Oulanka1 | 0.2 | clear-cutting, ploughing in the 1980s | 7.6 ± 2.1 | 165.90 ± 17.70 | 849 ± 79 | 4.4 ± 0.3 *a* |
| Oulanka2 | | untouched forest | 0.1 ± 0.1 | 193.60 ± 13.49 | 222 ± 57 | 3.3 ± 0.4 *b* |
| Kevo1 | 0.2 | clear-cutting, soil removal in the 1990s | 16.9 ± 3.4 | 84.10 ± 7.78 *b* | 709 ± 112 | 2.1 ± 0.2 |
| Kevo2 | | grazing, trampling | 1.8 ± 1.2 | 119.80 ± 11.23 *a* | 441 ± 98 | 3.4 ± 0.4 |
| Kilpisjärvi1 | 0.1 | road construction in the 1990s | 7.0 ± 2.6 | 129.00 ± 16.15 | 868 ± 119 | 2.8 ± 0. |
| Kilpisjärvi2 | | untouched forest | 0.0 ± 0.0 | 165.83 ± 5.53 | 745 ± 124 | 2.5 ± 0.1 |
| $\chi^2$ environment (d.f. = 2) | | | 3.296 | 22.077 *** | 4.580 | 12.250 ** |
| $U_{site}$ | (d.f. = 1) | | 72.5 *** | | 14.355 *** | |

## Material and Methods

### *HOST PLANT*

Ramets of *V. vitis-idaea* are shoots branching from a bud on a belowground stem. Ramets consist of several annual shoot generations, belowground stem, and roots. Within ramets lateral buds can produce vegetative shoots or remain dormant, while terminal buds may produce vegetative shoots, inflorescences, or remain dormant (Shevtsova et al., 1995; Tolvanen 1995). Propagation of new *V. vitis-idaea* ramets occurs mainly vegetatively. Ramets remain connected through the belowground stem, but the connection can decay after a few years (personal observation). Apart from abundant seed production, the seedling establishment is impeded by dense vegetation under undisturbed conditions (Eriksson and Fröborg, 1996; Hautala et al., 2001).

### *PATHOGEN*

The genus *Exobasidium* Woron. (Basidiomycotina) consists of highly host- and tissue-specific biotrophic pathogens, which infect immature plant tissues (Sundström, 1964; Nannfeldt, 1981). *E. splendidum* infects and kills current year's shoots of *V. vitis-idaea*. It is a monocyclic fungus, i.e., it reproduces only once during the growing season (Nannfeldt, 1981). Dormant spores probably overwinter on vegetative buds and infect the newly developing shoots in spring. Symptoms become visible after a couple of weeks as red coloration and hypertrophic growth of annual shoots and leaves (Savile, 1959; Nannfeldt, 1981). Diseased annual shoots die after sporulation.

### *RESEARCH SITES*

The research was carried out in northern boreal Oulanka (66°20′N, 29°20′E), subarctic Kevo (69°45′N, 27°01′E), and subarctic-subalpine Kilpisjärvi (69°01′N, 20°50′E) from 1998 to 2002. The study sites were relatively dry heath forests of type EM (*Empetrum-Myrtillus*) or the subalpine variant of the same type, sEM (Kalliola, 1973). *V. vitis-idaea* dominates in these forests along with dwarf shrubs *Empetrum nigrum* subsp. *hermaphroditum* (Hagerup) Böcher and *Vaccinium myrtillus* L.

In each environment, two neighboring sites of different disturbance histories were chosen at 20–50 m apart (Table 1). At each site, 10 (Oulanka and Kevo) or 6 (Kilpisjärvi) plots of 1 $m^2$ were randomly laid in the field at least 5 m apart in 1998. In each plot, the percentage cover of bare ground and vegetation was estimated using the point frequency method. The percentage of bare ground was used as a coarse estimate of disturbance level at the sites. The vegetation cover may exceed 100%, since all vegetation layers were recorded in the cover measurements.

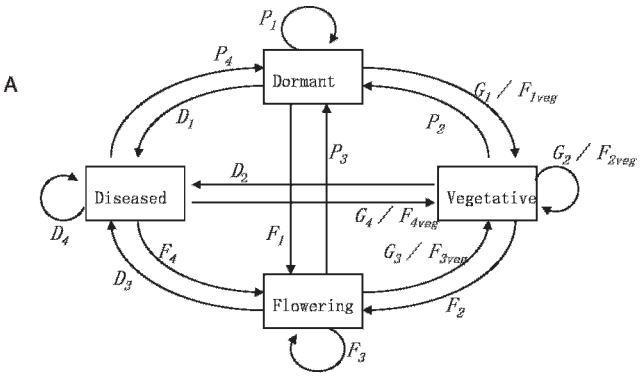
### *MEASUREMENTS ON CLIMATE AND SOIL*

To evaluate the relationship between temperature and precipitation and the frequencies of *E. splendidum* infections, monthly and annual temperature and precipitation data in the three environments were obtained from nearby weather stations, which are located 9, 1, and 10 km from Oulanka, Kevo, and Kilpisjärvi research sites, respectively (Finnish Meteorological Institute, 1998, 1999, 2000, 2001, 2002).

To investigate the relationships between soil organic matter (SOM) characteristics and the infection frequencies, SOM samples were taken from five randomly chosen study plots at Oulanka and Kevo, and from four study plots at Kilpisjärvi in 1998. Four subsamples per study plot were taken with soil cores from the organic layer down to the depth of the mineral soil. The thickness of the samples varied from 2 to 5 cm due to the difference in thickness of the SOM layer. The subsamples were pooled for the analyses of pH, conductivity, and the concentrations of $NH_4^+$, $NO_3^-$, and P. Nutrient concentrations were analyzed colorimetrically. The analysis of P was carried out according to John (1970), and analyses of $NH_4^+$ and $NO_3^-$ according to Page et al. (1982).

### *MEASUREMENTS ON PLANT DEMOGRAPHY*

A total sample of 520 healthy *V. vitis-idaea* ramets, 10 per plot, were tagged at the study sites in 1998, while all diseased ramets, altogether 1220, were tagged annually during 1998–2002. In each year, the tagged ramets were measured for growth and classified into four life-cycle stages according to the stage of the shoots: dormant,




| | Dormant | Vegetative | Flowering | Diseased |
|---|---|---|---|---|
| Dormant | $P_1$ | $P_2$ | $P_3$ | $P_4$ |
| Vegetative | $G_1 / F_{1veg}$ | $G_2 / F_{2veg}$ | $G_3 / F_{3veg}$ | $G_4 / F_{4veg}$ |
| Flowering | $F_1$ | $F_2$ | $F_3$ | $F_4$ |
| Diseased | $D_1$ | $D_2$ | $D_3$ | $D_4$ |

**FIGURE 1. Life-cycle graph of a *V. vitis-idaea* ramet (A). Stage-classified projection matrix based on the life-cycle graph (B). $P_i$ denotes dormancy, $G_i$ growth, $F_i$ flowering, and $D_i$ disease. $F_{i\ veg}$ denotes asexual reproduction, which is incorporated into all stages by dividing the number of newly emerging vegetative ramets by all older ramets, since the maternal ramets could not be detected.**

vegetative, flowering, and diseased. Dormancy indicated that the buds produced during autumn of the previous year were alive but did not open during the year of observation. Dead shoots were distinguished from dormant shoots by their shrunken size and brownish color. A ramet was classified dead if all of its shoots had died during the winter season. Vegetative and flowering ramets carried at least one vegetative or flowering current-year shoot, respectively. Diseased ramets had at least one vegetative or flowering shoot which showed symptoms of *E. splendidum* infection.

Since all healthy ramets could not be tagged due to the high ramet densities, corrections were later needed in order to estimate the actual densities of dormant, vegetative, and flowering ramets. Detailed density measurements carried out in each plot in 2000 were used as an estimate for the ramet density. According to an earlier study, annual *V. vitis-idaea* ramet densities vary approximately ±15% (Tolvanen et al., 1995). Hence the use of the densities measured in 2000 as a reference has to be regarded cautiously in this study.

The annual survival rate of the ramets was calculated as the percentage of tagged ramets surviving each year. The annual birth rate of the ramets was measured by counting the number of new ramets emerging asexually from belowground stems. The censuses were made during late June to mid August during 1998–2002.

## *MATRIX MODEL*

Twenty-four 4 × 4 stage-based population projection matrices were built to describe ramet demography at the six populations during four transitions (1998–1999, 1999–2000, 2000–2001, and 2001–2002). For each matrix, we determined the probabilities of dormancy ($P_i$), growth ($G_i$), flowering ($F_i$), and shoot disease ($D_i$) (Fig. 1). $P_i$ was calculated as the proportion of ramets at stage $i$ that survived but did not grow, $G_i$ was the proportion of the ramets at stage $i$ that produced new vegetative shoots, $F_i$ was the proportion of flowering ramets at stage $i$, and $D_i$ the proportion of ramets carrying diseased shoots at stage $i$. Asexual reproduction, i.e., the number of new vegetative ramets ($F_{i\ veg}$) emerging from belowground stems was divided among all other ramet types. Sexual reproduction was not included in the model, as seedlings were not found at any of the study sites. Sexual reproduction was therefore assumed to be of minor importance in the model.

Matrix calculations were run using a Fortran-based program (Tolvanen et al., 2001, 2002). The program pooled the transition frequency data from each plot and calculated summary matrices for each population, after which the population growth rates (λ), stable stage distributions (**w**), and elasticities ($\mathbf{e}_{ij}$) were calculated (described in detail in Caswell, 2001). A bootstrap analysis was done by the program to account for the statistical differences between the plant populations. The bootstrap sample size was 2000. Confidence limits of 95% (95% CI) were used to detect significant differences between the populations (Caswell, 2001).

## *STATISTICAL ANALYSES*

We first investigated whether environmental variables and plant characteristics are different between the environments and sites, as the differences might account for the variation in infection frequencies. We therefore compared plant and SOM characteristics between the three environments and the two sites within each environment. SOM characteristics (pH, conductivity, concentrations of nutrients) measured in study plots in 1998 were compared using the nested ANOVA, in which the sites were nested within the environments. The difference in plant characteristics (total vegetation cover, density, and growth of *V. vitis-idaea*) and the cover of bare ground were compared using the Kruskall-Wallis test and Mann-Whitney *U*-test, since the data were neither normal nor homoscedastic. If the difference was significant between environments, the comparison between sites was carried out separately for each environment. To test whether infection frequencies differ between years, the three environments, and the sites within each environment, we used Repeated Measures ANOVA, in which year was the within-subjects factor.

To investigate the relationship between infection frequencies and climate, SOM, and plant characteristics, we first calculated Spearman's correlations. Since the tested variables were interacting, partial correlations were used thereafter. Partial correlation holds all other variables constant except for those two that are being compared (Zar, 1984). Correlations with annual climate variables were calculated for data pooled over sites within each environment.

To detect whether the probability of *V. vitis-idaea* staying healthy or becoming infected is related to its life-cycle stage in the previous year, we used odds ratios (OR). The odds is the ratio of the probability that the event of interest (i.e., a ramet stays healthy) occurs to the probability that it does not (i.e., a ramet becomes infected). An odds ratio is calculated by dividing the odds in the target group by the odds in the reference group (e.g., Christensen, 1990). For example, OR for dormant ramets were calculated as: odds of dormant ramets/odds of diseased ramets. Confidence limits of 95% were calculated using SPSS to account for statistical significances of OR. If the 95% CI does not overlap 1 then it indicates that the event of staying healthy is significantly less ($0 \leq \text{OR} < 1$) or more ($\text{OR} > 1$) likely to happen in dormant ramets than in diseased ramets. OR are generally used in case-control studies where the disease prevalence is not known: the apparent frequency depends on the ratio of sampling cases to controls, which is artificial. To use a measure which is altered by frequency would be incorrect, so OR are the ideal choice (Deeks, 1996).

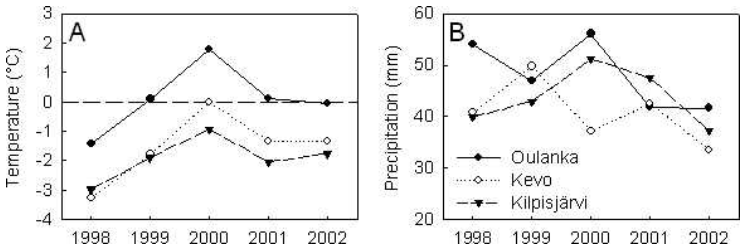



**FIGURE 2. Mean annual temperatures (A) and precipitation (B) at boreal Oulanka, subarctic Kevo, and subarctic-subalpine Kilpisjärvi in 1998–2002.**

## Results

### *DIFFERENCES IN ENVIRONMENTAL CONDITIONS AND PLANT CHARACTERISTICS BETWEEN ENVIRONMENTS AND SITES*

The mean annual temperatures decreased from boreal Oulanka towards subarctic Kevo and subarctic-subalpine Kilpisjärvi, whereas no consistent difference occurred in the precipitation between the environments (Fig. 2). SOM pH values were similar between the three environments and the two sites within each environment (nested ANOVA, Table 2). The conductivity and the concentrations of nutrients were highest in the study plots of Oulanka and lowest either in Kevo ($NH_4^+$) or both Kevo and Kilpisjärvi (conductivity, $NO_3^-$, and P) (Table 2). Since the concentration of P differed both between environments and sites within the environments, further testing was carried out separately for each environment to detect for differences between the sites. The concentration of P was only different between the clear-cut forest and untouched forest at Oulanka ($F = 8.101$, d.f. $= 1$, $p = 0.022$).

The total vegetation cover and the mean annual growth of ramets were highest in the study plots of Oulanka (Kruskal-Wallis test, Table 1). In all environments, there was a significant difference in the cover of bare ground and density of *V. vitis-idaea* ramets between sites (Table 1). Sites with a higher cover of bare ground (and a higher disturbance level) had a higher density of *V. vitis-idaea* ramets than sites with a lower cover of bare ground. Annual population growth rate values (λ) generated by the projection matrices ranged between 1.09 and 1.22 at boreal Oulanka, between 0.98 and 1.14 at subarctic Kevo, and between 0.91 and 1.08 at subarctic-subalpine Kilpisjärvi. Although there was a decreasing trend of λ-values from Oulanka to Kevo and Kilpisjärvi environments, the difference was not significant according to 95% CI. Population growth rate neither differed significantly between the two sites within each environment nor among years within each site. There were neither consistent differences in sensitivity nor elasticity values between the environments or sites (data not shown).

### *FREQUENCIES OF* E. SPLENDIDUM *INFECTIONS*

Infection frequency of *E. splendidum*, i.e. the percentage of diseased ramets among all ramets, was in general low in the study plots, ranging from 0.0% at Oulanka2 to 4.8% at Kilpisjärvi1 (Fig. 3). The frequencies varied among years, environments, and sites, being highest at subarctic-subalpine Kilpisjärvi throughout the study (Repeated Measures ANOVA; $F_{year} = 13.233$, d.f. $= 4$, $p < 0.001$; $F_{environment} = 19.727$, d.f. $= 2$, $p < 0.001$; $F_{site} = 20.551$, d.f. $= 1$, $p < 0.001$. Tukey's test, $p < 0.05$, Fig. 3). When the three environments were tested separately, the infection frequencies differed between the sites only at Oulanka, where *E. splendidum* was only found in the clear-cut forest (ANOVA, $F_{site} = 47.784$, d.f. $= 1$, $p = 0.001$).

### *CORRELATIONS BETWEEN INFECTION FREQUENCIES, POPULATION GROWTH RATES, AND ENVIRONMENT*

Frequency of *E. splendidum* infections correlated negatively with monthly summer temperatures, whereas there was no correlation with precipitation (Table 3). Positive correlations were significant or almost significant between λ and monthly temperatures from March to July, mean annual temperature, and precipitation in May and June (Table 3). The correlation between the frequency of *E. splendidum* infections and λ was insignificant (Spearman's rho $= -0.227$, N.S., $n = 24$ comprising 3 environments × 2 sites × 4 transitions).

Frequency of *E. splendidum* infections correlated positively with the concentration of $NH_4^+$ and negatively with the concentration of P (partial correlation, all other variables were held constant, Table 4). The cover of bare ground and the density of *V. vitis-idaea* showed a positive correlation with the frequency of *E. splendidum* infections (Table 4).

### *LIFE-CYCLE STAGE AND PATHOGEN INFECTIONS*

At Oulanka, odds ratios (OR) for staying healthy were usually higher in vegetative and flowering ramets than in diseased

**TABLE 2**

**Characteristics of soil organic matter (SOM) (mean ± SE) measured in 1998. Asterisks indicate differences between environments and sites significant at the 0.1% (***), 1 % (**) and 5 % (*) levels (nested ANOVA; site within environment). Different letters indicate significant differences between the three environments at the 5% level (Tukey's test). Number of measured study plots: $n = 5$ at Oulanka and Kevo, $n = 4$ at Kilpisjärvi, total $n = 28$.**

| Site | pH | Conductivity ($\mu S/cm^2$) | $NH_4^+$ ($\mu g\ L^{-1}$) | $NO_3^-$ ($\mu g\ L^{-1}$) | P ($\mu g\ L^{-1}$) |
|---|---|---|---|---|---|
| Oulanka1 | 4.5 ± 0.2 | 41.1 ± 5.0 *a* | 718.7 ± 2.1 *a* | 51.8 ± 5.9 *a* | 297.7 ± 69.0 *a* |
| Oulanka2 | 4.3 ± 0.1 | 77.5 ± 12.2 *a* | 621.8 ± 6.6 *a* | 48.6 ± 0.5 *a* | 823.2 ± 58.8 *a* |
| Kevo1 | 4.8 ± 0.2 | 29.0 ± 9.7 *b* | 300.1 ± 4.7 *b* | 44.7 ± 1.0 *b* | 66.2 ± 14.7 *b* |
| Kevo2 | 4.4 ± 0.1 | 28.6 ± 4.9 *b* | 388.9 ± 59.2 *b* | 44.0 ± 0.5 *b* | 153.6 ± 47.8 *b* |
| Kilpisjärvi1 | 4.9 ± 0.1 | 22.7 ± 3.9 *b* | 638.7 ± 117.1 *a* | 34.4 ± 7.3 *b* | 111.9 ± 22.9 *b* |
| Kilpisjärvi2 | 4.6 ± 0.1 | 23.5 ± 3.9 *b* | 553.8 ± 29.4 *a* | 35.6 ± 7.4 *b* | 155.9 ± 44.0 *b* |
| *F* environment (d.f. = 2) | 3.397 | 12.142 *** | 10.382 ** | 32.232 *** | 18.277 *** |
| $F_{site}$ (d.f. = 1) | 2.185 | 0.778 | 1.061 | 0.750 | 3.837 * |

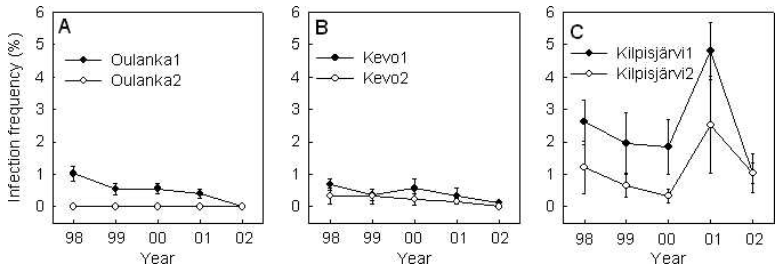



**FIGURE 3. Infection frequencies (± SE) in three environments, the two sites within each environment, and during five years.**

ramets, whereas at Kevo, OR of all healthy ramets (i.e., dormant, vegetative and flowering) for staying healthy were always higher than those of the diseased ramets (Figs. 4A, 4B). At Kilpisjärvi, there were no consistent differences in OR of healthy and diseased ramets for staying healthy (Fig. 4C).

## Discussion

According to our results, low mean air temperature, high concentration of $NH_4^+$, low concentration of P, high coverage of bare ground, and the high density of *V. vitis-idaea* were positively associated with the frequency of *E. splendidum* in *V. vitis-idaea* populations. In addition, plant life-cycle stage had an impact on the vulnerability of *V. vitis-idaea* to *E. splendidum* infections, as the diseased ramets were at a greater risk of becoming reinfected than healthy ramets. Although a five-year study gives a reasonably good view on the variability of *E. splendidum* infections in *V. vitis-idaea* populations and on possible factors influencing the variability, there may be underlying causes for the observed relationships, which could not be investigated in this study. Hence experimental work will be needed to confirm the causes of the patterns found in the field.

The negative correlation between the infection frequencies and the temperatures across environments reflects the northern and high elevation distribution of the pathogen. *Exobasidium* fungi have relatively low maximum temperature limits for growth, above which they are not able to grow (Sundström, 1964). The artificial inoculation of *E. splendidum* has succeeded only after cold treatment of the basidiospores in natural conditions (Pehkonen et al., unpublished ms). The higher infection frequencies at Kilpisjärvi relative to the other sites can apparently be explained by the direct positive effects of colder climate on germination and growth of *E. splendidum*. In addition, a colder environment may cause more stress on the host plant, which increases the susceptibility towards pathogen infections. Precipitation had no apparent impact on the infection frequency of *E. splendidum*. However, a peak in the pathogen frequency in Kilpisjärvi observed in 2001 may be due to high precipitation in 2000 and 2001, and the collapse in the pathogen frequency in 2002 may be due to low precipitation in 2002. Interannual variability in precipitation is high compared with temperature, and thus a longer time than five years of observations may be needed to investigate the association between *E. splendidum* frequency and precipitation. Pathogen frequencies often increase with increasing precipitation (Strengbom et al., 2006), which implies the sensitivity of the pathogens to humidity. Moreover, annual pathogens, such as *E. splendidum,* need to reinfect their host plant every season unlike perennial systemic pathogens, which stay alive inside their hosts for years (Wennström 1994). Hence annual pathogens are likely to be especially sensitive to environmental conditions.

The positive correlation between the infection frequency and SOM $NH_4^+$ may indicate that the availability of N influences pathogen attacks on *V. vitis-idaea*. Increased N has been observed to increase the infection frequencies of *Exobasidium vaccinii* Woron. in our experimental fertilization study (T. Pehkonen and A. Tolvanen, unpublished data). Higher availability of N may increase the amino acid concentrations of plant tissues, which can result in higher infection frequencies of plants (Strengbom et al., 2002). Despite the increased disease levels, the relative impact of the pathogen is still suggested to be lower under good nutrient status relative to poor conditions, and the plant growth is enhanced (Wennström and Ericson, 1992). Unlike $NH_4^+$, the relationship between the frequency of *E. splendidum* infections and the concentration of P was negative. This pattern might partially be explained by the contrasting impact of N and P deficiency on possible defense of the plant against the pathogen. For example in mature tomato plants, the deficiency of P does not influence the flavonoid biosynthesis in leaves, contrary to the deficiency of N, which promotes flavonoid biosynthesis (Stewart et al., 2001). Flavonoids are phenolic compounds that are involved in

**TABLE 3**

**Spearman's correlation coefficients (*rho*) between annual frequencies of *E. splendidum* infections (*E. splend.* %) and annual population growth rate values (λ) with respective temperature and precipitation values during 1998–2002. Asterisks indicate correlations significant at the 1% (**), 5% (*), and 10% ((*)) levels. The sites within environments were pooled, since there was only one weather station per environment. For *E. splend.* %, $n = 15$ indicating five years and three environments. For λ, $n = 12$ indicating four transitions and three environments.**

| Month | *E. splend.* % vs. temperature | *E. splend.* % vs. precipitation | λ vs. temperature | λ vs. precipitation |
|---|---|---|---|---|
| March | −0.413 | −0.114 | 0.509 (*) | −0.107 |
| April | −0.583 * | 0.197 | 0.582 * | 0.193 |
| May | −0.530 * | −0.107 | 0.411 | 0.637 * |
| June | −0.658 ** | 0.152 | 0.580 * | 0.561 (*) |
| July | −0.606 * | −0.107 | 0.804 ** | −0.018 |
| August | −0.479 (*) | −0.136 | 0.358 | −0.095 |
| Annual | −0.411 | 0.318 | 0.834 ** | 0.053 |

**TABLE 4**

**Partial correlation coefficients *r* between the infection frequency of *E. splendidum*, and SOM and plant characteristics during 1998 (ramet density was measured in 2000). Asterisks indicate correlations significant at the 1% (**) and 5% (*) levels. Number of measured study plots: $n = 5$ at Oulanka and Kevo, $n = 4$ at Kilpisjärvi, total $n = 28$, d.f. = 16.**

| Variable | *r* |
|---|---|
| pH | 0.204 |
| Conductivity (μS/cm2) | 0.274 |
| $NH_4^+$ (μg/l) | 0.666 ** |
| $NO_3^-$ (μg/l) | −0.382 |
| P (μg/l) | −0.480 * |
| Cover of bare ground (%) | 0.555* |
| Total cover of vegetation (%) | −0.217 |
| Density of *V. vitis-idaea* /$m^2$ | 0.490* |
| Growth (mm/yr) | 0.380 |

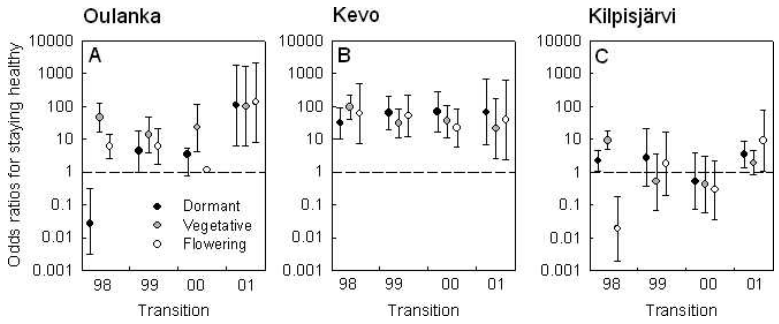



**FIGURE 4. Odds ratios and their 95 % confidence limits (95% CI) for dormant, vegetative, and flowering ramets relative to diseased ramets to stay healthy in Oulanka (A), Kevo (B), and Kilpisjärvi (C). Values pooled for the two sites within each environment, except at Oulanka, where there were no diseased ramets at Oulanka2 in any year. Cases where the 95% CIs do not overlap 1 indicate that the event of staying healthy is significantly more likely to happen (OR $>$ 1) or less likely happen (0 $\leq$ OR $<$ 1) relative to diseased ramets. Note the logarithmic scale of the y axis.**

many biological functions in plants, including protection against pathogen attack (Treutter, 2006). Our recent study shows that *V. vitis-idaea* may be able to defend against pathogen attacks through flavonoid biosynthesis (Pehkonen et al., unpublished ms), but information concerning the impact of N and P availability on the defense is still lacking.

Disturbance such as clear cutting increases the amount of soluble nutrients, which are leached from the soil surface (Piirainen, 2002; Piirainen et al., 2004). Disturbance can therefore increase the deficiency of P, as was observed indirectly also in this study. Disturbance also activates the vegetative production of new *V. vitis-idaea* ramets and increases ramet densities (Hautala et al., 2001). Dense patches of ramets may be easier for *E. splendidum* to infect, as the density is known to have an impact on host-pathogen interactions (Burdon and Chilvers, 1982). Our earlier study shows an increase in infection frequencies in *V. vitis-idaea* populations after disturbance (Pehkonen et al., 2002). We could not directly study the impact of disturbance in this study due to the difference in disturbance histories and environmental variables between sites. Nevertheless, the positive correlation between infection frequencies, the cover or bare ground (estimating disturbance level), and the density of *V. vitis-idaea* refers to an amplifying influence of disturbance on the infection frequency of *E. splendidum* in the present study.

The plant life-cycle stage had an impact on the vulnerability of *V. vitis-idaea* to *E. splendidum* infections. The odds ratios (OR) of healthy ramets for staying healthy were usually higher than those of the diseased ramets at Oulanka and Kevo. This indicates that certain ramets becoming infected for several seasons may be functioning as a disease source within these populations. Whether the ramets belong to the same genotype or not could not be verified in a field study. There can be significant variation between plant genotypes in the level of pathogen infections, which indicates that the genetic diversity of plants can influence pathogen levels in populations (Schmid, 1994). There was also a high degree of consistency in the pattern of disease prevalence between years at the site level; sites of the highest infection frequency in the first year of the study had the highest infection frequency across the study. These patterns might be explained by distance-to-source effect, as there seems to be no variation in the susceptibility between *V. vitis-idaea* populations according to artificial *E. splendidum* infections (Pehkonen et al., unpublished ms).

The present study shows that both environmental factors and host plant characteristics can constrain the infections by *E. splendidum* in *V. vitis-idaea* populations. The possible defense of *V. vitis-idaea* against fungal attacks (Pehkonen et al., unpublished ms) may be a further explanation for the low frequency of *E. splendidum* in nature. Based on our results, we can suppose a further decrease in the frequency of *E. splendidum* infections under warming temperatures, since pathogens restricted to high latitudes and elevations are expected to suffer from warming and the decreasing duration of snow cover (Kurkela, 1969; Nissinen, 1996; Wu et al., 1998). Although the predicted increase in precipitation might have an increasing effect on *E. splendidum* frequencies, changes in the yearly distribution of the rainfall may counteract this effect. The direct effect of the increasing soil nitrogen availability through enhanced nutrient mineralization (e.g., Dormann and Woodin, 2002) can be positive on *E. splendidum*. Nevertheless, enhanced N levels may increase the abundance of graminoids relative to slow-growing dwarf shrubs at least at fertile sites (Mäkipää, 1994; Olsson and Kellner, 2006), which results in reduced cover of the host plant. In that case, higher nutrient availability may have an indirect, negative impact on the frequency of *E. splendidum*. Experimental studies will be crucial in estimations of the complex relationships between the host plant *V. vitis-idaea*, its fungal pathogens, and the environment.

## Acknowledgments

We are grateful to Michael Colborn, Taina Hanhimäki, Ronja Kyrö, Carolin Nuortila, Elina Pehkonen, Erja Pehkonen, Riikka Savolainen, and Pauliina Varpunen for assisting in the field, to the staff of Subarctic Research Station Kevo, Oulanka Research station, and Kilpisjärvi Biological Station for help and technical support, to Dr. Jyrki Schroderus for the Fortran programming of the matrix calculations, and to Professor Kari Laine for making this research possible. We also thank the two anonymous referees for constructive comments on the manuscript. This study was financially supported by the Academy of Finland, Thule Institute of the University of Oulu, Environmental Graduate Net School, Graduate School of Evolutionary Ecology, Finnish Konkordia Fund, and Oulu University Scholarship Foundation.

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*Ms accepted February 2008*