

***Lophodermium piceae* and *Rhizosphaera kalkhoffii* in Norway spruce: correlations with host age and climatic features**

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Summary. The study was carried out in 4 similar Norway spruce stands and it demonstrated that the spreading structures produced by *Lophodermium piceae* and *Rhizosphaera kalkhoffii* are not correlated. The two fungi were always detected with opposite growth trends, probably due to different needle colonization and spreading strategies, and this was most likely also why they were able to co-exist, colonizing different parts of the needle. Independently of the year, site, sampling period and amount of precipitation, the two fungi were significantly less common in saplings and more common in mature trees, with frequencies also depending on both the minimum and the maximum temperatures.

Key words: endophytism, antagonism, temperature, precipitation, *Picea abies*.

Introduction

According to the recent literature, an “endophyte” is a micro-organism spending all or most of its life cycle within plant tissues and acting in a *continuum* from neutralism to mutualism to parasitism. At least in the fungi, many species inhabiting forest trees are normally asymptomatic but they have the ability to gradually shift to parasitism when features of the environment or the host partner’s features (e.g. the host’s suboptimal ecological conditions, or cell age) change to cause the phenotypically plastic fungus to become a real pathogen (Jumpponen, 2001; Sieber, 2002; Ragazzi *et al.*, 2003; Causin *et al.*, 2004; Schulz and Boyle, 2005; Scattolin and Montecchio, 2007; Montecchio and Mutto Accordi, 2007). However, this change

in the nature of a fungus is far from being the rule as it depends not only on the fungus and host involved, but also on the genotype and age of the host, on environmental characteristics (Rodrigues *et al.*, 2004; Schulz and Boyle, 2005), and on the relationship of the fungus with other fungi in the fungal community. Variations occur in the host (e.g. its phenological status), the site and climatic features (moisture, temperature, rainfall; Bernstein and Carroll, 1977; Carroll and Carroll, 1978; Petriani and Carroll, 1981; Johnson and Whitney, 1989; Suske and Acker, 1990). Furthermore, the occurrence of an endophyte in individual host cells does not necessary mean that the endophyte has turned into a parasite infecting the whole plant. It could also mean that the host is merely mobilising itself against possible changes from the pathogen (Horsfall and Cowling, 1980; Van Driesche and Bellows, 1996; Sticher *et al.*, 1997). It is also likely that an endophyte affects the virulence of a fungal parasite when these two fungi occur together in a plant.

The genus *Lophodermium* Chevall contains one

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species, *Lophodermium seditiosum* Minter, Staley & Millar, which is a distinct parasite, but for the rest it comprises widespread endophytic species inhabiting *Pinus*, *Abies* and *Picea*. This genus thus offers a good opportunity to study the possible correlations between endophytes and pathogens in the needles (Stone *et al.*, 2000; Ortiz-García *et al.*, 2003).

In *Picea abies* (L.) Karst., *L. piceae* (Fuckel) Höhn. is a very frequent endophytic and asymptomatic needle colonizer. It occurs inter- and intracellularly in up to 90% of needles in all the host's growth stages, with easily recognizable anamorphic and teleomorphic structures, and it is thought to cause resistance to needle parasites (Butin, 1986; Barklund, 1987; Suske and Acker 1987; Sieber, 1989; Osorio and Stephan 1991a,b; Livsey 1995; Stone and Petrini 1997, Schulz *et al.* 1999; Lehtijärvi and Barklund, 2000). Of these needles parasites, *Rhizosphaera kalkhoffii* Bubák causes widespread premature needle loss in nurseries, Christmas tree plantations and forests. It mainly attacks needles in the lower portion of the crown, and is easily recognized by the fruiting bodies (pycnidia) emerging from the stomata or "breathing" pores of infected needles (Maresi *et al.*, 2001; Lorenzi *et al.*, 2006; Müller *et al.*, 2007).

As information on the *L. piceae* – *R. kalkhoffii* relationship and ecology is still incomplete (Livsey and Barklund, 1992; Maresi *et al.*, 2001), the main goals of this study were to detect whether the occurrence of these two fungi in Norway spruce is correlated, and whether the production of fruit bodies of the fungi is associated with host age, precipitation or temperature.

Materials and methods

Studied sites and plots

The study was carried out in four high-mountain stands of Norway spruce (*Picea abies* [L.] Karst.) growing in the Province of Trento (northern Italy). Stands were representative of the spruce stands in the area (Anonymous, 2001), with similar climatic and site features, soil processes, silvicultural treatment and productivity (ArcExplorer software; ESRI Institute Inc., ArcExplorer, 2.0.800-version, Redlands, USA).

The forest sites selected (Malé [A], Tione [B], Cavalese [C] and Cles [D]; 5.122.408-5.151.385 N,

1.638.049-1.704.852 E, Gauss-Boaga] are located at 1620–1870 m a.s.l., with mean annual precipitations of 1020–1080 mm, and a mean annual temperature of +3–5°C (Provincia Autonoma di Trento, 2001).

Monthly environmental data (precipitation [Prec] in mm, minimum temperature [Tmin], average temperature [Tmed], and maximum temperature [Tmax] in °C of the four sites are shown in Fig. 1 (Provincia Autonoma di Trento, 2006).

In each site, three 200×200 m plots, each representing a forest stage: sapling (SP; mean age 4–5 yrs), thicket stand (TH; mean age 14–16 yrs) and mature (MT; mean age 155–175 yrs) were selected. In each plot, 10 healthy Norway spruce trees, undamaged by climatic events and with a fully developed crown, were chosen for the study.

Sampling procedure and laboratory observations

To take into account seasonal variations in plant physiology and climate, on the first Monday of January, March, May, July, September and November 2003, a sieve (40×40 cm; mesh 5×5 mm) was placed for 20 days below each tree at a random point along 10, 50 or 100 cm away from the stem for SP, TH and MT respectively, and 10 or 50 cm above the ground. From each sieve, 100 undamaged needles were randomly collected and air dried at 18±1°C for 24 hrs.

Each distal half, which is usually more intensely colonized by endophytes (Deckert and Peterson, 2000; Lorenzi *et al.*, 2006), was first checked using a dissecting microscope and then inspected on the basis of the available literature, for *L. piceae* (Fuckel) Höhn. and *R. kalkhoffii* Bubak according to their morphological features (Minter, 1981; Osorio and Stephan, 1991b; Livsey and Barklund, 1992; Stephan and Osorio, 1995; Ortiz-García *et al.*, 2003). *L. piceae* infections are distinguished as: "I" (conidiomata), and "L" (ascomata) and *R. kalkhoffii* conidiomata are coded as "Rh", and referred to as "fructifications".

All specimens were stored in the herbarium of the Te.S.A.F. Dept., University of Padova, Italy.

Data analysis

The total number of needles infected by *L. piceae* (independently of fructifications) and *R. kalkhoffii* in the 4 sites was calculated every two-months for 2 years, summing the data of each plant from each chronological forest stage (n=30).

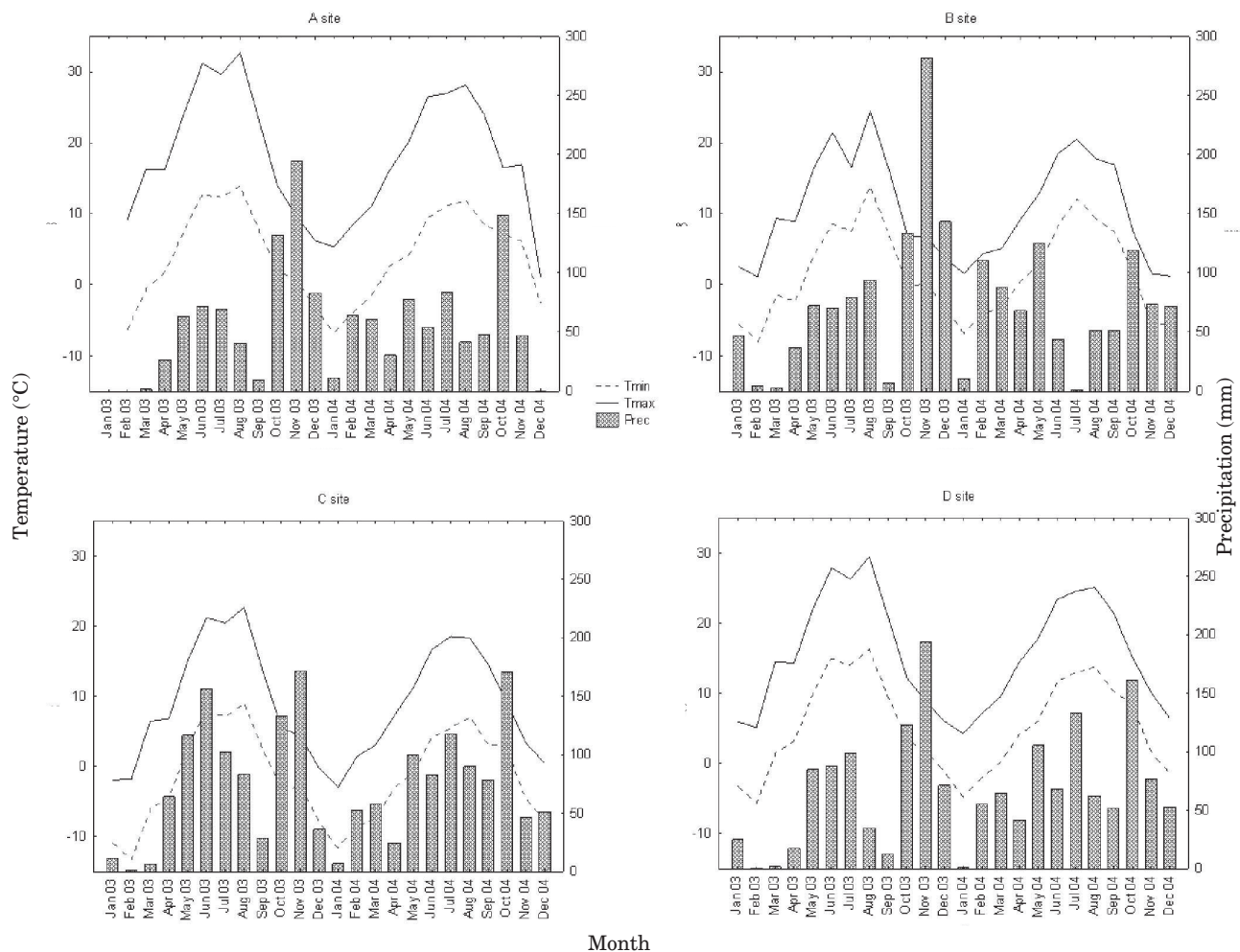


Fig. 1. Minimum and maximum temperature (Tmin, Tmax respectively) and precipitation (Prec) at the 4 sites during 2003 and 2004.

As the 3 fructifications did not present a normal distribution in either the sites or at any growth stage, the correlation among *L. piceae* (“I” and “L”) and *R. kalkhoffii* (“Rh”) was assessed through the non-parametric Spearman correlation test ($P < 0.05$; Siegel and Castellan, 1998; StatSoft, Inc. Textbook, 2007).

The mean frequency of needles infected with the 3 fructifications was then calculated in the 12 plots, every 2 months for 2 years ($n = 10$).

Significant differences among mean frequencies of the 3 fructifications in each of the 3 growth stages were determined by the non-parametric Kruskal-Wallis test ($P < 0.05$, $n = 30$) after verifying the normality of the data distribution with the ANOVA

Test, 1-factor, and Levene’s test for the homogeneity of variances ($P < 0.05$; Levene, 1960).

Relationships between each of the 3 fructifications, Tmin, Tmax, Tmed and Prec were investigated by calculating the relative percent frequency of a fructification (mean percent frequency in all the studied plants, frequency in 100 needles per plant), to avoid data not normally distributed, and/or influenced by possible outliers (StatSoft, Inc. Textbook, 2007), and performing a linear correlation and regression analysis. For each site, a rectangular matrix of the correlation coefficients was calculated with “I”, “L”, “I or L” (*L. piceae* occurrence independently of fructification), “Rh” (4 dependent variables) and Tmax, Tmin, Tmed and

Prec (4 independent variables). The variables identified as significantly correlated by means of the r Pearson index of simple linear correlation ($P < 0.05$; Pearson, 1896; StatSoft, Inc. Textbook, 2007) were then studied through subsequent linear univariate regression analysis. The independent variables that were significant in the models ($P < 0.05$) were used in a multivariate regression model (StatSoft, Inc. Textbook, 2007). Because of a possible relationship between temperature and precipitation, the environmental variable with R^2 higher than the correlated one was taken to explain the variability of the dependent variable, and used to draw the model, the significance of which was confirmed by the ratio of explained and residual variability (F test, $P > 0.05$). The model was also checked by the non-violation of the model validity assumptions:

i.e. no outliers in the residuals and with a normal distribution of the residuals. When assumptions were violated and a highly significant correlation occurred between 2 variables, a logarithmic transformation of the dependent variable was applied, according to StatSoft, Inc. Textbook (2007).

All the statistical analyses were performed by means of Statistica software (Vers. 7.1; StatSoft Inc., Tulsa, OK, USA).

Results

The frequency of needles infected with *L. piceae*, independently of the fructification, and with *R. kalkhoffii* was very similar in all 4 sites and in both years. The trend associated with site A is shown as a representative example (Fig. 2).

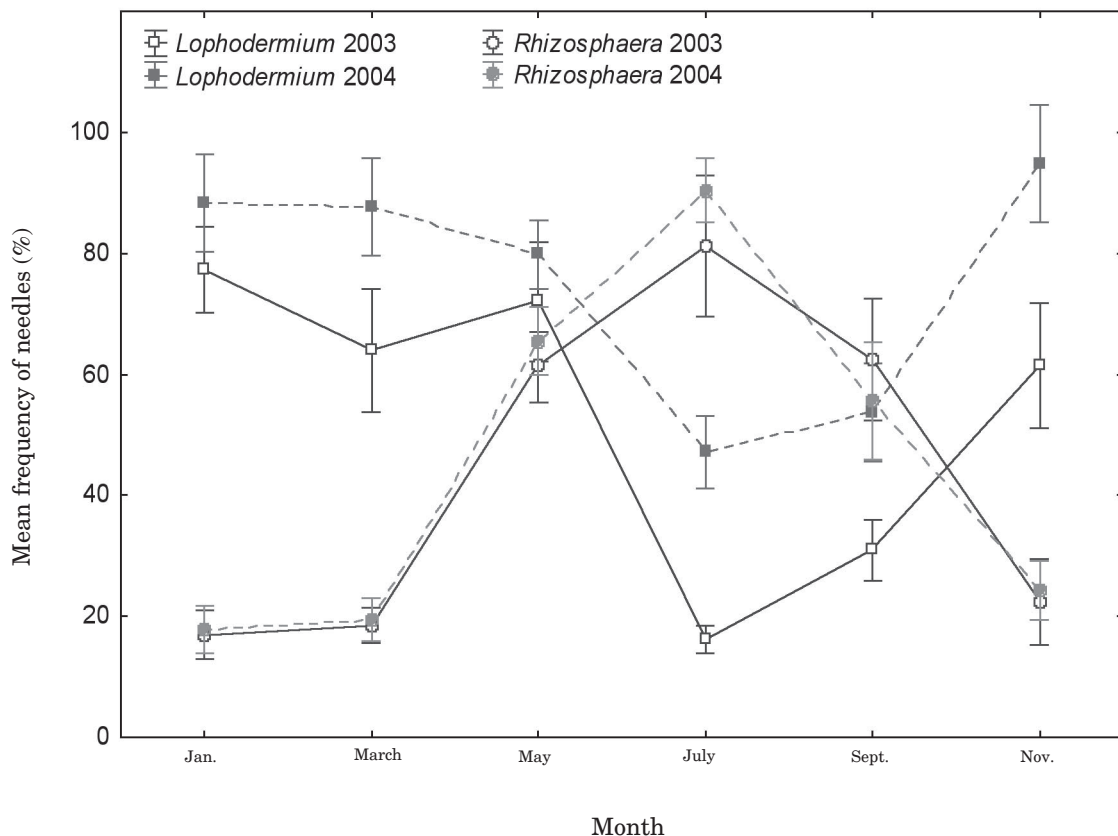


Fig. 2. Frequency of needles infected with *Lophodermium piceae* and *Rhizosphaera kalkhoffii* at site A, during 2003 and 2004. Fungal trends are the means of 60 trees, calculated every two months. The data point values of a month, with standard errors indicated by whiskers, relate to that and the following month.

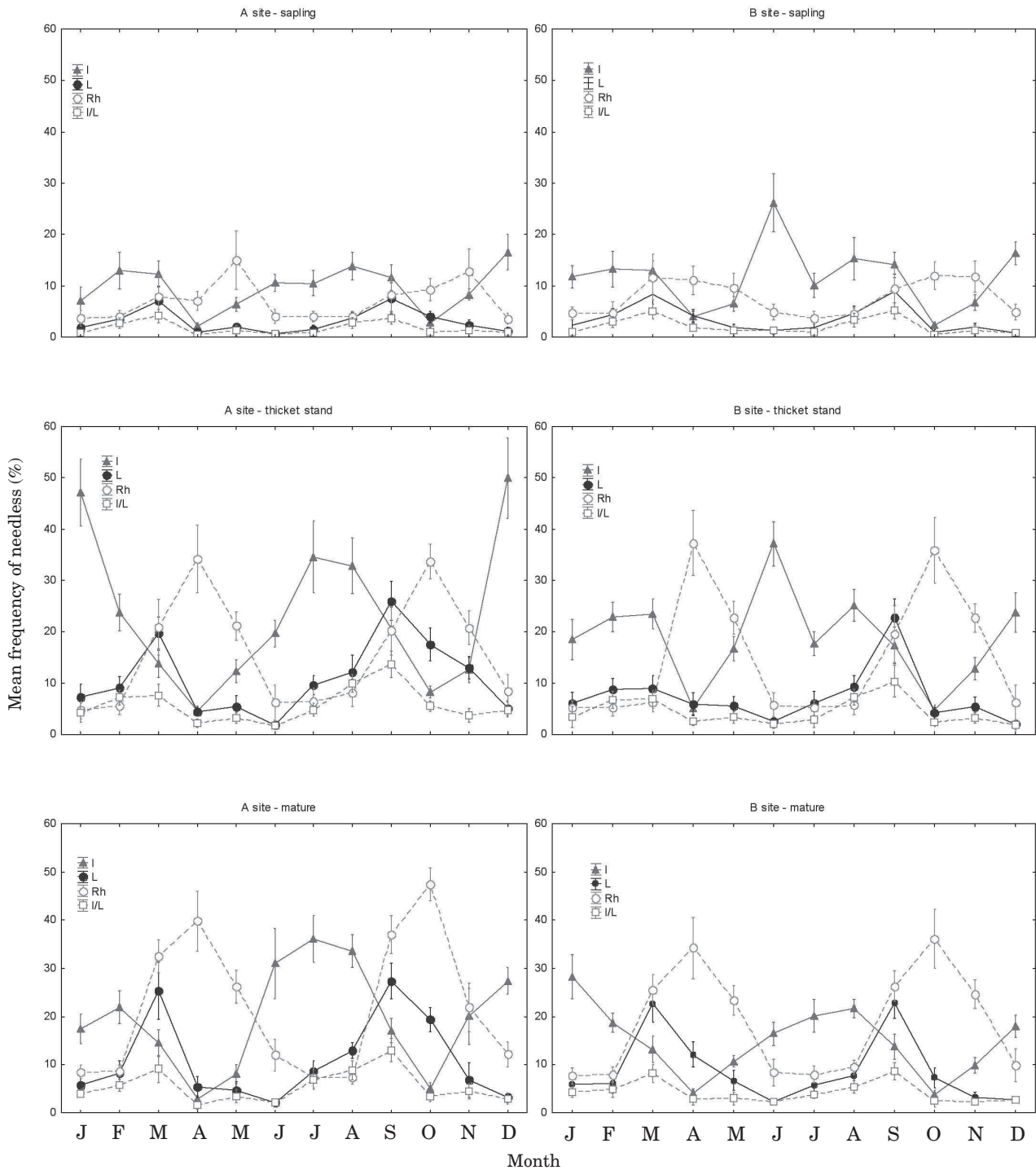


Fig. 3a. Frequency of needles infected with *Lophodermium piceae* (checked as: conidiomatal stage “I”, ascomatal stage “L”) and *Rhizosphaera kalkhoffii* (Rh) at sites A and B. Fungal trends are the means of 10 trees in each plot, calculated every two months. The simultaneous occurrence of the conidiomatal and the ascomatal stage of *L. piceae* is represented by the “I/L” line. The data point values of a month, with standard errors ($P < 0.05$) indicated by whiskers, relate to that and the following month.

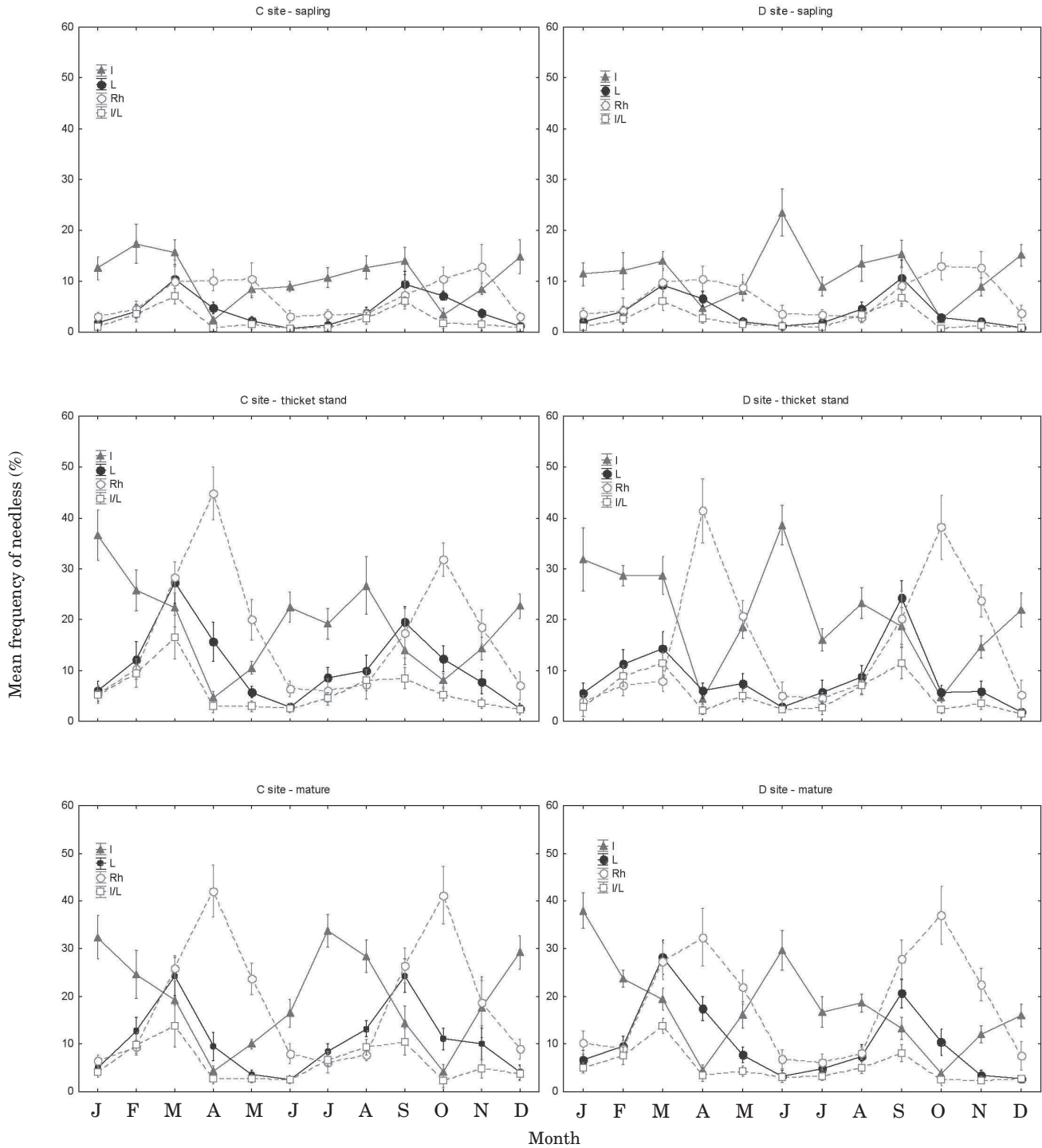


Fig. 3b. Frequency of needles infected with *Lophodermium piceae* (checked as: conidiomatal stage “I”, ascomatal stage “L”) and *Rhizosphaera kalkhoffii* (Rh) at sites C and D. Fungal trends are the means of 10 trees in each plot, calculated every two months. The simultaneous occurrence of the conidiomatal and the ascomatal stage of *L. piceae* is represented by the “I/L” line. The data point values of a month with standard errors ($P < 0.05$) indicated by whiskers, relate to that and the following month.

Although significant differences between years were found, *L. piceae* fructifications were detected in the majority of needles examined from January–February to May–June, with a rapid reduction from May–June to July–August, followed by a trend inversion. *R. kalkhoffii* instead was less common from January–February to March–April, with a sudden increase from May–June to July–August, followed by a trend inversion, comparable to the patterns for Tmin, Tmax and Prec. The mean frequencies of needles infected with the single studied fructifications in the 4 sites are shown in Fig. 3a, b.

After verifying the data not normally distributed and the significant ($P < 0.05$) Levene’s Test for Homogeneity of Variances ($F = 3.40$, $P = 0.048$), the Kruskal-Wallis non-parametric test ($P < 0.05$, $n = 30$) (Table 1) showed:

- significantly fewer needles with “I” in saplings than in mature and thicket stands;
- significantly fewer needles with “L” in saplings than in mature and thicket stands;

- significantly fewer needles with “I” or “L” in saplings than in mature and thicket stands;
- a significantly fewer needles with “Rh” in saplings than in mature and thicket stands.

The mean frequencies of the needles, from trees of the 3 growth stages, infected simultaneously with “I” and “L” (I/L); “Rh” and “I” (Rh/I); “Rh” and “L” (Rh/L); and “Rh”, “I” and “L” are shown in Fig. 4.

The Spearman correlation test did not give significant differences between sites and between years when comparing “I” or “L” with “Rh” ($R = -0.53$, $P = 0.075$).

Both correlation (Table 2) and regression analysis comparing the mean frequency of the 4 fructifications with Tmin, Tmax, Tmed and Prec, gave significant results which demonstrated that none of the 4 fructifications was correlated with Tmed or with Prec, and that only the anamorphic stage (“I”) of *L. piceae* and the pycnidial occurrence (“Rh”) of *R. kalkhoffii* was correlated with the temperature ranges recorded. The significant results of the univariate and multivariate regression analysis, summarized in Tables 3–7, made it possible to

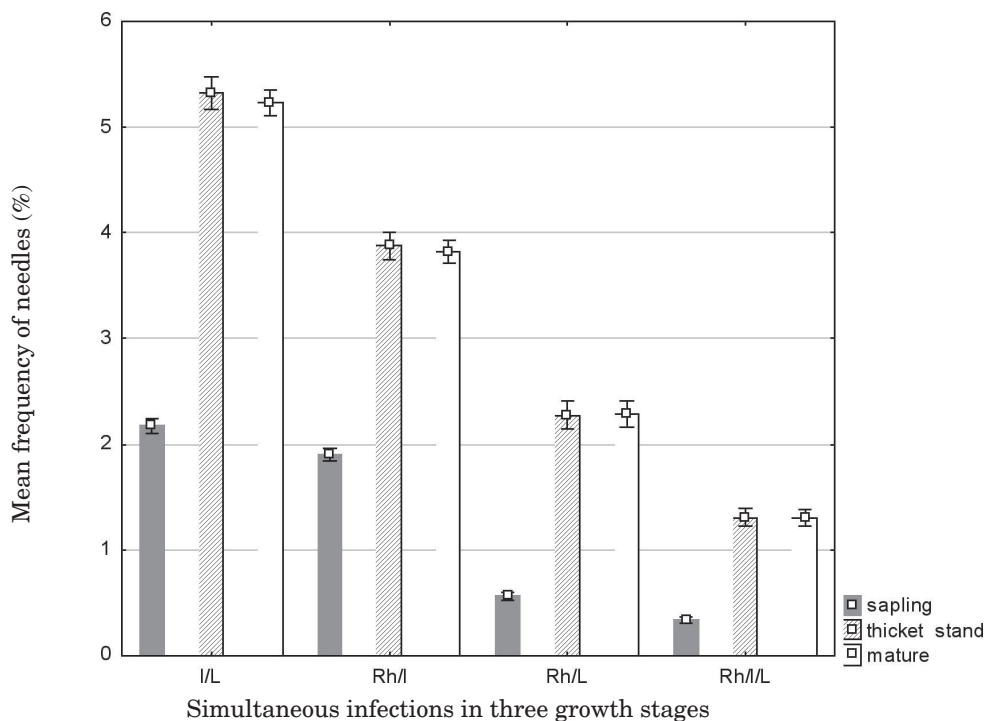


Fig. 4. Mean frequency of needles, from trees of 3 growth stages, infected simultaneously with “I” and “L” (I/L); “Rh” and I (Rh/I); “Rh” and “L” (Rh/L); “Rh”, “I” and “L”. Whiskers represent standard errors ($P < 0.05$).

characterize the following models:

“I” (in sites A, B, C, respectively):

$l=0.326413-0.008734 \cdot T_{\max}$, $R^2=0.78$, $F=36.19931$ ($P=0.00013$);

$l=0.1639-0.0087 \cdot T_{\min}$, $R^2=0.61$, $F=15.70374$ ($P=0.00267$);

$l=0.148907-0.009195 \cdot T_{\min}$ ($R^2=0.68$, $F=21.02582$, $P<0.002$),

and “Rh” (in sites A, B, C, D, respectively):

$Rh=0.103999+0.013045 \cdot T_{\min}$ ($R^2=0.86$, $F=61.03345$, $P<0.00001$);

$Rh=\exp(-2.2891+0.09809 \cdot T_{\min})$, ($R^2=0.92$, $F=242.8883$; $P<0.000001$);

$Rh=0.040646+0.011417 \cdot T_{\max}$ ($R^2=0.91$, $F=99.73046$, $P<0.000001$);

$Rh=0.069505+0.012613 \cdot T_{\min}$ ($R^2=0.84$, $F=51.90108$, $P<0.00004$).

Discussion

The study examined whether the frequencies of spreading structures produced by the endophyte *L. piceae* (ascomata and conidiomata) and by the parasite *R. kalkhoffii* (conidiomata) in Norway spruce needles were correlated with each other, and also whether these frequencies were correlated with the sampling period, host age, and climatic features such as temperature (minimum, maximum and average) and precipitation.

On the whole, the spreading structures produced by the two fungi were not correlated with each other in the experimental conditions, with slight changes between years, probably due to yearly differences in the weather in both years, but always with opposite trends within the year (*L. piceae* being more common in the coldest, and *R. kalkhoffii* in the warmer periods), probably as a result of different needle colonization and different spreading strategies (Livsey and Barklund, 1992), which may have been related to phenological differences in the needle contents (Gruber, 1990).

The co-occurrence of the two fungi indicated that these species did not exclude one another from the same tissue, suggesting that the localized (non-systemic) colonization by an endophyte allows the co-occurrence of a number of endophytic taxa, as has been demonstrated for the binomials *Rhabdocline parkeri* - *Phyllosticta* sp. in needles of *Pseudotsuga menziesii* and *L. piceae* - *Chrysomyxa abietis* in

Picea abies (Petrini, 1986; Stone, 1987; Lehtijärvi *et al.*, 2001).

Lophodermium piceae, which directly penetrates the cuticle (Osorio and Stephan, 1991b) and produces its thalli within the epidermis, hypodermis and near-surface mesophyll tissue (Suske and Acker, 1990), spreads mainly in the coldest months, whereas *R. kalkhoffii* grows in dead and damaged needles, colonizes the mesophyll (Diamandis, 1978) and mainly spreads in the warmest months. These different strategies probably permit both fungi to co-exist in different parts of the needle. It seems plausible, but still remains to be demonstrated that these fungi interact at later stages of the *R. kalkhoffii* infection, close to needle fall, when *R. kalkhoffii* colonizes most of the mesophyll, and when direct competition may well take place, as is thought to happen between *L. piceae* and *C. abietis*, as has been shown to happen between *L. pinastri* and some other fungi within individual needles (Minter and Millar, 1980; Kowalski, 1990).

The weather varied substantially in Europe between the 2 years studied (Kosatsky, 2005). Nevertheless, the two fungi were significantly less common in needles fallen from saplings, than in those fallen from thicket and mature trees, independently of the year, the site, and the month of sampling. This could be explained by the trophic contents of needles which becomes more favourable to infection as tree ages (Mishra and Dickinson, 1981; Gruber, 1990; Helander *et al.*, 2006).

An examination to detect correlations between the occurrence of fruit bodies and precipitation revealed that, in the experimental conditions, the occurrence of spreading structures was never correlated with precipitation. *Picea abies* is a mesophylic species that needs medium-high air and soil moisture, with frequent rainfall mainly in the early growing period (Pignatti, 1982; Gellini and Grossoni, 1996). As it is known that fruit bodies production by *L. piceae* depends on soil moisture, according to Lehtijärvi and Barklund (1995) it could be supposed that there is a correlation with the total amount of rainfall, but the water available to the root tips (mainly varying with the weather and with pedological features; Scattolin *et al.*, 2008) is a factor limiting the spread of *L. piceae* and most likely, *R. kalkhoffii* too.

From the available literature, the typical temperature ranges for *L. piceae* and *R. kalkhoffii*

Table 1. Significant results of the Kruskal-Wallis non parametric test ($P < 0.05$, $n = 30$). Only significant differences among the mean values of the frequencies of 3 fructifications, according to the 3 tree growth stages, are given.

I	
Sapling Mean=343.6 SD=54.63	Mature Mean=860.3; SD=58.628 $P=0.0118$
$H=22.8, P < 0.000001$	Thicket stands Mean=983.5; SD=88.283 $P=0.000007$
L	
Sapling Mean=177.2 SD=37.646	Mature Mean=486.6; SD=31.394 $P=0.000045$
$H=20.4, P < 0.000001$	Thicket stands Mean=448.8; SD=50.161 $P=0.003013$
I or L	
Sapling Mean=595.7 SD=55.2350	Mature Mean=486.6; SD=31.394 $P=0.000045$
$H=20.72, P < 0.000001$	Thicket stands Means=1117; Std.dev=90.454 $P=0.0000033$
Rh	
Sapling Mean=343.6 SD=66.697	Mature Mean=924.6; SD=76.564 $P=0.000002$
$H=24.6; P < 0.00001$	Thicket stands Mean=757.1; SD=80.6301 $P=0.022959$

are $0 \pm 27^\circ\text{C}$ and $0 \pm 24^\circ\text{C}$ respectively (Diamandis, 1979; Osorio and Stephan, 1991a). As expected, therefore, in the experimental conditions both the minimum and the maximum temperatures recorded sometimes become limiting factors for both fungi, since these ranges were exceeded in sites A and D, in both years.

Even if fungal endophytes have been shown to have ecological functions, their full amplitude is still unclear. In summary, however, in the experimental conditions, *L. piceae* and *R. kalkhoffii* were not correlated. Furthermore, in both fungi the fructification capacity changed with host age, probably due to changes in the needle contents, and also with

Table 2. Rectangular matrix of the correlation coefficients between “I”, “L”, “I or L”, “Rh” and Tmax, Tmin, Tmed and Prec, in each of the four sites (A, B, C, D). Significant values of the r Pearson Index are marked in bold ($P < 0.05$, $n = 12$).

Variable	Tmed	Tmin	Tmax	Prec
A site				
I or L	-0.5337 $P=0.104$	-0.6481 $P=0.023$	-0.6495 $P=0.022$	-0.2214 $P=0.513$
Rh	0.4321 $P=0.793$	0.9269 $P=0.000$	0.8897 $P=0.000$	0.1432 $P=0.674$
L	0.5103 $P=0.078$	0.4481 $P=0.144$	0.5439 $P=0.068$	-0.1471 $P=0.666$
I	-0.6219 $P=0.097$	-0.8537 $P=0.000$	-0.8852 $P=0.000$	-0.1947 $P=0.566$
B site				
I or L	-0.4971 $P=0.100$	-0.5639 $P=0.056$	-0.4631 $P=0.129$	0.3298 $P=0.295$
Rh	0.9001 $P=0.000$	0.9430 $P=0.000$	0.8729 $P=0.000$	-0.0389 $P=0.905$
L	0.4718 $P=0.121$	0.3888 $P=0.212$	0.5278 $P=0.078$	-0.1201 $P=0.710$
I	-0.7434 $P=0.006$	-0.7816 $P=0.003$	-0.7293 $P=0.007$	0.3547 $P=0.258$
C site				
I or L	-0.04963 $P=0.108$	-0.5418 $P=0.069$	-0.4527 $P=0.139$	-0.4484 $P=0.144$
Rh	0.5587 $P=0.346$	0.9404 $P=0.000$	0.9533 $P=0.000$	0.6565 $P=0.020$
L	0.3899 $P=0.121$	0.5751 $P=0.050$	0.6678 $P=0.018$	0.4502 $P=0.142$
I	-0.5361 $P=0.123$	-0.8232 $P=0.001$	-0.778 $P=0.003$	-0.6429 $P=0.024$
D site				
I or L	-0.4212 $P=0.173$	-0.4476 $P=0.145$	-0.4212 $P=0.173$	-0.0465 $P=0.886$
Rh	0.9088 $P=0.000$	0.9157 $P=0.000$	0.9088 $P=0.000$	0.4077 $P=0.188$
L	0.6047 $P=0.037$	0.5507 $P=0.064$	0.6047 $P=0.037$	0.0643 $P=0.843$
I	-0.7609 $P=0.017$	-0.6782 $P=0.015$	-0.6634 $P=0.019$	-0.1022 $P=0.552$

temperature, with minimum and maximum temperatures acting as limiting factors in both years (including the abnormally hot 2003), suggesting that this variable could play an important role, also within the context of global climate change. To explore the effect of climatic features over a longer period, further studies are in progress.

Table 3. Regression summaries (univariate models) for the dependent variables “Rh” and “I” in site A. The significant models are marked in bold ($P < 0.05$, $n = 12$).

Dependent variable	Regression summary values	Independent variable	β	SE of β	β coeff.	SE of β coeff.	T(10)	P-level
Rh	$R = 0.92694$; $R^2 = 0.85922126$; <i>Adj</i> $R^2 = 0.84514338$;	Intercept	0.10399	0.01187	0.01187	0.01187	8.75978	0.000005
	$F(1,10) = 61.033$; $P < 0.00001$; SE of estimate = 0.032602	Tmin	0.92694	0.11865	0.01304	0.00167	7.81239	0.000014
		Intercept	0.32641	0.02811	0.02811	0.02811	11.6108	0.000000
I	$R = 0.88518165$; $R^2 = 0.78354655$; <i>Adj</i> $R^2 = 0.76190121$;	Intercept	-0.88518	0.14712	-0.00878	0.00146	-6.0165	0.000129
	$F(1,10) = 36.199$; $P < 0.00013$; SE of estimate = 0.04360	Tmax						

Table 4. Regression summaries (univariate models) for the dependent variables “Rh” and “I” in site B. The significant models are marked in bold ($P < 0.05$, $n = 12$).

Dependent variable	Regression summary values	Independent variable	β	SE of β	β coeff.	SE of β coeff.	T(10)	P-level
Rh	$R = 0.90013074$; $R^2 = 0.81023535$; <i>Adj</i> $R^2 = 0.79125888$;	Intercept	0.07674	0.01462	0.07674	0.01462	5.24772	0.000375
	$F(1,10) = 42.697$; $P < 0.00007$; SE of estimate = 0.03934	Tmed	0.90013	0.12775	0.01195	0.00183	6.54428	0.000066
		Intercept	0.11741	0.00894	0.11741	0.00894	13.3005	0.000000
Rh	$R = 0.94303100$; $R^2 = 0.88930746$; <i>Adj</i> $R^2 = 0.87823820$;	Intercept	0.94303	0.10521	0.01332	0.00148	8.96328	0.000004
	$F(1,10) = 80.340$; $P < 0.00000$; SE of estimate = 0.03004	Tmin						
		Intercept	0.02290	0.02383	0.02290	0.02383	0.96097	0.359224
Rh	$R = 0.87289404$; $R^2 = 0.76194401$; <i>Adj</i> $R^2 = 0.73813841$;	Intercept	0.87289	0.15429	0.01094	0.00193	5.65746	0.000210
	$F(1,10) = 32.007$; $P < 0.00021$; SE of estimate = 0.04406	Tmax						
		Intercept	0.19024	0.01766	0.19024	0.01766	10.76988	0.000001
I	$R = 0.74343017$; $R^2 = 0.55268842$; <i>Adj</i> $R^2 = 0.50795726$;	Intercept	-0.74343	0.21149	-0.00777	-0.00221	-3.51508	0.005584
	$F(1,10) = 12.356$; $P < 0.00558$; SE of estimate = 0.04751	Tmed						
		Intercept	0.16386	0.01318	0.16386	0.01318	12.42396	0.000000
I	$R = 0.78163392$; $R^2 = 0.61095158$; <i>Adj</i> $R^2 = 0.57204674$;	Intercept	-0.78163	0.19724	-0.00868	0.00219	-3.96279	0.002674
	$F(1,10) = 15.704$; $P < 0.00267$; SE of estimate = 0.04431	Tmin						
		Intercept	0.22607	0.02629	0.22607	0.02629	8.59656	0.000006
I	$R = 0.72926430$; $R^2 = 0.53182642$; <i>Adj</i> $R^2 = 0.48500906$;	Intercept	-0.72926	0.21637	-0.00719	0.00213	-3.37040	0.007117
	$F(1,10) = 11.360$; $P < 0.00712$; SE of estimate = 0.04861	Tmax						

Table 5. Multivariate models Rh vs. Tmed, Tmin and Tmax in site B: regression summary for the dependent variable “Rh”. The significant model is marked in bold ($P < 0.05$, $n = 12$).

Dependent variable “Rh”	Independent variable	β	SE of β	β coeff.	SE of β coeff.	T(8)	P-level
R=0.97091555; $R^2=0.94267701$; Adj $R^2=0.92118089$; F(3,8)=43.853; $p < 0.00003$; SE of estimate=0.02417	Intercept		0.120515	0.07040	1.71167	0.125319	
	Tmed	-3.82136	2.49184	-0.050771	0.03310	-1.53355	0.163684
	T min	3.18770	1.00978	0.045040	0.01426	3.15683	0.013459
	Tmax	1.57671	1.60677	0.019774	0.02015	0.98129	0.355191

Table 6. Regression summaries (univariate models) for the dependent variables “I” and “Rh” in site C. The significant models are marked in bold ($P < 0.05$, $n = 12$).

Dependent variable	Regression summary values	Independent variable	β	SE of β	β coeff.	SE of β coeff.	T(10)	P-level
I	R=0.64290400; $R^2=0.41332555$; Adj $R^2=0.35465811$; F(1,10)=7.0452; $P < 0.02413$; SE of estimate=0.05937	Intercept		0.25096	0.03682	6.81457	0.000047	
		Prec	-0.64290	0.23221	-0.03546	0.01336	-2.65428	0.024135
		Intercept		0.148907	0.01314	11.32563	0.000001	
I	R=0.83421795; $R^2=0.67768779$; Adj $R^2=0.64545656$; F(1,10)=21.026; $P < 0.00100$; SE of estimate =0.04401	Tmin	-0.82321	0.17953	-0.00919	0.00200	-4.58539	0.001002
		Intercept		0.22834	0.02155	10.59493	0.000001	
		Tmax	-0.77776	0.19876	-0.00752	0.00184	-3.91293	0.002899
Rh	R=0.65646887; $R^2=0.43095138$; Adj $R^2=0.37404652$; F(1,10)=7.5732; $P < 0.02041$; SE of estimate =0.07533	Intercept		0.02773	0.04672	0.59352	0.566012	
		Prec	0.65646	0.23854	0.04665	0.01695	2.75194	0.020410
		Intercept		0.16442	0.01014	16.20995	0.000000	
Rh	R=0.94043906; $R^2=0.88442563$; Adj $R^2=0.87286819$; F(1,10)=76.524; $P < 0.00001$; SE of estimate =0.03395	Tmin	0.94043	0.10750	0.01353	0.00154	8.74782	0.000005
		Intercept		0.04064	0.01333	3.047917	0.012295	
		Tmax	0.95334	0.09546	0.01141	0.00114	9.98651	0.000002

Table 7. Regression summaries (univariate models) for the dependent variables “I” and “Rh” in site D. The significant models are marked in bold ($P < 0.05$, $n = 12$).

Dependent variable	Regression summary values	Independent variable	β	SE of β	β coeff.	SE of β coeff.	T(10)	P-level
I	R=0.67820424; $R^2=0.45996099$; Adj $R^2=0.40595708$; F(1,10)=8.5172; $P < 0.01534$; SE of estimate=0.06142	Intercept	0.20917	0.002293	0.20917	0.002293	9.11875	0.000004
		Tmin	-0.67820	0.23238	-0.00814	0.00279	-2.91842	0.015342
I	R=0.66343166; $R^2=0.44014157$; Adj $R^2=0.38415573$; F(1,10)=7.8617; $P < 0.01867$; SE of estimate =0.06253	Intercept	0.27034	0.04113	0.27034	0.04113	6.57151	0.000063
		Tmax	-0.66343	0.23661	-0.00668	0.00238	-2.80386	0.018670
I	R=0.66343166; $R^2=0.44014157$; Adj $R^2=0.38415573$; F(1,10)=7.8617; $P < 0.01867$; SE of estimate =0.06253	Intercept	0.23567	0.03002	0.23567	0.03002	7.85029	0.000014
		Tmed	-0.67092	0.23449	0.23449	0.00254	-2.86118	0.016923
Rh	R=0.91567021; $R^2=0.83845193$; Adj $R^2=0.82229712$; F(1,10)=51.901; $P < 0.0003$; SE of estimate =0.03854	Intercept	0.06950	0.01439	0.06950	0.01439	4.82887	0.000693
		Tmin	0.91567	0.12710	0.01261	0.00175	7.20424	0.000029
Rh	R=0.89622897; $R^2=0.80322636$; Adj $R^2=0.78354900$; F(1,10)=40.820; $P < 0.0008$; SE of estimate =0.04253	Intercept	-0.02533	0.02798	-0.02533	0.02798	-0.90538	0.386558
		Tmax	0.89622	0.14027	0.01035	0.01035	6.38903	0.000079
Rh	R=0.90883782; $R^2=0.82598619$; Adj $R^2=0.80858481$; F(1,10)=47.467; $P < 0.0004$; SE of estimate =0.04000	Intercept	0.02810	0.01937	0.02810	0.01937	1.45070	0.177496
		Tmed	0.90883	0.13191	0.01129	0.00163	6.88960	0.000042

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