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Dothistroma septosporum: spore production and weather conditions

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Abstract

Dothistroma septosporum, the causal agent of Dothistroma needle blight is a widespread fungus which infects more than 80 species of coniferous trees through the entire world. Spreading of the infection is strongly affected by climatic factors of each locality where it is recorded. We attempt to describe the concrete limiting climatic factors necessary for the releasing of conidia of *D. septosporum* and to find out the timing of its spore production within the year. For this purpose we used an automatic volumetric spore trap and an automatic meteorological station. We found that a minimum daily average temperature of 10 °C was necessary for any spore production, as well as a long period of high air humidity. The values obtained in the present study were a little bit higher than those previously published, which may arise questions about a possible changing trend of the behaviour in the development of the Dothistroma needle blight causal agent. We used autoregressive integrated moving average (ARIMA) models to predict the spore counts on the base of previous values of spore counts and dew point. For a locality from Hackerovka, the best ARIMA model was 1,0,0; and for a locality from Lanzhot, the best was 3,1,0.

Key words: Red band needle blight; Dothistroma needle blight; automatic volumetric spore trap; spore production; climatic conditions.

Resumen

***Dothistroma septosporum*: producción de esporas y condiciones climáticas**

El *Dothistroma septosporum*, el agente causal del tizón Dothistroma de las acículas, es un hongo ampliamente distribuido que infecta más de 80 especies de coníferas en el mundo. La propagación de la infección está fuertemente afectada por factores climáticos de cada localidad donde se registra. Tratamos de describir los factores limitantes necesarios para la liberación de los conidios de *D. septosporum* y averiguar el momento de la producción de esporas en el año. Para este fin se utilizó una trampa de esporas volumétrica y una estación meteorológica automáticas. Se ha encontrado que fue necesaria una temperatura media mínima diaria de 10 °C para cualquier producción de esporas, así como un largo período de alta humedad del aire. Los valores obtenidos en el presente estudio fueron un poco más altos que los publicados anteriormente, que pueden surgerir preguntas acerca de una posible tendencia cambiante de la conducta en el desarrollo del agente causal del tizón Dothistroma de las acículas. Se utilizaron modelos autorregresivos integrados media móvil (ARIMA) para predecir los conteos de esporas sobre la base de los valores anteriores de los recuentos de esporas y del punto de rocío. Para una localidad de Hackerovka, el mejor modelo ARIMA es 1.0.0 y para una localidad de Lanzhot, el mejor fue 3.1.0.

Palabras clave: tizón rojo de acículas; tizón Dothistroma de aciculas; trampa de esporas volumétrico automático; producción de esporas; condiciones climáticas.

Introduction

Dothistroma needle blight (DNB), also known as red band needle blight, is a very serious needle disease of conifers caused by fungi *Dothistroma septosporum* (Dorog.) Morelet. and *Dothistroma pini* Hulbary (Barnes *et al.,* 2004). It primarily affects pine species (*Pinus spp.*), and only on occasions other conifers. A total of 89 host species has been described previously (Watt *et al.,* 2009). Needles of all ages are commonly

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affected by this disease, which can cause a total defoliation and death of trees in severe cases (Gibson *et al.,* 1964). Economic damage from DNB in forest plantations results mainly from severe growth losses (Gibson, 1972), in addition to the loss of aesthetic value resulting from defoliation of ornamental and Christmas trees.

The global climatic change has been discussed to be an important factor of the rapid spreading of the disease. It may enhance the vulnerability of trees, especially the non-native and may offer more appropriate conditions for the pathogens (Woods *et al.,* 2005). In the case of DNB the increasing frequency of long periods of rain during warm days has been assumed as the key factor of changing climatic conditions in temperate zone (Woods *et al.,* 2005). Generally is known that the severity of attack remains low except at warm temperatures (15-20 °C) under regimes of continuous moisture (Peterson, 1967; Gadgil, 1974; Harrington and Wingfield, 1998) with at least three days of rain (Woods, *et al.* 2005). But also a small amount of rain is enough for a successful infection progress (Gadgil, 1974). However the incidence of infection is highly sensitive to the annual variation in weather (Peterson, 1973).

The aim of this study was to describe the temporal spore dispersal pattern of *D. septosporum* in two years, 2010 and 2011, in relation with the local weather conditions in a selected area strongly infected by the pathogen. We attempted to predict spore releasing events and their relation with the measured meteorological variables.

Materials and methods

Spore trapping was done two years, 2010 and 2011, during a period from March to December in localities from South Moravia (South-east of the Czech Republic). In 2010 the spore trap was placed in a 18 year-old plantation of Austrian pine (*Pinus nigra* Arnold), located in Lanzhot (48º41’16”N, 16º56’9”E, 141 m a. s. l.). This plantation has been strongly infected by *Dothistroma septosporum* for a few years. In 2011 the spore trapping was replaced in a new stand with artificial infection of various species of pines, spruce and larch seedlings (three years old) with fully developed symptoms. This new stand was located in Hackerovka (49º19’23.962”N, 16º44’39.419”E, 470 m a. s. l.).

For trapping the Dothistroma spores the automatic volumetric spore trap of usual Hirst, Burkard or Lanzoni VPPS construction was used, described by Hirst (1952), Portnoy *et al.* (2000) or Konopinska (2004). Our spore trap (AMET Velké Bílovice, Czech Republic) was placed inside the plantation with the orifice 0.3 m above ground. Close to the spore trap, a SIGNALIZATOR automatic climatic station (AMET Velké Bílovice, Czech Republic) was installed 2 m above ground. The weather station made hourly records of temperature, relative humidity and dew point during the entire spore trapping period. In 2011 the leaf wetness was also measured. It was not performed for the whole measuring season, but just from June 24 to the end of season (October 24).

The spore trap disc (driven by a clock machine) was covered by a melinex film coated by medical vaseline, an appropriate way for the adsorption of spores drawn through a rectangle shaped orifice in the outer cylinder. The film was changed regularly after each seven days and checked by a transmission microscope. The underpressure in the disc chamber was made by a 12V DC ventilator, placed in the bottom of the chamber, bellow the cylinder bearing. The motor of the ventilator was supplied by batteries and by a 38W solar power panel.

A Spearman’s rank correlation analysis was applied in order to quantify the influence of meteorological factors (the maximum, minimum and mean temperatures (°C), relative humidity (%), dew point (°C) and leaf wetness (V – difference of voltage measured between electrodes connected by a filter paper)) on the daily spore counts. A nonparametric statistical analysis was used due to the nonexistence of normality in the data.

The daily number of spores and the corresponding weather variables were time series, characterized by a temporal autocorrelation. Autoregressive integrated moving-average (ARIMA(p,d,q)) model was used to describe these time series.

In the model, the three ARIMA parameters ‘autoregression’, ‘differentiation’ and ‘moving average’ were tested. Autoregression (p) is defined as the number of autoregressive parameters of the model; each parameter measures the independent effect of the values with a specified delay. It means that the current value, y_t, of a series $\{y_t\}$ is related linearly to previous values (i.e. y_{t-1}, y_{t-2}, etc.). A random variable, a_t, is added to the autoregressive (p) component to account for any residual error; successive values of the series $\{a_t\}$ are assumed to be uncorrelated. Differentiation (d) is the number of times that a time series was transformed; this factor accounts for the differences between the values of the series and their predecessors. Trends can often be removed by differencing the series. The new series generated by first-order differencing has the current value y_t - y_{t-1} in place of y_t; second-order differencing has the current value $(y_t - y_{t-1}) - (y_{t-1} - y_{t-2})$, and so on. An ARIMA model is one in which a differenced series follows an autoregressive moving-average (ARMA) model; ARIMA models can be extended to describe periodic patterns. The moving average (q) is the order of the moving average of the process, it means that y_t is related linearly not only to previous values (the AR component) but also to the current and previous values of the random variable $\{a_t\}$, i.e. the moving-average (MA) component.

The best possible model was chosen by a BIC criterion (smaller is better), R-squared – coefficient of determination (higher is better) and RMSE (root mean square error – smaller is better). The SPSS 18.0 software package was used for all statistical analyses.

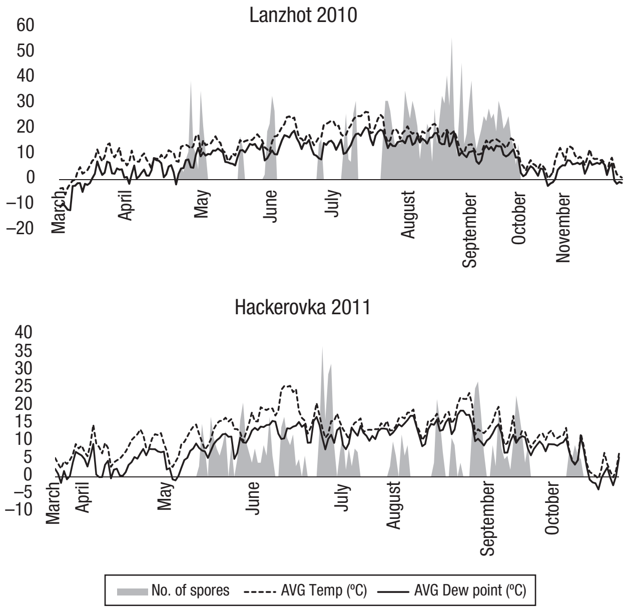

Figure 1. Course of daily average temperature, daily average dew point and spore counts in Lanzhot plot in 2010 (up) and Hackerovka plot in 2011 (down).

Results

Spore films from the trap showed remarkable differences between years and localitions. The period of sporulation of each year/location can be observed in Table 1.

A visible fact was that the production of spores occurred solely during the days with a daily average temperature above 10 °C and only in the part of the period without frost days. Such conditions started in 2010 in the third decade of April and the spores began to spread on April 24 (Fig. 1). The last spores were caught on October 6 because of the rapid decline of daily mean temperatures (below the limit of 10 °C). The reincrease of temperatures at the end of October was not followed by any spore production, because the period before was characterized by a high frequency of frost days (min. temperatures around –4 °C.) During the spore-active season some periods of interruptions were observed. The longest period without any spore observation was recorded at the first and the second decade of June 2010. It was characterized by an average daily temperature above 18 °C and an average daily relative humidity under 75%. After such dry conditions, the spore production stopped after few days. On the other hand, the optimal weather conditions were represented by an average daily temperature of 15-20 °C and an average daily relative humidity above 90%. This optimal period was taken from end July to middle September. A similar pattern was noticed in 2011 in Hackerovka (Fig. 1), although due to the higher altitude, the sporulation began nearly three weeks later than in Lanzhot.

Spearman's rank correlation analysis took into account the number of spores and the weather parameters recorded on the same date. Correlation coefficients were significant in most of cases (with the only exception of AVG RH). The highest correlation was found for AVG Dew point for both localities (Table 2). All corellation are relatively low (usually below 0,5), it means that dependance of count of spores on studied factors is only moderate. In Hackerovka site, average

Table 1. Spore production

	Lanzhot 2010	Hackerovka 2011
Elevation (m a. s. l.)	141	470
Start of sporulation	April 24	May 13
End of sporulation	October 6	October 11
Length of sporulation season (days)	166	152
Min. temperature for sporulation (°C)	approx. 10	approx. 10

Table 2. Spearman's rank correlations between number of spores and main climatic factors

Correlation	Hackerovka		Lanzhot	
	Spearman	p-value	Spearman	p-value
No. spores & AVG T(°C)	0,364	0,000	0,417	0,000
No. spores & MAX T(°C)	0,277	0,000	0,353	0,000
No. spores & MIN T(°C)	0,354	0,000	0,423	0,000
No. spores & AVG RH(%)	0,127	0,078	0,204	0,002
No. spores & AVG Dew point(°C)	0,387	0,000	0,448	0,000

temperature was the second most important weather variable, while in the case of Lanzhot, the correlation with minimal temperature was found as the second most important. Maximum temperature had a higher correlation in Lanzhot in comparison with Hackerovka (Table 2). The best adjusted ARIMA time-series model for predicting spores releasing was an ARIMA (1,0,0) for Hackerovka and an ARIMA (3,1,0) for Lanzhot. In the case of Hackerovka, the model had a R^2 of 0.375 and spore releasing was only affected by the previous spore releasing (formula 1). In the case of Lanzhot, the R^2 was 0.623 and the spore releasing was also affected by the dew point (formula 2). Comparison of measured and modeled counts of spores for Hackerovka and Lanzhot can be observed in Figure 2.

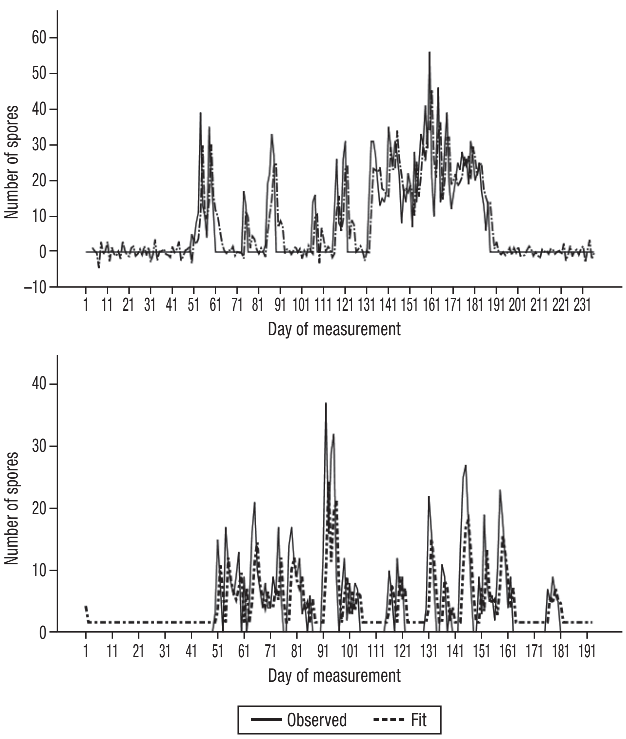

Figure 2. Daily numbers of spores modelled with ARIMA for Lanzhot (up) and Hackerovka (down) site.

[formula 1]

$$y_t = 4{,}313 + 0{,}611 y_{t-1} + \varepsilon$$

y_t – number of spores at time t.

y_{t-1} – number of spores at time ($t-1$)

ε – random error

[formula 2]

$$y_t = y_{t-1} - 0{,}311(y_{t-1} - y_{t-2}) - 0{,}308\,(y_{t-2} - y_{t-3}) - 0{,}274\,(y_{t-3} - y_{t-4}) - 0{,}485\,(DP_t - DP_{t-1}) - 0{,}467(DP_{t-1} - DP_{t-2}) + \varepsilon$$

y_t – number of spores at time t.

y_{t-n} – number of spores at time ($t-n$), where n is time lag

DP_t – dew point at time t

DP_{t-n} – dew point at time ($t-n$), where n is time lag

ε - random error

Discussion

According to Karadzic (1989) conidia are active for the DNB spreading from early May to end October. In average our observations can support this period. The beginning of spore production in 2010 was noticed a little bit earlier, but in 2011 was a little bit later. This fact could be clarified by the different elevation, which seems to be an important factor.

On the other hand Bednarova (2010) obtained a spore production period from middle April to October. In this period, the day temperatures above 10 °C and the high air humidity determined the best conditions for the spreading of conidia according to Bednarova (2010), which was in agreement with our observations.

A lower limit of air temperature was stated by Gilmour (1981). He noticed the average daily temperature 7 °C as critical, but he added another limiting condition – the leaf wetness period, which should not be less than 10 hours for a successful releasing of spores. Sinclair *et al*. (1987) mentioned also a lower limit of air temperature, 5 °C, as long as a sufficient humidity occurred.

Our records of leaf wetness data cannot support the statement of Gilmour (1981). We did not found any correlation between spore counts and leaf wetness.

Values of lowest temperature limits mentioned by Gilmour (1981) and Sinclair *et al.* (1987) are a few Celsius degrees lower. This observation may show a trend of the DNB causal agent to change its environmental limits and to adapt to the global climate change. Unfortunately we do not have enough long-term data for more robust conclusions. We also cannot neglect the ability of occurrence of various ecotypes of DNB causal agent, which will be discovered by more particular molecular screening among isolates of entire world. These screenings are currently in progress and also some differences between behaviour of *D. septosporum* and *D. pini* could be revealed.

The differences between correlations of meteorological parametres and spore releasing could be probably due to the different mesoclimatic conditions between localities. The main difference is the elevation. The altitude of Hackerovka was 329 m higher than that of Lanzhot. Also the geomorphology is different, Lanzhot was situated in a plane forest landscape and Hackerovka in sligthly inversion valley. Difference of elevation may cause the different length of the sporulation season (14 days according to Table 1).

Aerobiological studies generally use linear logistic models to predict spore concentrations (Rodríguez-Rajo *et al.,* 2002). These linear regression models, using only weather variables for prediction, yield results showing a low predictive capacity (Escuredo *et al.,* 2011). The ARIMA time-series model presents a high accuracy in the forecasting of the spore or pollen counts (Cotos-Yañez *et al.,* 2004, Escuredo *et al.,* 2011). We have tried many models, both 'pure' ARMA and ARIMA models and also models with additional predictors (weather variables). We use 'Expert Modeller' in SPSS 18. This module enables comparison of many candidate models and can choose the best one according several criterions (e.g. R-squared, root mean square error and Bayesian information criterion).

In this paper ARIMA models were used for the first time in modelling and predicting of spore counts of *D. septosporum*. Our spore count forecasting models may demonstrate that the dependence of spore count on weather variables is not so strong. Delimitation of the sporulation season by the air temperature is clear, but the amount of spores during the sporulation season seems to be driven more by the stage of the pathogen and the host, and only occasionally by the weather.

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