Host specificity of *Hypoxylon fuscum:* A statistical approach to the problem

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Summary. – The hypothesis that ascospore size in *Hypoxylon fuscum* may be correlated with the host on which the fungus is growing has been tested on 32 collections of *H. fuscum* from 5 hosts. Discriminant analysis and analysis of variance procedures have shown that collections of *H. fuscum* from different hosts can be distinguished on the basis of the size of their ascospores. The taxonomic and the philogenetic implications of these findings are briefly discussed.

Introduction

Hypoxylon fuscum (Pers.: Fr.) Fr. (Xylariaceae, Ascomycetes) is a widespread species whose distribution ranges from the temperate to the tropical regions of the world. The host range of this fungus is restricted to species of Betulaceae and only few collections are known from hosts belonging to other families (Miller, 1961; Petrini & Müller, 1986).

H. fuscum is characterized by purple to red-brown stromata and ellipsoid to asymmetrically ellipsoid spores bearing a clearly recognizable germ slit with a "knick" which allows to distinguish it from the morphologically closely related *H. macrocarpum* Z. Pouzar, *H. vogesiacum* (Pers.) Sacc., and *H. rubiginosum* (Pers.: Fr.) Fr. (Petrini & Müller, 1986).

During an extensive study of European *Hypoxylon* spp. (Petrini, 1985) a considerable variation in the ascospore size among collections of *H. fuscum* growing on different hosts was noticed. The observation by Enderle (1982) that collections of *H. fuscum* from *Carpinus* had ascospores that are smaller than the ones from other hosts led to the assumption that ascospore size may be correlated with the host on which the fungus is growing.

Materials and methods

A total of 32 collections of *H. fuscum* growing on *Alnus incana* (L.) Moench., *A. viridis* (Chaix) Dc., *Betula* sp., *Carpinus betulus* L., and *Corylus avellana* L. were used in this study. Within each collection a total of 50 spores and some asci from ten perithecia

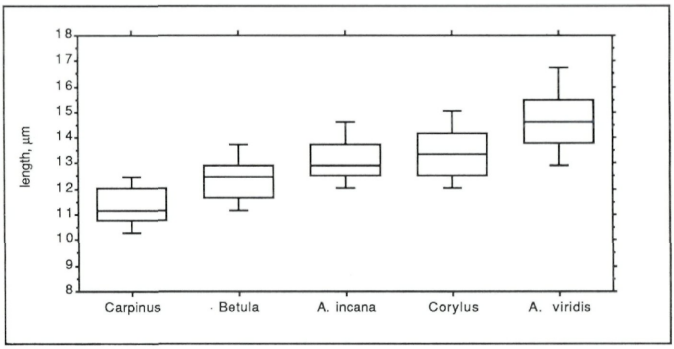

Fig. 1. *Hypoxylon fuscum:* Boxplots of ascospore length. Line within box: median; upper and lower lines: 75th, resp. 25th percentile. Length of vertical bar indicates the 80% confidence interval of the median.

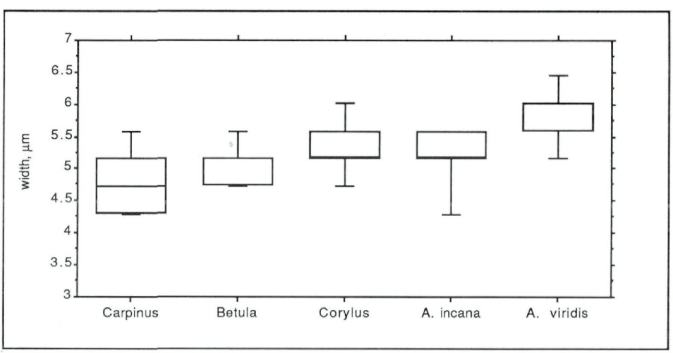

Fig. 2. *Hypoxylon fuscum:* Boxplots of ascospore width. Line within box: median; upper and lower lines: 75th, resp. 25th percentile. Length of vertical bar indicates the 80% confidence interval of the median.

taken from five stromata were chosen at random, mounted in water and their length and width measured at 1000X magnification.

To test for normality distribution, probability plots and a Kolmogorov-Smirnov test were used (WILKINSON, 1986). For the graphical display of the ascospore length and width boxplots, a graphical analogue to one-way analysis of variance (TUKEY, 1977) were chosen. The Bartlett test (SOKAL & ROHLF, 1981) was used to test for homogeneity of group variances; as this assumption was not con-

firmed, a Kruskal-Wallis one-way analysis of variance was performed on the ascospore length and width. A parametric one-way analysis of variance, combined with a multiple comparison testing by the Scheffé linear contrasts method (SOKAL & ROHLF, 1981) was also used but the results have to be interpreted with some caution because of missing homogeneity of group variances.

A multivariate discriminant analysis was performed on the ascospore length and width to test the hypothesis that the five groups can be distinguished on the base of these two variables.

No statistical analysis was performed on the measurements of the asci: the sample size was not large enough to allow it. For the asci, thus, only mean, standard deviation and 95% confidence intervals of ascus length are tabulated.

The statistical analyses were computed in double precision on an Apple MacIntosh Plus™ using the statistical packages SYSTAT 3.01 (WILKINSON, 1986) and STATVIEW 512+ (BRAINPOWER, INC., Calabasas CA, USA).

Results

Collections of *H. fuscum* growing on different hosts can be distinguished on the basis of the size of their ascospores (Tab. 1; Fig. 1, 2).

Although some overlapping values of the 95% confidence intervals can be seeen, the mean lenghts and widths of the spores are different for all hosts. The size of the ascospores of *H. fuscum* from *C. betulus* is mostly within the range 9.3 – 13.3 × 3.8 – 5.8 µm; ascospores from *A. viridis* are clearly larger and measure 11.7 – 17.7 × 4.8 – 6.8 µm. Ascospores from collections derived from other hosts occupy intermediate positions (Fig. 1, 2).

This observation is confirmed by the discriminant analysis and the analysis of variance for the spore length and width (Tab. 2–5; Fig. 1, 2). No statistically significant difference can be found between the colletions of *H. fuscum* on *C. avellana* and those on *A. incana*. Collections from other hosts, however, are different with regard to their spore length. The box plots (Fig. 1, 2) depict the data and show that the spores of the specimens on *C. betulus* are clearly different from those on *A. viridis, C. avellana* and *A. incana,* whereas they overlap for collections on *C. betulus* and *Betula* sp. The ascospore length of collections of *H. fuscum* growing on *A. viridis* is generally larger than that of the same fungus growing on *C. avellana, A. incana* and *Betula* sp. The specimens growing on *A. incana* and *C. avellana,* on the other hand, can not be distinguished by this variable, as shown by the 95% confidence intervals and the analysis

Tab. 1. Means, standard deviations and 95% confidence intervals of the spore length (L) and width (W) of *Hypoxylon fuscum* on the various hosts.
n: number of spores measured.

Host		mean, standard dev. µm	95% confidence interval µm
Carpinus betulus	L	11.3 ± 1	9.3 – 13.3
(n = 200)	W	4.8 ± 0.5	3.8 – 5.8
Alnus viridis	L	14.7 ± 1.5	11.7 – 17.7
(n = 250)	W	5.8 ± 0.5	4.8 – 6.8
Corylus avellana	L	13.4 ± 1.3	10.8 – 16.0
(n = 899)	W	5.3 ± 0.5	4.3 – 6.3
Alnus incana	L	13.0 ± 1.4	10.2 – 15.8
(n = 149)	W	5.3 ± 0.6	4.1 – 6.5
Betula sp.	L	12.3 ± 1.6	9.1 – 15.5
(n = 99)	W	5.0 ± 0.7	3.6 – 6.4

Tab. 2. Results of the discriminant analysis, using the hosts as the discriminator. Dependent variable canonical coefficients standardized by conditional (within hosts) standard deviations

	Factors	
	1	2
Length	0.807	-0.669
Width	0.397	0.970

Tab. 3. Results of classification analysis from discriminant functions based on spore length and width, using the host as the discriminator variable

	assigned group					
	Cb	Av	Ca	Ai	Bet	Total no of cases
Actual group						
Cb	147	2	6	9	36	200
Av	3	176	36	14	21	250
Ca	101	209	177	241	171	899
Ai	14	21	40	42	32	149
Bet	31	5	8	24	31	99
Total	296	413	267	330	291	1597
% correctly assigned:	73.5	70.4	19.7	28.1	31.3	35.9

Cb: *Carpinus betulus;* Av: *Alnus viridis;* Ca: *Corylus avellana;* Ai: *Alnus incana;* Bet: *Betula* sp.

Tab. 4. Results of the one-way analysis of variance for the spore length. Analysis of variance table

Source	DF	Sum square	Mean square	F-test
Between groups	4	1365.536	341.384	233.255
Within groups	1592	2330.048	1.464	
Total	1596	3695.584		

$p < 0.001$

Model II estimate of between component variance = 84.98

Kruskal-Wallis test statistic = 559.14, $P < 0.001$

Multiple comparison testing (Scheffé F-test)

	Alnus incana	*Alnus viridis*	*Betula* sp.	*Corylus avellana*	*Carpinus betulus*
Alnus viridis	*	–			
Betula sp.	*	*	–		
Corylus avellana	–	*	*	–	
Carpinus betulus	*	*	*	*	–

* = different at $p < 0.05$, – = not significant

of variance (Tab. 1, 4; Fig. 1). Similar results are obtained if the analysis is performed on the ascospore width (Tab. 1, 5; Fig. 2).

The results of the classification analysis from the discriminant functions performed using the host as the discriminator variable illustrate these findings. The samples of the *H. fuscum* on *C. betulus* and *A. viridis* have been identified to a higher percentage correctly by the analysis (73.5% for *C. betulus* and 70.4% for *A. viridis*). On the other hand, samples on *C. avellana, A. incana* and *Betula* sp. can not be distinguished clearly and the prediction power is very weak (Tab. 3).

Inspection of Fig. 1 and 2 as well as observation of the standardized canonical discriminant function coefficient (Table 2) show that the spore length is mainly responsible for the discrimination of the sample groups.

Probably as a consequence of the differences discussed above, also the ascus length, particularly the spore bearing part and the total length are different on the various hosts (Tab. 6).

Tab. 5. Results of the one-way analysis of variance for the spore width. Analysis of variance table

Source	DF	Sum square	Mean square	F-test
Between groups	4	117.248	29.312	113.243
Within groups	1592	412.079	0.259	
Total	1596	529.327		

$p < 0.001$
Model II estimate between component variance = 7.263
Kruskal-Wallis test statistic = 352.42, $P < 0.001$

Multiple comparison testing (Scheffé F-test)

	Alnus incana	*Alnus viridis*	*Betula* sp.	*Corylus avellana*	*Carpinus betulus*
Alnus viridis	*	–			
Betula sp.	–	*	–		
Corylus avellana	–	*	*	–	
Carpinus betulus	*	*	*	*	–

* = different at $p < 0.05$, – = not significant

Tab. 6. Means, standard deviations and 95% confidence intervals of the length of the spore bearing part (sp) and stipe (st) as well as of the total ascus (tot) of *Hypoxylon fuscum* on the various hosts.

Host		mean, standard dev. µm	95% confidence interval µm
Carpinus betulus	sp	77.6 ± 7.5	62.6 – 92.6
(n = 130)	st	53.0 ± 14.3	24.4 – 81.6
	tot	131.0 ± 14	103.0 – 159.0
Alnus viridis	sp	92.4 ± 10.2	72.0 – 112.8
(n = 51)	st	69.3 ± 12.5	44.3 – 94.3
	tot	161.6 ± 18.8	124.0 – 192.2
Corylus avellana	sp	88.6 ± 9.8	69.0 – 108.2
(n = 63)	st	55.7 ± 15.4	25.3 – 86.5
	tot	144.4 ± 20	104.4 – 184.4
Alnus incana	sp	85.7 ± 7.9	69.9 – 101.5
(n = 35)	st	49.4 ± 12.7	24.0 – 74.8
	tot	135.0 ± 16.2	102.6 – 167.4
Betula sp.	sp	82.4 ± 6.3	69.8 – 95.0
(n = 16)	st	51.0 ± 17.2	16.6 – 85.4
	tot	133.4 ± 15.6	102.2 – 164.6

Discussion

The average dimensions of the ascospores of *H. fuscum* from *Carpinus betulus* are clearly smaller and those from *Alnus viridis* larger than on the remaining hosts. The variability of the spore size related to the fungus host seems not to be restricted to *H. fuscum*. Within the Xylariaceae FRENCH & al. (1969) already reported a similar phenomenon for *H. mammatum* (WAHL.) J. H. MILLER; they refrained, however, from describing infraspecific taxa within this species because of the rather evident overlap of dimensions we observed also for *H. fuscum*.

With the exception of *Alnus viridis*, which usually occurs in higher altitudes in the Alps (1500–2300 m a. s. l.), all hosts studied grow under similar climatic conditions: the variation in ascospore size is thus unlikely to be related with the climate. The results of this study support the hypothesis of an ongoing process of differentiation of subspecies within the large *H. fuscum* – complex rather than an adaptation to ecological or physiological conditions related to the growth substrate. It can also be assumed that the biochemical composition of the substrates studied is quite similar.

From a taxonomic point of view, thus, the question of the erection of varieties arises. Comparative studies of cultures and of the anamorph of *H. fuscum* have brought up no differences among isolates from different hosts (PETRINI, 1985). Therefore, a subdivision of this species based only on the spore size seems not appropriate. Moreover, specimens growing on other recorded hosts, e.g. *Alnus rubra* BONG. or *A. glutinosa* (L.) GAERTN. have not yet been conclusively examined. Inoculation studies would add to these data and might allow differentiation of infraspecific taxa.

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