Each Isolate of *Hymenoscyphus fraxineus* is Unique as Shown by Exoenzyme and Growth Rate Profiles

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Abstract

Isolates of *Hymenoscyphus fraxineus,* the causal agent of ash dieback, were analyzed for growth rates and production of the exoenzymes β-glucosidases, amylases, cellulases, lipases, laccases, phenoloxidases, peroxidases and tyrosinases. None of the 176 isolates from diverse locations in Germany, Poland and Sweden secreted all of these enzymes. Growth rates also varied considerably, under the conditions studied between 0.03 and 0.28 cm / day. Additionally, we found inter- and intrapopulation diversity regarding exoenzyme profiles and growth rates among and between the isolates from the Elm forest and a tree nursery in Ellerhoop, both locations in northern Germany. There were no correlations between number of enzymes an isolate produces and growth rate, nor between either growth rate or number of enzymes and virulence, virulence having been determined in a previous study. These phenotypic results concur with previous genetic analyses that have found significant inter- and intrapopulation variability at the molecular level. Only considerable sexual reproduction can explain these results. We conclude that each isolate of *H. fraxineus* is an individualist.

Keywords: Exoenzymes, growth rate, ash dieback, *Hymenoscyphus fraxineus,* inter- and intrapopulation variation

Introduction

Hymenoscyphus fraxineus is the causal agent of ash dieback (Kowalski 2006, Kowalski and Holdenrieder 2009, Queloz et al. 2011, Gross et al., 2014a) and is endangering the existence of *Fraxinus excelsior* in Europe. The pathogen was first observed in the 1990s in Poland (Kowalski 2006, Kowalski and Holdenrieder, 2009) and has been rapidly spreading across Europe, arriving in Great Britain in 2012 (http://www.forestry.gov.uk/chalara). The pathogen is assumed to be native to Asia (Zhao et al. 2012), where *H. fraxineus* does not cause disease on native species of *Fraxinus* and was probably introduced once by at least two individuals (Gross et al. 2014b). The disease manifests itself in wilting of young shoots, and necroses on leaves, twigs and branches (Gross et al. 2014a). Within the host tissues, the hyphae grow both inter- and intracellularly (Figure 1a and 1b) as previously shown by Schumacher et al. (2009), apothecia developing on the rachises of the previous year's leaves (Figure 1c and 1d). The disease spreads primarily via ascospores (Gross et al. 2014a).

The isolates of *H. fraxineus* can differ considerably in their macroscopic morphologies (Kowalski and Bartnick 2010) and in their molecular identities, analyses having shown significant intra- and interpopulation diversity. Rytkoenen et al. (2012) reported the identities of 14 haplotypes among 32 isolates from three countries, i.e. Finland, Estonia and Latvia. Kraj and Kowalski (2014) and Nguyen et al. (2015) found significant genetic intrapopulation variability among *H. fraxineus* isolates from Poland and Germany, respectively; Haňáčková et al. (2015) also found interpopulation variability between isolates from the Czech Republic, Switzerland and Norway. In the same leaflets of *F. excelsior* from Britain, Cross et al. (2016) found two genets of *H. fraxineus.* The analyses of Bengtsson et al. (2012) revealed that there has been a high degree of gene flow, suggesting that *H. fraxineus* is a sexually out-crossing species.

Virulence factors of phytopathogens include phytotoxins and exoenzymes (Agrios 1997). Viridiol is a phytotoxin of *H. fraxineus* and when applied to leaves of *F. excelsior* causes necroses (Andersson et al 2010, Cleary et al. 2014). The lactone, 3,4 dimethylpentan-4-olid, another phytotoxin of *H. fraxineus*, inhibits germination and causes necroses on germinating seedlings (Citron et al. 2014). In culture, concentrations of viridiol and the lactone vary considerably among the various isolates (Junker et al. 2014; Citron, Junker, Schulz and Dickschat, unpublished). Hymenosetin is an antibacterial toxin that is also only produced by some isolates of *H. fraxineus* (Halecker et al. 2014). Considering the fact that isolates of *H. fraxineus* can vary morphologically and molecularly, as well as in concentrations of the secondary metabolites they produce, we here determined to what extent the growth rates of 176 isolates of *H. fraxineus* differed and if the exoenzyme profiles of the individual isolates of *H. fraxineus* varied. Subsequently, we determined whether there are correlations between exoenzyme profiles and another phenotypic characteristic, i.e. growth rate, but also of exoenzyme profiles and growth rates with virulence factors of *H. fraxineus* (disease symptoms following inoculation into *F. excelsior* and synthesis of the phytotoxin viridiol) as determined in previous research. Additionally, we were interested in determining whether there is inter- and/or intrapopulation variability of exoenzyme profiles and growth rates among isolates of *H. fraxineus*, and in particular among and between those from the locations Ellerhoop and Erkerode.

Material and Methods

Isolates

The 176 strains of *H. fraxineus* used in this study had been isolated from diseased *F. excelsior* growing in various locations in Germany, Sweden and Poland (Supplementary Table 1). Larger numbers of isolates from one single location were either from the Elm forest (23 isolates; 52.204514, 10.719210°) or from a tree nursery in Ellerhoop (100 isolates; Landwirtschaftskammer Schleswig-Holstein; 53.716928, 9.770588°), two locations in northern Germany. C398 – C548 were isolated at the Julius Kühn-Institute Braunschweig and identified by Jörg Schumacher between 2007-2009, with the exception of the three strains from Poland (C428-C430), which were provided by Tadeusz Kowalski and C401 from Sweden, provided by Rimvys Vasaitis (Schumacher et al. 2009). Those from the Elm forest near Erkerode were isolated and identified by Siegfried Draeger and authors of this paper in 2009. The studies reported here were conducted in 2010.

Growth rates

The growth rates of all 176 isolates were determined following inoculation of a culture plug (ca. 0.5 x 0.5 cm) onto potato – carrot agar medium (20 g l^{-1} cooked and mashed potatoes, 20 g l^{-1} cooked and mashed carrots, 12 g l^{-1} agar) and incubation at 20° C for 25 days or until the culture had reached the boundaries of the Petri dish (9 cm). Tests were run in triplicate. The growth rate was determined to be the average of the colony radius (measured from the boundary of the inoculated culture plug) in four directions / number of days of culture, i.e. cm/day.

Exoenzymes

The capability of the isolates to secrete exoenzymes that could be utilized for infection and colonization of *F. excelsior* was analyzed using established methodologies for cellulases, β-glucosidases, amylases, lipases, laccases, phenoloxidases, peroxidases and tyrosinases. Positive controls were *Chaetomium globosum* for cellulases and laccases, *Schizophyllum commune* for β-glucosidases, *Xylaria hypoxylon* for peroxidases, *Phialocephala fortinii* for polyphenoloxidases, *Penicillium chrysogenum* for amylases and *Aspergillus niger* for lipases. The control isolates were from our institute's culture collection and had been isolated and identified by Siegfried Draeger from our institute.

The activities of cellulases and β-glucosidases, both involved in the degradation of cellulose, were determined according to the methods of Thorn (1993) and Ng and Zeikus (1980), and Falbe and Regitz (1996) and Nakatsubo (2001), respectively. Starch is degraded by amylases, the activity of which was determined using a starch-nitrite agar medium according to Krainsky (1914). A medium containing Tween-20 (Sierra, 1957) was used to determine the activity of lipases. Laccases and peroxidases are necessary for the degradation of lignins; the methods of Pointing (2000) were used for measuring their activities. The activities of phenoloxidases and tyrosinases, which are also involved in the degradation of lignin as well as other phenolic compounds, were determined according to Bavendamm (1928) and Lyr (1958), respectively. Tests were run in duplicate. Since the tests were conducted on agar media, an objective quantification of dry weight and activity were not possible. Thus, a color change indicated a positive reaction and the respective exoenzyme was evaluated as active.

Statistical analyses

Normality was tested using a Shapiro-Wilk Test (Shapiro and Wilk, 1965). Since the isolates from Ellerhoop were not normally distributed, a Mann-Whitney U test was used to test for significant differences between growth rates in Erkerode vs. Ellerhoop (Mann and Whitney, 1947). Correlation between number of enzymes and growth rate and of both of these parameters with virulence and viridiol concentration were computed using Spearman's rank correlation coefficient or Spearman's rho, a non-parametric parameter of rank correlation for the ranking of two variables (Spearman, 1904). A perfect Spearman correlation of +1 or −1 occurs when each of the variables is a perfect monotone function of the other. Virulence in seedlings of *F. excelsior* and concentration of viridiol in culture extracts of *H. fraxineus* had been determined previously (Junker et al., 2014). All statistical analyses were performed using R version 3.3.2 (R Core Team 2016).

Results

Growth rates of H. fraxineus have inter- and intrapopulation variability

The growth rates of the *H. fraxineus* isolates varied considerably (Supplementary Table 1), ranging from 0.03 to 0.28 cm / day. Exemplary for the interpopulation variability of growth rates are the differences in growth rates of isolates from Erkerode and from Ellerhoop. Whereas the growth rates of the 23 isolates from the Elm Forest near Erkerode varied between 0.03 and 0.19 cm / day with an average of 0.09 cm / day, the growth rates of the 100 isolates from 3-year old seedlings that had all been isolated from a tree nursery in Ellerhoop, Germany, ranged from 0.05 to 0.28 cm / day with an average of 0.15 cm / day (Supplementary Table 1, Figure 2).

To compare the interpopulation variance between growth rates of *H. fraxineus* isolates from Ellerhoop and those from Erkerode, normality was tested using a Shapiro-Wilk Test (Shapiro and Wilk, 1965). The growth rates of *H. fraxineus* isolated from ash in Erkerode followed a normal distribution (p = 0.3795), while the growth rates of *H. fraxineus* isolated from ash in Ellerhoop did not (p = 0.0008666). We, hence, performed a Mann-Whitney *U* test (Mann and Whitney, 1947) and found that the growth rates of *H. fraxineus* isolates from Ellerhoop (average 0.15 ± 0.06 cm / day) were significantly higher ($p = 1.332 \times 10^{-5}$) than the growth rates of isolates from Erkerode (average 0.09 ± 0.04 cm / day; Figure 2).

Exoenzyme profiles of isolates of H. fraxineus vary

The proportion of the isolates that secreted the eight exoenzymes tested varied between 17 % for cellulases and 98 % for tyrosinases (Figure 3). Lipases (95 %) and β-glucosidases (91 %) were the second and third most frequently secreted enzymes. None of the isolates produced all eight of the tested enzymes. All of the isolates had at least two of the enzymes characteristic for fungi that colonize woody hosts (Dashtban et al 2009; Supplementary Table 1). However, they differed considerably in their individual exoenzyme profiles, as demonstrated by the 100 isolates from Ellerhoop, e.g. the enzyme profiles of C461, C477, C484 and C506 (Figure 4). Whereas, C461 secreted all the enzymes with the exception of peroxidases and C484 had all but peroxidases and lipases, C506 produced only lipases and tyrosinases. Others such as C477 secreted four enzymes – tyrosinases, laccases, lipases and amylases (Figure 4). As exemplified in Figure 4, there was also no obvious link between morphology and exoenzyme profile.

In spite of the fact that lipases, tyrosinases and β-glucosidases were the most frequently secreted enzymes of isolates from both the locations Ellerhoop and Erkerode, the proportion of isolates that secreted the exoenzymes varied between these locations (Figure 5). For example, none of the isolates from Erkerode secreted cellulases in contrast to 15 % of those from Ellerhoop. Whereas only 9 % of the Erkerode isolates secreted polyphenoloxidases, 33 % of those from Ellerhoop did.

Correlations between parameters involved in infection

Table 1 shows the parameters and isolates used to calculate correlation coefficients between growth rates and exoenzyme profiles with virulence of *H. fraxineus*, using data from our previous results (disease symptoms following inoculation of *F. excelsior* with *H. fraxineus* and concentration of viridiol in cultures extracts of *F. excelsior,* Junker et al., 2014)*;* Table 2 shows the calculated Spearman coefficients. With a Spearman coefficient of rho = -0.1935709 and p-value = 0.5466, it is clear that there was no correlation between growth rate and disease symptoms in *F. excelsior* following inoculation with *H. fraxineus.* There was also no correlation between growth rate and synthesis of viridiol by *H. fraxineus,* as shown by a coefficient of rho = -0.01967954 and p-value = 0.9516. Nor was there a correlation between number of enzymes / isolate and virulence, the Spearman coefficient between number of enzymes / isolate and disease symptoms in *F. excelsior* following inoculation with *H. fraxineus* being rho = 0.03194128 and p = = 0.9215, and between number of enzymes / isolate and synthesis of viridiol by *H. fraxineus* being rho = -0.2839943 and p = 0.371.

Discussion and Conclusions

Exoenzymes can be virulence factors of pathogenic fungi, enabling infection and colonization of the plant host (Agrios 1997). As tree pathogens, the isolates of *H. fraxineus* secreted exoenzymes for degrading lignin: 98 %

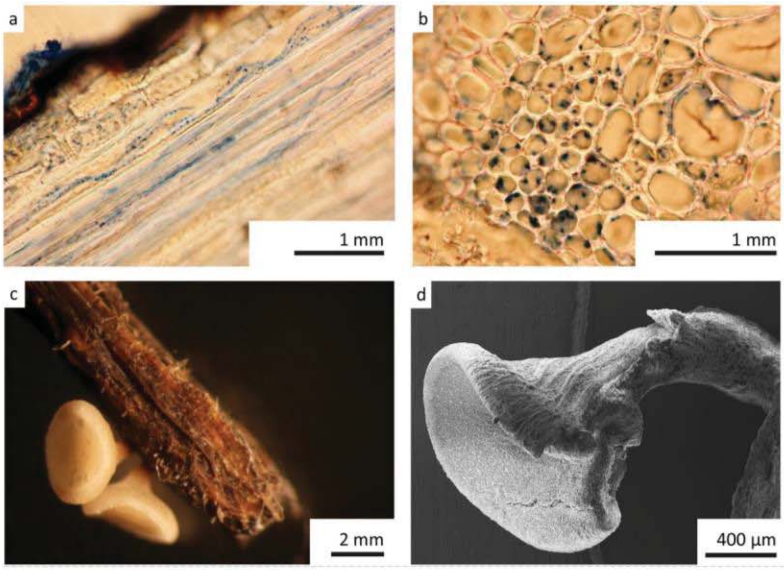

Figure 1. a) and b): inter- and intracellular growth of *Hymenoscyphus fraxineus* in *Fraxinus excelsior,* c) and d): Apothecia of *H. fraxineus* on rachises of *F. excelsior*

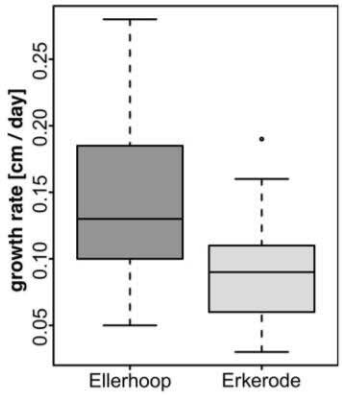

Figure 2. Box-plots displaying the interquartile range (50 ± 25%) of the growth rates of 100 *H. fraxineus* isolates from Ellerhoop and 23 *H. fraxineus* isolates from Erkerode. The thick horizontal lines in each box mark the median (50%) and the whiskers extend to the furthest data points within the 1.5 times interquartile range; the circle above the right box-plot marks the outlier EL 101 with a growth rate of 0.19 cm / day

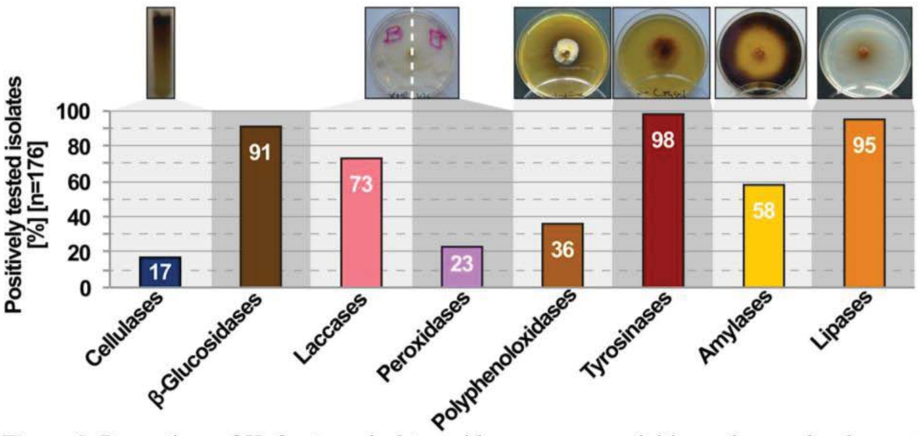

Figure 3. Proportions of *H. fraxineus* isolates with exoenzyme activities and examples demonstrating positive detection of exoenzymes on special media. Culture was for about 21 days at 20°C. Positive reaction (dark brown) with β-glucosidase is not shown

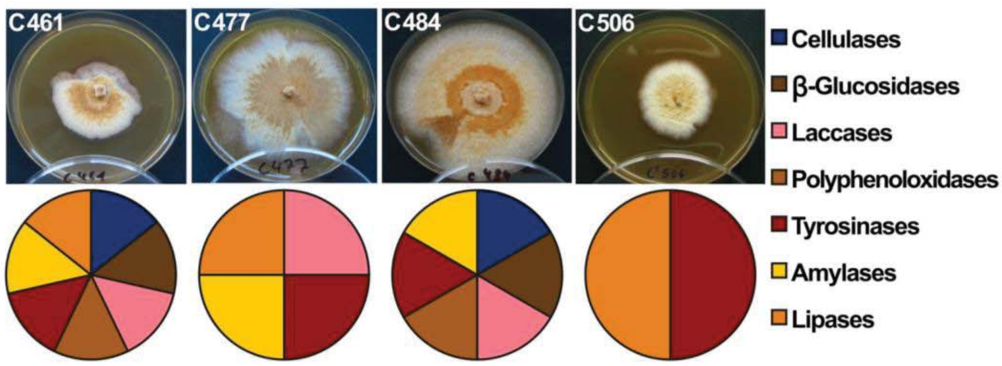

Figure 4. Exoenzyme profiles and morphologies of isolates of *H. fraxineus* demonstrate the diversity of exoenzyme profiles and culture morphology. Exoenzyme activity was evaluated as present or absent. All isolates are from the same origin, i.e. Ellerhoop, Germany. Culture was for two to three weeks at 20°C on biomalt medium.

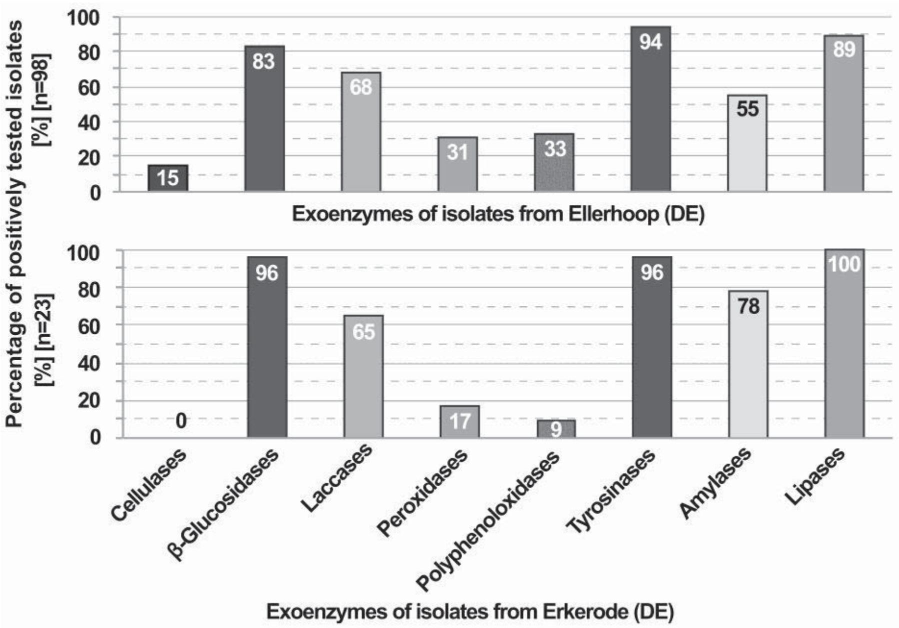

Figure 5. Proportions of *H. fraxineus* isolates isolated from *Fraxinus excelsior* from a tree nursery in Ellerhoop and from the Elm forest near Erkerode that produce each of the tested exoenzymes

Table 1. Strains of *Hymenoscyphus fraxineus* for which Spearman's coefficients were computed for number of enzymes / isolate and growth rate with virulence in seedlings and with concentration of viridiol. For coefficients see Table 2 and the main text

	Enzymes								no. enzymes	Growth rate [ø cm/day]	[1]Virulence in seedlings	[2]Viridiol [µg/mg fungal culture extract]
	Cellulase	β-Glucosidase	Laccase	Peroxidase	Polyphenol-oxidase	Tyrosinase	Amylase	Lipase				
C 403	-	+	+	-	+	+	+	+	6	0.13	2	0
C 411	-	+	+	-	-	+	+	+	5	0.15	1	77.2
C 421	+	+	+	-	-	+	+	+	6	0.09	1	43.5
C 428	-	+	+	-	-	+	+	+	5	0.13	2	2.33
C 429	+	+	+	-	+	+	+	+	7	0.22	1	0
C 444	+	+	-	-	+	+	-	+	6	0.28	2	80.6
C 472	-	+	+	-	+	+	+	+	6	0.23	2	0
C 489	-	-	+	+	+	+	-	+	5	0.08	3	0.02
C 492	-	+	+	-	+	+	+	+	6	0.22	3	0.55
C 502	-	+	+	-	-	+	+	+	5	0.28	0	0
C 509	+	-	+	-	-	+	-	+	4	0.24	2	24.9
EL 120	-	+	+	-	-	+	+	+	5	0.16	1	3.82

Activity of exoenzymes was either present (+) or absent (-).Previously determined (Junker et al., 2014): [1]Virulence of *H. fraxineus* in seedlings of *F. excelsior* (0 = no disease symptoms, 1 = mild disease symptoms, 2 = moderate disease symptoms, 3 = severe disease symptoms) and [2]Concentration of viridiol in culture extracts of *H. fraxineus*

of the isolates produced tyrosinases, 73 % laccases, 36 % polyphenoloxidases and 23 % peroxidases. Most of the isolates (95 %) secreted lipases, which are required for degradation of the leaf cuticle. Lipases could be a necessity for *H. fraxineus*, because initial infection presumably occurs primarily through the leaves and petioles (Cleary et al. 2013, Gross et al. 2014a). Even though most of the isolates secreted tyrosinases and lipases, the enzyme profiles of the individual isolates differed considerably (Supplementary Table 1). It is probable that other enzymes for which we did not test were involved and that isolates such as C506, which only secreted lipases and tyrosinases, attain their nutrients primarily from sugars present in the vascular bundles and might secrete, for example, invertases.

Table 2. Spearman correlation coefficients (rho-value) between number of exoenzymes / isolate and growth rates of *H. fraxineus* and virulence parameters involved in the infection of *F. excelsior* by *H. fraxineus*

Parameter	Growth rate	[1]Virulence: Infection ash seedlings	[1]Viridiol concentration
Number of exoenzymes	0.01895	0.03194128	-0.2839943
Growth rate	n.a.	-0.1935709	-0.01967954

Number of exoenzymes was for the individual isolates.
[1]Previously determined for isolates of *H. fraxineus* (Junker et al., 2014): Virulence evaluated as disease symptoms following inoculation of axenically cultured ash seedlings with isolates of *H. fraxineus,* and concentration of viridiol in culture extracts of *H. fraxineus.* n.a.=not applicable.

Fungi that grow predominantly in the apoplast can survive only on the nutrients present there (Boyle et al. 2001).

With respect to growth rate, our results corroborate with those of previous studies, (Kowalski 2006, Kowalski and Bartnik 2010, Kirisits et al. 2013). The growth rates of the 176 isolates tested here at 20° C on potato – carrot medium averaged 0.14 cm / day, though with considerable variation, i.e. between 0.03 and 0.28 cm / day, showing that the range of growth rates is even broader than previously measured. Kirisits et al. (2013) measured the growth rates of four isolates of *H. fraxineus* at 20° C, though on a malt extract medium. Nevertheless, although their measured growth rates were relatively low in comparison to ours, i.e. between 0.03 and 0.08 cm / day with an average of 0.05 cm / day, their values fit within the range that we measured and might have covered a broader range if they had included more isolates.

The production of a broad spectrum of exoenzymes should enable degradation of a broad range of nutrients and thus improve growth on complex substrates. Indeed, in the case of the four exemplary isolates (Figure 4) such a correlation is suggested. On potato – carrot medium, the growth rate of C461 with seven exoenzymes was fastest at 0.23 cm / day, that of C484 which produces six exoenzymes was 0.17 cm / day, that of C477 with four exoenzymes was 0.11 cm / day and that of C506 with only two exoenzymes was slowest with 0.05 cm / day. We, however, found that the number of exoenzymes per isolate did not correlate with growth rate ($\rho = 0.01895$). This fact is underscored by counter-examples of isolates that produce seven of the eight tested exoenzymes with growth rates of only 0.07 cm / day (e.g. C423, C532; Supplementary Table 1). In contrast, C409 produced only two of the exoenzymes, but had a growth rate of 0.20 cm / day. Thus, the results of this study revealed no correlation between number of exoenzymes an isolate secretes and growth rate.

Gross et al. (2014a) suggested that there may be a correlation between growth rate and virulence, the slower growing isolates being more virulent. However, we found no correlation between growth rate with our previous results regarding virulence, i.e. disease symptoms following inoculation into axenically cultured *F. excelsior* seedlings and synthesis of the phytotoxin viridiol by *H. fraxineus*.

Of interest is the finding that the two populations from the Elm forest near Erkerode and the tree nursery in Ellerhoop differ both in their growth rates and in their exoenzyme profiles. For example, none of the 23 isolates from Erkerode produced cellulase. And, the average growth rate of those from Erkerode was significantly lower than that of the isolates from Ellerhoop (Figure 2). Thus, there is interpopulation variation between the isolates from Erkerode and Ellerhoop, both located in northern Germany. Nevertheless, there is diversity within each of these populations. For example, not all of the isolates from Ellerhoop produced viridiol and not all of them were pathogenic when inoculated into axenically cultured seedlings of *F. excelsior* (Junker et al. 2014; Table 1). Junker et al. (2014) had also previously shown that there is no correlation between virulence and concentration of viridiol in the culture extract.

Previously, it was known that isolates of *H. fraxineus* can vary in morphology (Kowalski, 2006, Kowalski and Bartnik 2010), growth rate (Kowalski and Bartnik 2010, Kirisits et al. 2013, Gross et al. 2014a), virulence (Junker et al. 2014), and synthesis of phytotoxins and secondary metabolites, e.g. viridiol (Junker et al. 2014), hymenosetin (Halecker et al. 2014, Halecker, personal communication), and the lactone, 3,4 dimethylpentan-4-olid (Citron et al. unpublished). Results presented here revealed that there is also great variability in the individual exoenzyme profiles, providing further evidence for the uniqueness of each of the 176 isolates of *H fraxineus*. Additionally, we have shown that there is no correlation between number of exoenzymes / isolate and growth rate, between number of exoenzymes / isolate and the two parameters for virulence, and between growth rate and the two parameters for virulence (virulence as determined by Junker et al., 2014; Table 2).

Our results demonstrate diversity of the studied phenotypic parameters for individual isolates, but also inter- and intrapopulation variability. Only considerable sexual reproduction can explain these results, corroborating with genetic analyses that have found significant inter- and intrapopulation variability at the molecular level (Bengtsson et al. 2012, Rytkoenen 2011, Kraj and Kowalski 2014, Nguyen 2015, Haňáčková et al. 2015, Cross et al., 2016). These findings underpin the necessity to study isolates both from diverse as well as from confined sampling sites in future studies. It will be a challenge to recognize whether certain phenotypic characteristics other than those studied here and / or molecular markers are correlated with virulence. Whether or not such correlations are found, on the basis of phenotypic diversity we can nevertheless conclude: Each isolate of *H. fraxineus* is an individualist.

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References

Agrios, G.H. 1997. Plant Pathology, Academic Press, San Diego.

Andersson, P.F., Johansson, S.B.K, Stenlid, J. and Broberg, A. 2010. Isolation, identificationand necrotic activity of viridiol from *Chalara fraxinea,* the fungus responsible for dieback of ash. *Forest Pathology* 40: 43-46.

Bavendamm, W. 1928.Über das Vorkommen und den Nachweis von Oxydasen bei holzzerstoerenden Pilzen. *Zeitschrift fuer Pflanzenschutz* 38: 257-276.

Bengtsson, S.B.K., Vasaitis, R., Kirisits, T. Solheim, H. and Stenlid, J. 2012. Population structure of *Hymenoscyphus pseudoalbidus* and its genetic relationship to *Hymenoscyphus albidus. Fungal Ecology* 5: 147-153.

Boyle, C., Goetz, M., Dammann-Tugend, U. and Schulz, B. 2001. Endophyte - host interactions III. Local vs. systemic colonization. *Symbiosis* 31: 259-281.

Citron, C.A., Junker, C., Schulz, B.and Dickschat, J.S. 2014. A volatile lactone of *Hymenoscyphus pseudoalbidus,* pathogen of European ash dieback, inhibits host germination. *Angewandte Chemie International Edition* 53: 4346-4349.

Cleary, M.R., Andersson, P.F., Broberg, A., Elfstrand, M., Daniel, G. and Stenlid, J. 2014. Genotypes of *Fraxinus excelsior* with different susceptibility to the ash dieback pathogen *Hymenoscyphus pseuoalbidus* and their respone to the phytotoxins viridiol – A metabolomic and microscopic study. *Phytochemistry* 102: 115-125.

Cleary, M.R., Daniel, G. and Stenlid, J. 2013. Light and scanning electron microscopy studies of the early infection stages of *Hymenoscyphus pseudoalbidus* on *Fraxinus excelsior. Plant Pathology* 62: 1294-1301.

Cross, H., Sǿnstebǿ, J.H., Nagy, N.E., Timmermann, V., Solheim, H., Bǿrja, I., Kauserud, H., Carlsen, T., Rzepka, B., Wasak, K., Vivian-Smith, A. and Hietala, A.M. 2016. Fungal diversity and seasonal succession in ash leaves infected by the invasive ascomycete *Hymenoscyphus fraxineus. New Phytologist*: doi: 10.1111/nph.14204.

Dashtban, M., Schraft, H. and Qin, W. 2009. Fungal bioconversion of lignocellulosic residues; Opportunities and perspectives. *International Journal of Biological Science* 5(6):578-595.

Falbe, J. and Regitz, J. 1996. *Roempp Chemie Lexikon, Thieme Verlag.*

Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V., and Sieber, T.K. 2014a. *Hymenoscyphus pseudoalbidus,* the causal agent of European ash dieback. *Molecular Plant Pathology* 15: 5-21.

Gross, A., Hosoya, T. and Queloz, V. 2014b. Population structure of the invasive forest pathogen *Hymenoscyphus pseudoalbidus. Molecular Ecology* 23: 2943-2960.

Halecker, S., Surup, F., Kuhnert, E., Mohr, K.I., Brock, N.L., Dickschat, J.S., Junker, C., Schulz, B. and Stadler, M. 2014. Hymenosetin, a 3-decalinoyltetramic acid antibiotic from cultures of the ash dieback pathogen, *Hymenoscyphus pseudoalbidus. Phytochemistry* 100: 86-91.

Haňáčková, Z., Koukol, O., Hardova, L. and Gross, A. 2015. Local population structure of *Hymenoscyphus fraxineus* surveyed by an enlarged set of microsatellite markers. *Forest Pathology* 45: 400-407.

Junker, C., Mandey, F., Pais, A., Ebel, R. and Schulz, B. 2014. *Hymenoscyphus pseudoalbidus* and *Hymenoscyphus albidus:* viridiol concentration and virulence do not correlate. *Forest Pathology* 44: 39-44.

Kirisits, T., Daempfle, L. and Kraeutler, K. 2013. *Hymenoscyphus albidus* is not associated with an anamorphic stage and displays slower growth than *Hymenoscyphus pseudoalbidus* on agar media. *Forest Pathology* 43: 386-389.

Kowalski, T. 2006. *Chalara fraxinea* sp. nov. associated with dieback of ash (*Fraxinus excelsior*) in Poland. *Forest Pathology* 36: 64-270.

Kowalski, T. and Bartnick, C. 2010. Morphological variation in colonies of *Chalara fraxinea* isolate from ash (*Fraxinus excelsior* L.) stems with symptoms of dieback and effects of temperature on colony growth and structure. *Acta Agrobotanica* 63: 99-106.

Kowalski, T. and Holdenrieder, O. 2009. Pathogenicity of *Chalara fraxinea. Forest Pathology* 39: 1-7.

Krainsky, A. 1914. Die Aktinomyceten und ihre Bedeutung in der Natur. *Zentralblatt für Bakteriologie, Parasitenkunde und Hygiene. Abteilung II* 41: 649-688.

Kraj, W. and Kowalski, T. 2014. Genetic variability of *Hymenoscyphus pseudoalbidus* on ash leaf rachises in leaf litter of forest stands in Poland. *Journal of Phytopathology* 162: 218-227.

Lyr, H. 1958. Über den Nachweis von Oxydasen und Peroxydasen bei hoeheren Pilzen und die Bedeutung dieser Enzyme für die Bavendamm-Reaktion. *Planta* 50: 359-370.

Mann, H.B. and Whitney, D.R. 1947. On a test of whether one of two random variables is stochastically larger than the other. *Annals of Mathematical Statistics* 1: 50–60.

Nakatsubo, F. 2001. Chemical synthesis of cellulose. In: Hon, D.N.S. and Shiraishi, N. (Editors) Wood and Cellulosic Chemistry (2nd Edition). Marcel Dekker, Inc. New York, pp. 627-654.

Ng, T.K. and Zeikus, J.G. 1980. A continuous spectrophotometric assay for the determination of cellulase solubilizing activity. *Analytical Biochemistry* 102: 42-50.

Nguyen, D.T., Cleary, M.R., Enderle, R., Berlin, A. and Stenlid, J. 2015. Analyses of the ash dieback pathogen, *Hymenoscyphus fraxineus,* suggest role of tree species diversity on colonization and population structure differentiation. *Forest Pathology* doi: 10.1111. efp.12236

Pointing, S.B., Jones, E.B.G. and Vrijmoed, L.L.P. 2000. Optimization of laccase production by *Pycnoporus sanguineus* in submerged culture. *Mycologia* 92: 139-144.

Queloz, V., Grünig, C.R., Berndt, R., Kowalski, T., Sieber, T.N. and Holdenrieder, O. 2011. Cryptic speciation in *Hymenoscyphus albidus. Forest Pathology* 41: 133-142.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rytkoenen, A., Lilja, A., Drenkhan, R., Gaitnieks, T. and Hantula, J. 2011. First reconrd of *Chalara fraxinea* in Finland and genetic variation among isolates sampled from Åland, mainland Finland, Estonia and Latvia. *Forest Pathology* 41: 169-174.

Shapiro, S.S. and Wilk, M.B. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52: 591–611

Schumacher, J., Kehr, R. and Leonhard, S. 2009. Mycological and histological investigations of *Fraxinus excelsior* nursery saplings naturally infected by *Chalara fraxinea. Forest Pathology* 40: 419-429.

Sierra, G. 1957. A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of contact between cells and fatty substrates. *Antonie van Leeuwenhoek* 23: 15-22.

Spearman, C. 1904. The proof and measurement of association between two things. *American Journal of Psychology* **15**: 72–101.

Thorn, G. 1993. The use of cellulose azure agar as a crude assay of both cellulolytic and ligninolytic abilities of wood inhabiting fungi. *Proceedings of the Japanese Academy of Science* 69: 29-34.

Zhao, Y.-J., Hosoya, T., Baral, H.-O., Hosaka, K. and Kakishima, M. 2012. *Hymenoscyphus pseudoalbidus* is the correct name for *Lambertella albida* reported from Japan. *Mycotaxon* 122: 25-41.

Supplementary material

Supplementary Table 1. Strains of *Hymenoscyphus fraxineus* with their origins, exoenzymes and growth rates. Activity of exoenzymes was either present (+) or absent (-). The isolates were from Germany, unless otherwise noted

Strain no.	Origin			Identified by	Enzymes								Growth rate [ø cm / day]
					Cellulase	β-Glucosidase	Laccase	Peroxidase	Polyphenol-oxidase	Tyrosinase	Amylase	Lipase	
C 398	Hamburg	2007	*F. excelsior* (received from the University of Hamburg)	J. Schumacher	-	+	+	+	+	+	-	+	0.14
C 399	Hamburg	2007	*F. excelsior* (received from the University of Hamburg)	J. Schumacher	+	+	+	-	-	+	-	-	0.14
C 400	Hamburg	2007	*F. excelsior* (received from the University of Hamburg)	J. Schumacher	-	+	-	-	+	+	-	+	0.27
C 401	Uppsala, Sweden	2007	*F. excelsior*	R. Vasaitis	-	+	-	-	-	+	+	+	0.15
C 402	Hamburg	2007	*F. angustifolia*, shoot	G. Hilfert	+	+	+	-	-	+	+	+	0.14
C 403	Hamburg	2007	*F. angustifolia*, shoot	J. Schumacher	-	+	+	-	+	+	+	+	0.13
C 404	Tree nursery Grelck, Haltenbeck, Pinneberg	2007	*F. excelsior*, 3 year old seedlings 1/2	J. Schumacher	-	+	+	-	-	+	-	+	0.16
C 405	Tree nursery Grelck, Haltenbeck, Pinneberg	2007	*F. excelsior*, 3 year old seedlings 1/2	J. Schumacher	-	+	+	-	+	+	-	+	0.21
C 406	Tree nursery Grelck, Haltenbeck, Pinneberg	2007	*F. excelsior*, 3 year old seedlings 1/2	J. Schumacher	-	+	+	-	-	+	+	-	0.08

Supplementary Table 1. (Continued)

Strain no.	Origin			Identified by	Enzymes								Growth rate [ø cm / day]
					Cellulase	β-Glucosidase	Laccase	Peroxidase	Polyphenol-oxidase	Tyrosinase	Amylase	Lipase	
C 407	Tree nursery Grelck, Haltenbeck, Pinneberg	2007	*F. excelsior*, 3 year old seedlings 1/2	J. Schumacher	-	+	-	+	-	+	-	-	0.16
C 408	Uelzen	2007	*F. excelsior*, branch with necroses	J. Schumacher	-	+	-	-	-	+	+	+	0.15
C 409	Uelzen	2007	*F. excelsior*, branch with necroses	J. Schumacher	-	-	-	-	-	+	-	+	0.20
C 410	Uelzen	2007	*F. excelsior*, branch with necroses	J. Schumacher	-	+	+	-	-	+	+	+	0.13
C 411	Uelzen	2007	*F. excelsior*, branch with necroses	J. Schumacher	-	+	+	-	-	+	+	+	0.15
C 412	Uelzen	2007	*F. excelsior*, branch with necroses	J. Schumacher	+	+	+	-	-	+	-	-	0.16
C 413	Uelzen	2007	*F. excelsior*, branch with necroses	J. Schumacher	-	+	+	-	-	+	+	+	0.11
C 414	Uelzen	2007	*F. excelsior*, branch with necroses	J. Schumacher	-	+	-	-	+	+	-	+	0.20
C 415	Uelzen	2007	*F. excelsior*, branch with necroses	J. Schumacher	-	+	+	-	+	+	-	+	0.05
C 416	Uelzen	2007	*F. angustifolia*, branch with necroses	J. Schumacher	+	-	-	-	+	+	-	+	0.17
C 417	Uelzen	2007	*F. angustifolia*, branch with necroses	J. Schumacher	+	+	+	-	+	+	-	+	0.18
C 418	Uelzen	2007	*F. angustifolia*, branch with necroses	J. Schumacher	-	+	+	-	+	+	+	+	0.23
C 419	Uelzen	2007	*F. angustifolia*, branch with necroses	J. Schumacher	-	+	+	-	+	+	-	+	0.16
C 420	Seeth Ekholt, Pinneberg	2008	*F. excelsior*, 1 year old seedling, shoot - transition brown to green, necroses	J. Schumacher	+	+	+	-	+	+	-	+	0.11
C 421	Seeth Ekholt, Pinneberg	2008	*F. excelsior*, 1 year old seedling, shoot - transition brown to green, necroses	J. Schumacher	+	+	+	-	-	+	+	+	0.09
C 422	Nursery Torsten Perrau, Rellingen	2008	*F. excelsior*, 1 year old seedling, shoot - transition brown to green, necroses	J. Schumacher	-	+	-	-	+	+	-	+	0.12
C 423	Nursery Torsten Perrau, Rellingen	2008	*F. excelsior*, 1 year old seedling, shoot - transition brown to green, necroses	J. Schumacher	-	+	+	+	+	+	+	+	0.07
C 424	Nursery Torsten Perrau, Rellingen	2008	*F. excelsior*, 1 year old seedling, thin brown shoot, necroses	J. Schumacher	-	+	+	-	-	+	-	+	0.13
C 425	Nursery Torsten Perrau, Rellingen	2008	*F. excelsior*, 1 year old seedling, thin brown shoot, necroses	J. Schumacher	-	+	+	-	-	+	+	+	0.17
C 426	Nursery Torsten Perrau, Rellingen	2008	*F. excelsior*, 4 year old seedling, brown wood after removal of bark	J. Schumacher	-	+	+	-	+	+	-	+	0.16

Supplementary Table 1. (Continued)

Strain no.	Origin			Identified by	Enzymes								Growth rate [ø cm / day]
					Cellulase	β-Glucosidase	Laccase	Peroxidase	Polyphenol-oxidase	Tyrosinase	Amylase	Lipase	
C 427	Nursery Torsten Perrau, Rellingen	2008	*F. excelsior*, 4 year old seedling, brown wood after removal of bark	J. Schumacher	-	+	+	-	-	+	+	+	0.13
C 428	Eastern Poland, Forest district Lublin, Forestry Mircze	2006	*F. excelsior*, necroses	J. Schumacher	-	+	+	-	-	+	+	+	0.13
C 429	Eastern Poland, Forest district Lublin, Forestry Mircze	2006	*F. excelsior*, necroses	T. Kowalski	+	+	+	-	+	+	+	+	0.22
C 430	Eastern Poland, Forest district Lublin, Forestry Mircze	2006	*F. excelsior*, necroses	J. Schumacher	-	+	+	-	-	+	+	+	0.05
C 431	Tree nursery Albert Brandt, Prisdorf, Hamburg	2008	*F. excelsior*, young seedlings	J. Schumacher	-	+	+	-	-	+	-	+	0.12
C 432	Ölper forest, Braunschweig	2008	*F. excelsior*, natural regeneration	J. Schumacher	-	+	+	+	+	+	+	+	0.24
C 433	Ölper forest, Braunschweig	2008	*F. excelsior*, natural regeneration	J. Schumacher	+	+	+	-	+	+	-	+	0.13
C 434	Elm Forest, near Königslutter	2008	*F. excelsior*, plantation	J. Schumacher	-	+	+	-	-	+	+	+	0.10
C 435	Elm Forest, near Königslutter	2008	*F. excelsior*, plantation	J. Schumacher	+	+	+	-	-	+	+	+	0.11
C 436	Richtenberg near Rostock	2008	*F. excelsior*	J. Schumacher	-	+	+	-	+	+	-	+	0.23
C 437	Richtenberg near Rostock	2008	*F. excelsior*	J. Schumacher	-	+	-	-	+	+	+	+	0.25
C 438	Hamburg (Billwerder)	2008	*Fraxinus* sp.	J. Schumacher	-	+	+	-	+	+	-	-	0.13
C 439	Berlin	2008	*F. excelsior*	J. Schumacher	-	+	-	+	-	+	-	-	0.22
C 441	Haiming, SE-Baveria	2008	*F. excelsior*, young trees	J. Schumacher	-	+	+	-	+	+	-	+	0.19
C 442	Haiming, SE-Baveria	2008	*F. excelsior*, young trees	J. Schumacher	+	+	+	-	+	+	-	+	0.10
C 443	Haiming, SE-Baveria	2008	*F. excelsior*, young trees	J. Schumacher	-	+	+	-	+	+	-	+	0.21
C 444	Tree nursery Hobohm, Nauen	2009	*F. excelsior*, "Westhof's Glory", grafting of a piece of stem	J. Schumacher	+	+	-	-	+	+	-	+	0.28
C 445	Tree nursery Hobohm, Nauen	2009	*F. excelsior*, young seedling, shoot tip, necrotic	J. Schumacher	-	+	+	-	+	+	-	+	0.15
C 446	Nauen, Am Kuhdamm	2009	*F. excelsior*, old tree population, sucker	J. Schumacher	-	+	+	-	-	+	+	-	0.19
C 447	Nauen, An den Rohrwiesen, Havelland	2009	*F. excelsior* "Westhof's Glory", 3 year old graft, necroses of cortex and sapwood	J. Schumacher	-	+	+	-	-	+	+	+	0.15
C 448	Cottbus	2008	*F. excelsior* "Pendula"	J. Schumacher	-	+	+	-	-	+	+	+	0.05

Supplementary Table 1. (Continued)

Strain no.	Origin			Identified by	Enzymes								Growth rate [ø cm / day]
					Cellulase	β-Glucosidase	Laccase	Peroxidase	Polyphenol-oxidase	Tyrosinase	Amylase	Lipase	
C 449	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings from a tree nursery	J. Schumacher	-	+	-	+	+	+	-	+	0.23
C 450	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	-	+	0.12
C 451	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings from a tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.13
C 452	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	-	+	0.12
C 453	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	+	+	-	+	+	+	0.07
C 454	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	+	+	-	+	0.23
C 455	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	-	+	0.17
C 456	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	-	+	-	+	0.15
C 457	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	-	-	-	+	-	+	0.20
C 458	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	-	-	-	+	-	-	0.07
C 459	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	+	-	-	+	+	+	0.13
C 460	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.16
C 461	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	+	-	+	+	+	+	0.23
C 462	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	-	+	0.12
C 463	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.18
C 464	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	+	+	+	-	+	0.14
C 465	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	-	-	+	+	+	+	0.20
C 466	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	-	+	0.09
C 467	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	+	-	+	+	+	+	0.11
C 468	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	+	+	0.21
C 469	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	-	+	-	+	0.12
C 470	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	+	+	-	+	0.17
C 471	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	-	+	0.13
C 472	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	+	+	0.23

Supplementary Table 1. (Continued)

Strain no.	Origin			Identified by	Enzymes								Growth rate [ø cm / day]
					Cellulase	β-Glucosidase	Laccase	Peroxidase	Polyphenol-oxidase	Tyrosinase	Amylase	Lipase	
C 473	Ellerhoop	2008	*F. excelsior*3 year old seedlings, tree nursery	J. Schumacher	-	-	-	-	+	+	-	-	0.13
C 474	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	+	+	-	+	+	+	0.18
C 475	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	-	+	0.25
C 476	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.13
C 477	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	+	-	-	+	+	+	0.11
C 478	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	-	+	0.12
C 479	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	+	-	+	-	+	0.07
C 480	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	-	-	+	-	+	-	+	0.24
C 481	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	+	-	+	+	+	+	0.15
C 482	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	+	-	+	-	+	0.10
C 483	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	+	-	-	+	-	+	0.11
C 484	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	+	-	+	+	+	-	0.17
C 485	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	+	-	-	+	-	+	0.10
C 486	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	-	-	-	+	+	+	0.07
C 487	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	-	+	+	+	-	+	0.22
C 488	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	+	+	-	+	+	+	0.16
C 489	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	+	+	+	+	-	+	0.08
C 490	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	-	+	0.09
C 491	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	-	+	0.24
C 492	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	+	+	0.22
C 493	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	+	-	-	+	-	+	0.12
C 494	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	+	+	-	-	+	+	0.08
C 495	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	-	+	0.17
C 496	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	-	+	+	+	0.07
C 497	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	+	+	+	+	+	0.28

Supplementary Table 1. (Continued)

Strain no.	Origin			Identified by	Enzymes								Growth rate [ø cm / day]
					Cellulase	β-Glucosidase	Laccase	Peroxidase	Polyphenol-oxidase	Tyrosinase	Amylase	Lipase	
C 498	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	-	+	0.21
C 499	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	-	+	-	+	0.18
C 500	Ellerhoop	2008	*F. excelsior*, forest plantation	J. Schumacher	-	+	-	-	-	+	-	+	0.14
C 501	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	+	-	+	-	-	+	0.18
C 502	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.28
C 503	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	-	+	0.22
C 504	Ellerhoop	2008	F. excelsior, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	-	+	0.16
C 505	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	-	+	0.12
C 506	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	-	-	-	+	-	+	0.05
C 507	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	+	+	-	+	+	+	0.15
C 508	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	-	+	+	+	0.27
C 509	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	-	+	-	-	+	-	+	0.24
C 510	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	+	-	+	+	+	+	0.12
C 511	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.12
C 512	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	-	+	+	+	0.27
C 513	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	-	+	+	0.11
C 514	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	-	+	0.19
C 515	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	+	+	-	-	0.06
C 516	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	-	+	+	+	0.09
C 517	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.12
C 518	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	-	+	+	+	0.12
C 519	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	-	+	+	+	0.12
C 520	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.09
C 521	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	-	+	+	+	0.20
C 522	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	-	+	+	0.12

Supplementary Table 1. (Continued)

Strain no.	Origin			Identified by	Enzymes								Growth rate [ø cm / day]
					Cellulase	β-Glucosidase	Laccase	Peroxidase	Polyphenol-oxidase	Tyrosinase	Amylase	Lipase	
C 523	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	-	-	-	+	0.18
C 524	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	-	+	+	-	0.15
C 525	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	-	+	-	+	+	+	0.10
C 526	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	+	+	+	+	0.10
C 527	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	-	-	-	-	0.24
C 528	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.08
C 529	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	+	+	-	+	+	-	0.13
C 530	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	-	+	+	-	0.08
C 531	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	-	+	-	+	0.13
C 532	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	+	+	+	+	0.07
C 533	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	-	+	+	+	0.07
C 534	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	+	-	+	-	+	0.18
C 535	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	+	+	0.09
C 536	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	-	0.25
C 537	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	-	-	-	-	+	+	+	-
C 538	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.14
C 539	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	-	0.10
C 540	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	-	+	+	-	0.13
C 541	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	+	-	+	+	+	0.22
C 542	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	-	+	+	+	0.17
C 543	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	+	+	-	+	+	+	+	+	0.18
C 544	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	-	-	+	+	+	+	0.14
C 545	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	+	+	+	-	+	0.08
C 546	Ellerhoop	2008	*F. excelsior*, 3 year old seedlings, tree nursery	J. Schumacher	-	+	+	-	+	+	+	+	0.13
C 547	Tree nursery Gera, Auenboden	2009	*F. excelsior*, 2-3 year old seedling	J. Schumacher	+	+	+	-	-	+	-	+	0.20

Supplementary Table 1. (Continued)

Strain no.	Origin			Identified by	Enzymes									Growth rate [ø cm / day]
					Cellulase	β-Glucosidase	Laccase	Peroxidase	Polyphenol-oxidase	Tyrosinase	Amylase	Lipase		
C 548	Tree nursery Gera, Auenboden	2009	*F. excelsior*, 2-3 year old seedling	J. Schumacher	-	+	+	-	-	+	+	+		0.25
Es 89a	Ellerhoop	2009	*F. excelsior,* seedling, tree nursery	S. Draeger	-	+	-	+	-	+	+	+		0.21
Es 60c	Ellerhoop	2009	*F. excelsior,* seedling, tree nursery	S. Draeger	-	+	+	-	+	+	-	+		0.09
Es 20d	Ellerhoop	2009	*F. excelsior,* seedling, tree nursery	S. Draeger	-	+	+	-	-	+	-	+		0.07
EL 100	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	-	+	-	-	+	-	+		0.13
EL 101	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	+	+		0.19
EL 102	Elm Forest, near Erkerode	2009	*F. excelsior*, , sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	-	-	-	+	+	+		0.06
EL 103	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	-	-	+	+	+	+		0.13
EL 104	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	-	-	-	+	+	+		0.06
EL 105	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	+	+		0.03
EL 106	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	+	+	+	+	+		0.07
EL 107	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	-	+		0.08
EL 108	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue e	S. Draeger	-	+	-	+	-	+	+	+		0.13
EL 109	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	+	-	-	+	+		0.09
EL 110	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	-	-	-	+	+	+		0.11
EL 111	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	-	+		0.03
EL 112	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	-	+		0.09
EL 113	Elm Forest, near Erkerode	2009	*F. excelsior* sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	-	-	-	+	+	+		0.09

Supplementary Table 1. (Continued)

Strain no.	Origin			Identified by	Enzymes								Growth rate [ø cm / day]
					Cellulase	β-Glucosidase	Laccase	Peroxidase	Polyphenol-oxidase	Tyrosinase	Amylase	Lipase	
EL 114	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	-	-	-	+	+	+	0.08
EL 115	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	+	+	0.05
EL 116	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	+	+	0.08
EL 117	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	+	+	0.04
EL 118	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	+	-	+	+	+	0.09
EL 119	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	-	-	-	+	+	+	0.06
EL 120	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	+	+	0.16
EL 121	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	+	+	0.10
EL 122	Elm Forest, near Erkerode	2009	*F. excelsior*, sapwood from transition healthy to necrotic tissue	S. Draeger	-	+	+	-	-	+	-	+	0.11