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Originalarbeit

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New disease on red flowering horse chestnut (*Aesculus* x *carnea* Hayne) and the molecular identification of the involved pathogens

Neue Krankheit der Rotblühenden Rosskastanie (*Aesculus* x *carnea* Hayne) und molekulare Identifizierung der beteiligten Schadorganismen

Abstract

A so far unknown disease which is found on red flowering horse chestnut (*Aesculus* × *carnea* Hayne) in the north-west of Germany has been investigated. It is characterized by the presence of fruiting bodies of *Flammulina velutipes* (Curtis) Singer and *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer, able to cause breakage of branches and stems by white rot. Additional infection by *Pseudomonas syringae* pv. *aesculi*, and cracks of the bark are found.

To get detailed knowledge of the disease, samples were taken from cross sections of four decayed red flowering horse chestnut stems. The investigation of individual tissues across the stem enables the assignment of the pathogens to the new disease. Sequencing of the rDNA-ITS identified *F. velutipes* and *P. ostreatus*, *Bjerkandera adusta* (Willd. ex Fr.) Karst. and *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar, four white rot fungi, as main pathogens and some ascomycetes in the infected and decaying stem tissues.

Our studies revealed the co-appearance of different basidiomycetes and ascomycetes in decaying tissue zones which emphasizes the complexity of the disease. It is also the first proof of involvement of *F. velutipes* as a parasite on red flowering horse chestnut. In addition, for the first time, *Pseudomonas syringae* pv. *aesculi* was detected in the inner bark and within the secondary xylem of natural infected red flowering horse chestnut.

Key words: *Aesculus* × *carnea*, new disease, *Pseudomonas syringae* pv. *aesculi*, *Flammulina velutipes*, *Pleurotus ostreatus*, *Bjerkandera adusta*, *Chondrostereum purpureum*, ascomycetes, rDNA-ITS

Zusammenfassung

In dieser Studie wurde eine in Nord-West Deutschland bisher nicht bekannte Krankheit der Rotblühenden Rosskastanie (*Aesculus* × *carnea* Hayne) untersucht. Die Krankheit ist durch Fruchtkörper der Weißfäulepilze *Flammulina velutipes* (Curtis) Singer und *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer, die Stämmlings- und Astabbrüche verursachen, einer Infektion mit *Pseudomonas syringae* pv. *aesculi* und Rindenrisse charakterisiert. Um die Hauptverursacher der Krankheit zu identifizieren, wurden vier Stammscheiben von zwei erkrankten Rotblühenden Rosskastanien untersucht und über den Querschnitt in Probenbereiche aufgeteilt. Die Untersuchung der einzelnen Gewebebereiche ermöglicht es, die Schadorganismen ihrer Rolle im Schadverlauf zuzuordnen. Durch die Sequenzierung der ITS Region der rDNA wurden vier Weißfäulepilze *F. velutipes*, *P. ostreatus*, sowie *Bjerkandera adusta* (Willd. ex Fr.) Karst. und *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar und einige Ascomyceten identifiziert. Die Verteilung der Pilze über den Querschnitt der Stammscheiben lässt es zu, diese als die Hauptverursacher

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des Schadens zu charakterisieren und diesen als Komplexkrankheit zu beschreiben.

In dieser Studie gelang der Nachweis von mehreren Basidiomyceten in einem Gewebe, was die Komplexität der Krankheit unterstreicht. Zudem wird *F. velutipes* zum ersten Mal molekular als Parasit an der Rotblühenden Rosskastanie beschrieben und *Pseudomonas syringae* pv. *aesculi* erstmals sowohl im inneren Phloem als auch im sekundären Xylem von natürlich infizierten Rotblühenden Rosskastanien nachgewiesen.

Stichwörter: *Aesculus* × *carnea*, neue Krankheit, *Pseudomonas syringae* pv. *aesculi*, *Flammulina velutipes*, *Pleurotus ostreatus*, *Bjerkandera adusta*, *Chondrostereum purpureum*, Ascomyceten, rDNA-ITS

Introduction

In autumn and winter 2011/2012, a new disease characterized by fruiting bodies of *Flammulina velutipes* (Curtis) Singer and *Pleurotus ostreatus* (Jacq. ex Fr.) Kummer and other fungi has been found on the stem and in the crown of horse chestnut (*Aesculus* sp.) in several locations in north-western Germany. Such a cumulative occurrence of various fungi on living horse chestnut has not been observed before (Gaiser, 2012). Other symptoms as cracks in the bark, breakage and dieback of branches and stems conditioned from white rot on trees with such infestation and an infection with *Pseudomonas syringae* pv. *aesculi* are remarkable.

Preliminary studies on red flowering horse chestnut trees at three different locations showed that *Flammulina velutipes* and *Pleurotus ostreatus* are often involved. However, other fungi as well as an infestation with the bacterium *Pseudomonas syringae* pv. *aesculi*, have been detected (Gaiser et al., 2013a).

Despite the importance of these damages and infestations, detailed knowledge about the interaction between *P. ostreatus* and *F. velutipes* and the distribution within the damaged and infected xylem is still missing. Both fungi are basidiomycetes and saprophytes or wound parasites (Kreisel, 1979; Jahn, 2005; Schmidt, 2006) but have not yet been described as parasites causing an extensive decay in standing horse chestnut.

In living trees wounds are compartmentalized by reactions summarized as the CODIT-Principle (compartmentalization of damage in trees; Dujesiefken and Liese, 2008). Within the wood, reactions of parenchyma cells form a dark colored reaction zone. This reaction zone separates infected from sound sapwood and thus inhibits spread of the microorganisms. Effective encapsulation of damage is additionally characterized by the closure of the wound by the formation of wound wood (Liese and Dujesiefken, 1996). In *Aesculus* sp., however, compartmentalization is weak (Dujesiefken, 2012), thus enabling the invasion of further pathogens, followed by a rapid spread of the decay. In case of the new disease of red flowering horse chestnut the damage caused by the multitude of fungi cannot be compartmentalized by tree reaction. The present study aims in shedding more light onto the organisms causing these damages and their spatial distribution within the trunk. Identification of the fungi involved is by sequencing of the internal transcribed spacer region of the rDNA (ITS) and of *P. syringae* pv. *aesculi* by detection of the specific gyrase-B-gene (Schmidt et al., 2009; Schmidt et al., 2012).

Material

Two approximately 80-years-old urban red flowering horse chestnut trees (*A.* × *carnea*) were felled in Hamburg, Germany in February 2012. At about 3 m height, the trunks divided into co-dominant stems, bearing fruiting bodies of *F. velutipes, P. ostreatus,* and *C. purpureum*. Additionally, necrotic lesions and bleeding spots were visible. Discs of 5 cm thickness were taken, from tree 1, from the lower (height of about 6 m; sample 1.1) and upper (height of about 8 m; sample 1.2) part of one stem in the areas where fungal fruiting bodies were present. From tree 2, discs were taken from two co-dominant stems in about 6 m height (samples 2.1, 2.2). All discs were surface sterilized by cutting of 0.5 to 1 cm with a sterilized chisel and subsequent freeze-dried. Decay covered one third of the cross section area in all discs, stretched from the outer most wood tissues towards the pith and was separated from the sound sapwood by a more or less dark reaction zone (reaction zone; Fig. 1). Based on these findings, eight different tissue zones were defined for further analyses and identification of fungi involved: adjacent to bark and cambium, outermost decayed sapwood samples were taken from the outermost middle part of the decay tissue (M) and from the reaction zones near the cambium (S1 and S2, respectively; Fig. 1). Additionally, samples from the inwards formed reaction zones (RS1 and RS2; Fig. 1), the central, innermost part of the reaction zone (sample name: RM) and the center of decayed wood (Z) were analyzed. Moreover, one sample of opposite, sound and not discolored tissue was used as reference (G). For analyzes of *P. syringae* pv. *aesculi*, material was collected from the discolored outermost xylem and innermost bark (P) adjacent to the decay of samples 1.1 and 2.2. All together more than 35 samples were taken.

Methods

The ITS region was used for the identification of the fungi and of the host tree (Schmidt et al., 2012). For this, DNA was extracted from 50 mg of homogenized wood tissue of the zones described using the DNeasy Plant Mini Kit, Qiagen (Hildesheim, Germany). PCR was in a total of 12.5 µl, using the Taq PCR Core Kit (Qiagen) by following the manufacturer's instructions in a Tpersonal cycler (Biometra, Göttingen, Germany). In order to amplify exclusively the ITS region from fungal DNA, the ITS1 and ITS2 subregions were amplified separately by using the

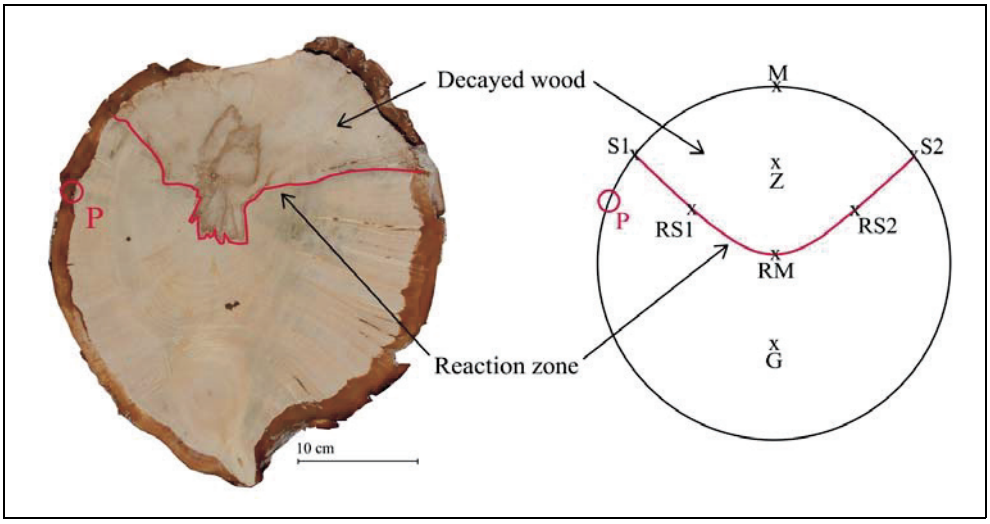

Fig. 1. Cross section of an infected stem showing decay in the upper part. Eight distinct zones were described and analyzed. The outermost middle part of the decay tissue (M), the outer (S1, S2) and inwards (RS1, RS2) formed, more or less dark reaction zones bordering sapwood, as well as the central, innermost part of the reaction zone (RM), the centre of decayed wood (Z) and outer discolored wood for *Pseudomonas* testing (P). Opposite, sound sapwood (G) was used as reference.

universal ITS1.1 and ITS4 primers (ITS1.1: GAACCTGCGGAAGGATCAT; ITS4: TCCTCCGCTTATTGATATGC; White et al., 1990) in combination with newly designed, modified ITS2 and ITS3 primers, with high specificity for the fungal 5.8S rDNA gene (ITS2.2: AACTTTCAACAACGGATCTCTT; ITS3.2: AAGAGATCCGTTGTTGAAAGTT). Additionally, for some samples, the complete ITS region was amplified by using the ITS primers ITS1.1-F and ITS4-B (ITS1.1-F: CTTGGTCATTAGAGGAAGTAA; ITS4-B: CAGGAGACTTGTACACGGTCCAG; Gardes and Bruns, 1993; Prewitt et al., 2008) or 18S and ITS4m (18S: CGCTACTACCGATTGAATG; and ITS4m: CCTCCGCTTATTGATATGC; Schmidt et al., 2012). Among 10 to 50 ng of total DNA was used as template in the PCR. The PCR was performed with an initial denaturation 94°C for 4 min, followed by 37 cycles of 30 sec at 94°C, 30 sec at 55°C for annealing, and 30 sec at 72°C for elongation, and a final end-elongation at 72°C for 7 min. Using the primer combination 18S and ITS4m, PCR conditions were changed to 52°C as annealing temperature and 60 sec for elongation.

Identification of *P. syringae* pv. *aesculi* was by amplifying fragments of the gyrase-B-gene using the primers G 5 (ATACCACCGGTACTCAGATCC) and G 6 (AATGCCGTCGTCGCGTTGAAT), employing PCR conditions outlined above for the ITS1 or ITS2 subregion (Schmidt et al., 2009).

Identification of the host tree was by producing amplicons of the ITS1 and ITS2 regions by using the primer combinations ITS1.1 (GAACCTGCGGAAGGATCAT)/ITS3.1 (GACTCTCGGCAACGGATATC), and ITS2.1 (CCCGTGAARCATCGAGTC)/ITS4 (GCATATCAATAAGCGGAGGA) showing high specificity for the plant 5.8S rDNA gene (Hansen et al., 2011).

PCR amplicons (2.5 µl) were checked on 2% agarose gels in TAE buffer (0.5 M TRIS, 10mM acetic acid, 1mM EDTA), separation at 135 V for 25 min and staining with ethidiumbromide. Purification of the amplicons was by the use of a QIAquickPCR Purification Kit (Qiagen). Sequencing of both strands of the purified PCR products was done by Eurofins Operon, Ebersberg, Germany (http://www.mwg-biotech.com).

In order to detect all fungi present within a sample, purified PCR-amplicons were cloned using the Qiagen PCR Cloning Kit (Qiagen, Hildesheim, Germany) following the manufacturer's instruction. Transformants were selected by blue-white screening and were directly used for PCR-amplification of the inserts using the primers M13-for (GTAAAACGACGGCCAGT) and M13rev (AACAGCTATGACCATG). Selection of the clones to be sequenced was by *Hae*III restriction analyzes (Appligen, Heidelberg, Germany) following the manufacturer's instruction.

Identification of the organisms was by comparison of the ITS sequences obtained with database sequences by BLAST (Basic local alignment search tool; http://blast.ncbi.nlm.nih.gov). To avoid misinterpretation, particular attention was drawn to the fact that unknown sequences reveal high homologies to reference datasets generated and deposited by different groups.

Results

Using the primers outlined a total of 13 fungal species were found. Most prominent were four basidiomycetes: *Flammulina velutipes* (13 findings, KF681358, 99% homology to published sequences), *Pleurotus ostreatus* (five findings, KF681359, 99% homology), *Chondrostereum purpureum* (six findings, KF681361, 99% homology) and *Bjerkandera adusta* (four findings, KF681360, 99% homology). Additionally, nine ascomycetes such as *Aspergillus* sp. (two findings, 99% homology), *Cadophora melinii* (one finding, 99% homology), *Cornuvesica falcata* (one finding, 98% homology), *Diplodia* sp. (two findings, 96% homology), *Fusarium* sp. (one finding, 96% homology), and its teleomorph *Nectria haematococca* (95% homology), *Neonectria punicea* and *N. faginata* (one finding each, 91 and 99% homology), *Phomopsis* sp. (two find-

ings, 87–99% homology), *Sclerotinia* sp. (one finding, 98% homology) and the bacterium *P. syringae* pv. *aesculi* (three findings, 100% homology) were molecularly identified. The localization of the individual fungi across the cross section within the infected and decayed wood tissues was highly stem-specific.

In the lower part of tree 1 (disc 1.1) fruiting bodies of *F. velutipes* and *C. purpureum* were found. The presence of *F. velutipes* within the wood was proven close to the emerging site of the respective fruiting body in S2 (Fig. 1; Tab. 1). *F. velutipes* was detected in the adjacent inwards directed reaction zone RS2 and in S1. In the outermost

Tab. 1. Distribution of the fungi detected within the stem of diseased red flowering horse chestnut trees. From tree 1, sections from the lower (1.1) and upper (1.2) part of one stem were analyzed. For tree 2, discs from two stems (2.1, 2.2) were investigated. Whereas x = one finding, x* = two findings

Tissue zone	Fungi	stem disc			
		1.1	1.2	2.1	2.2
M outermost middle part of the decay tissue	*Bjerkandera adusta*	×		×	
	Flammulina velutipes		×		
	Fusarium sp.				×
	Nectria haematococca				×
Z center of decayed wood	*Bjerkandera adusta*			×	
	Chondrostereum purpureum	×	×		
	Diplodia sp.				×
	Flammulina velutipes			×	×
RM innermost part of the reaction zone	*Aspergillus* sp.			×	
	Chondrostereum purpureum		×		
	Diplodia sp.				×
	Flammulina velutipes		×	×	
	Phomopsis sp.				×
S1 reaction zone near the cambium (left side)	*Flammulina velutipes*	×	×		
	Neonectria punicea/N. faginata				×
	Phomopsis sp.				×
	Pleurotus ostreatus	×		×	
RS1 inwards formed reaction zone (left side)	*Chondrostereum purpureum*	×	×		
	Nectria punicea				×
	Pleurotus ostreatus			×	
	Sclerotinia sp.				×
S2 reaction zones near the cambium (right side)	*Cadophora melinii*				×
	Chondrostereum purpureum		×		
	Flammulina velutipes	×	×		×
	Pleurotus ostreatus	×		×	
RS2 inwards formed reaction zone (right side)	*Bjerkandera adusta*			×	
	Flammulina velutipes	×	×		×
G reference	*Aspergillus* sp.		×		
	Cornuvesica falcata	×			
P samples under suspicion to be infected by *Pseudomonas syringae* pv. *aesculi*	*Pseudomonas syringae* pv. *aesculi*	×			×*

discolored tissues S1 and S2, *F. velutipes* was associated with *P. ostreatus*. *C. purpureum* was traced in the center parts of the decay (Z) inwards the insertion of the respective fruiting body and in the reaction zone RS1. Furthermore, *B. adusta* was involved in the decay processes in the outermost part of the stem (M).

In the upper part of the stem of tree 1 (disc 1.2) fruiting bodies of *F. velutipes* visualized the decay and emerged outwards the reaction zone S2. *F. velutipes* was widespread all over the affected stem and was characterized in the outer (S2, S1, and M), inner decayed (RM) and discolored tissues (RS2). In addition, in inner parts (Z, RS1, RM, S2) *C. purpureum* was found solitary or associated with *F. velutipes*.

In tree 2 stem disc 2.1 bore fruiting bodies of *P. ostreatus* and *F. velutipes*. Here, *P. ostreatus* was identified in the tissue from which the fruiting body emerged (S2) and the reaction zones (S1, and RS1). *F. velutipes* was present in the decayed wood areas adjacent to the fruiting body (Z, RM). Additionally, *B. adusta* accompanied *F. velutipes* in the centre part of the decay (Z) and was present in the outer decayed wood (M) as well as in the reaction zone RS2.

In disc 2.2 (tree 2) bearing fruiting bodies of *F. velutipes*, this fungus was identified in the wood beneath (S2) and bordering the areas where the fruiting bodies grew out (RS2, Z). In zone S2, *F. velutipes* was accompanied by the ascomycete *C. melinii* and in the central decayed tissue (Z) by *Diplodia* sp. (Tab. 1). In all other tissues, ascomycetes were detected, such as *N. punicea/N. faginata* and *Phomopsis* sp. in the outer part (S1), and *N. punicea* and *Sclerotinia* sp. in the inner reaction zone (RS1). In the center of the stem (RM), *Phomopsis* sp. and *Diplodia* sp. were present. In the outermost middle part of the infected stem (M), *Fusarium* sp. and its teleomorph *N. haematococca* were found.

In two discs (1.1 and 2.2), *Pseudomonas syringae* pv. *aesculi* (three findings) was present in discolored outermost xylem and phloem samples bordering and opposite of the decay zones.

The molecular identification of the two red flowering horse chestnut trees was shown by amplifying the ITS region from sound sapwood (zone G, Fig. 1). The nature of the hybrid was manifested by the presence of the ITS of both parents, *A. hippocastanum* and *A. pavia*. Moreover, in two out of four samples of sound wood contaminations with molds were detected (*Aspergillus* sp. and *Cornuvesica falcata*).

Discussion

Since 2011 severe damages by so far unknown symptoms are reported on red flowering horse chestnut. In the meantime many red flowering horse chestnuts are affected. In the present studies, 14 organisms involved in the damage of two trees were identified by molecular analyses. The fungi *F. velutipes*, *P. ostreatus* and *C. purpureum* have been detected often. Further species were *B. adusta*, *Diplodia* sp., *Phomopsis* sp. and miscellaneous species of *Nectria* as well as the bacterium *P. syringae* pv. *aesculi*. In former studies, infection by the bacterium *P. syringae* pv. *aesculi* is regarded as primary defect. The bacterium *P. syringae* pv. *aesculi* has also been proven three times in two stem discs of the two trees and thus occurred both in the phloem as well as in the outer xylem. Originally appearing on *Aesculus indica* in India *P. syringae* pv. *aesculi* has been found on *A. hippocastanum* in Germany for the first time in 2007 (Schmidt et al., 2008). The presence of *P. syringae* pv. *aesculi* on the red flowering horse chestnut is the first report of its appearance in North Germany. Moreover, it is also the first finding of *P. syringae* pv. *aesculi* within the outer wood of living, naturally diseased red flowering horse chestnut. Up to now, it was detected in the inner bark (Green et al., 2009; De Keijzer et al., 2012; Gaiser et al., 2013a) and the secondary xylem of artificially infected horse chestnut trees (De Keijzer et al., 2012). It is assumed that the infection of twigs and stems by *P. syringae* pv. *aesculi* takes place during dormancy. Necrosis of the phloem and sometimes also of the cambium are the consequence (Steele et al., 2010). Generally, *Aesculus* is a tree species with weak compartmentalization abilities (Dujesiefken and Liese, 2008). Larger phloem and cambium damages therefore lead to widespread discolorations and form potential habitats for different organisms, such as wood destroying fungi. The auburn-blackish bleeding spots of an early *Pseudomonas* infection are inconsiderable on trees and often appear in upper parts of the stem or crown and are thus often missed during tree inspections. Therefore, infections with *P. syringae* pv. *aesculi* often remain undetected for a long time and favour the establishment of wood destroying fungi.

In the investigated trees, none of the infection sites were effectively compartmentalized and thus encapsulated by the formation of wound wood. Thus, the stem and wood tissues inwards the damage were prone to invasion by wood destroying fungi. Based on the weak defence reactions and thus encapsulation of the pathogens, the decay expands thoroughly.

In the stems under investigation, several white rots are detected within the decaying stem tissues. Most frequent was *F. velutipes* with 13 findings in a total of 32 samples analyzed. This fungus has been identified in both trees and all stem discs. The main occurrence was along the reaction zones with ten detections. *F. velutipes* is known to be a saprobiont and weakening parasite and causes a white rot. Fruiting bodies preferentially appear on dead wood or stumps (Kreisel, 1979; Schlechte, 1986; Schmidt, 2006).

P. ostreatus occurred five times in both trees. It has been identified in one stem disc of each tree. Its spatial distribution was preferentially along the reaction zones. *P. ostreatus* is known to be a wound parasite causing a white rot (Kreisel, 1979; Jahn, 2005). Fruiting bodies of *P. ostreatus* have only been detected on tree 2.

C. purpureum has been identified in six samples of the two stem discs of tree 1. In the stem cross sections it was detected in the centre of the damage and along the reaction zones. *C. purpureum* is presumed to be a primary col-

oniser. It has been described by De Jong et al. (1998) and Jahn (2005) as a pioneer species spreading quickly without causing severe decay in the host. In the infected wood it causes a white rot (Schlechte, 1986; Krieglsteiner, 2000). Furthermore they found that the fungus in the parasitic state induces only weak defence reactions of the sapwood and therefore can spread quickly. Additionally, *C. purpureum* is known to kill the cambium near the wound (Jahn, 2005) which leads to further weakening of the tree.

B. adusta was in one stem cross section of each tree and predominantly in the centre of the damage and the reaction zones. It is regarded as a parasite of trunk wounds (Jahn, 2005) and often causes white rot hidden by dead bark (Kreisel, 1979; Seehann, 1979).

Further fungi, such as *Phomopsis* sp., *Diplodia* sp., *Neonectria* sp., *Nectria* sp. and *Fusarium* sp., as well as *Cadophora* sp. were found in one stem disc (2.2), often associated with *F. velutipes*. These fungi are known to induce necrosis of the cortex, cankers, wilts and dying back of shoots (Schütt, 1979; Kehr et al., 2003; Hartmann et al., 2007; Barreto et al., 2003) or are reported as pioneer organisms in wounds of trees (Shigo, 1977).

Thus, our results indicate that besides the fungi visible by their fruiting bodies, a huge variety of further fungi are additionally present within the decaying stem tissues.

Except *F. velutipes*, the basidiomycetes detected are known to cause white rots as parasites or wound parasites on trees. The distribution of the identified basidiomycetes across the cross sections of the stems permit the conclusion that *F. velutipes* and *P. ostreatus* together with *C. purpureum* and *B. adusta* are the main pathogens of the new disease. The conspicuous appearance of fruiting bodies of *F. velutipes* and *P. ostreatus* on red flowering horse chestnut can be taken as predictor of the disease. Additionally our investigations demonstrate the appearance of two white rot fungi in close proximity without inducing clearly defined demarcation lines (Tab. 1: disc 1.1, tissue zones S1 and S2; disc 1.2, tissue zone RM and S2; disc 2.1, tissue zone Z). Particularly among basidiomycetes causing white rot demarcation lines appear as dark lines separating colonies or/and individual species of white rot causing fungi (Rayner and Boddy, 1988). According to the great diversity of identified fungi within the red flowering horse chestnut trees we refer to former observations of naturally infected wood in which more than one basidiomycete and also several ascomycetes were detected (Chapela et al., 1988; Boddy, 2000; Teroh et al., 2007).

Inspections of the infected trees under investigation revealed a generally good vitality of the trees (according to Roloff, 2001). This leads to the assumption that *P. syringae* pv. *aesculi* has to be considered the primary pathogen as it is able to infect a tree even through intact cell structures (Mabbett, 2007; Gaiser et al., 2013b). A still open question is the span which passes between the bacteria infection, the appearance of fruiting bodies and tremendous losses of mechanical stability of the trees caused by rots. Nevertheless, it is assumed that it takes time. It is well known that small necrosis will be encapsulated and closed by wound wood (Dujesiefken and Liese, 2008) and are therefore of no risk of tree failure. It is not clear what size bark necrosis caused by bacteria, must reach before fungal organisms succeed to colonise the xylem underneath.

Our investigation revealed damage symptoms and mutual reactions of the different organisms in the xylem of red flowering horse chestnut which were not yet found or reported and clearly show that this new disease is characterized by the presence of saprobionts as well as parasites on and within the same tree. *F. velutipes* was present in all stem discs investigated what demonstrate its parasitic nature. In individual stem discs and tissue zones, *F. velutipes* was accompanied by other wood destroying white rot fungi such as *P. ostreatus, C. purpureum* or *B. adusta* or a number of deuteromycetes and ascomycetes. The distribution of fungi detected was different in all investigated cross sections. Whether this reflects a kind of succession of organisms during advancing of the disease or random co-appearance of several organisms, is focus of future research work. The assortment of molecular techniques used in this study enabled the elucidation of the fungi involved in this new disease. Moreover, it has to be considered that fruiting bodies present on branches or stems of a tree do not reveal the complexity and distribution of the fungal population within the infected tissue.

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