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Eutypa lata, the causal agent of dieback in red currant (*Ribes rubrum*) and gooseberry (*R. uva-crispa*) in the Netherlands

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Abstract Dieback of red currant (*Ribes rubrum*) and gooseberry (*Ribes uva-crispa*) is an increasing problem in commercial fields in the Netherlands. Field surveys were done in 2006–2007 and samples with dieback symptoms were analysed. In this study the causal agent was diagnosed as *Eutypa lata*, based on morphological characteristics and rDNA-ITS sequence data. The field surveys revealed the presence of the anamorph and teleomorph states of the fungus produced on dead infected currant wood. *Eutypa lata* is a vascular pathogen of many woody plants. Related fungi from the same family *Diatrypaceae* are difficult to distinguish from *E. lata* based on morphological features. The genetic variability of *E. lata* was compared by rDNA-ITS sequencing of isolates from different hosts and origins. Within the *E. lata* isolates little variability in the ITS sequences was observed. Phylogenetic analysis showed no clear subdivisions within the species. *Eutypa lata* strains isolated from the different hosts were closely related, indicating that there is no direct evidence for host specificity.

Keywords *Libertella* spp. · Grapevine · *Eutypella parasitica* · Molecular phylogeny

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Introduction

Since decades, growers in the Netherlands have encountered problems with a disease that causes dying branches and stem cankers in red currant (*Ribes rubrum*) and gooseberry (*R. uva-crispa*). For many years it was assumed that dieback in red currant was related to fungi such as *Nectria cinnabarina*, *Phomopsis* spp. or the insect *Synanthedon tipuliformis*. Control measures were therefore always focused on these (alleged) causal agents. However, control strategies against these pathogens and pests did not result in reduced dieback incidences in commercial red currant plantations.

Symptoms of dieback do normally not appear until plants are at least 4 years old. The first symptom is usually a sudden wilt of a branch during mid-summer. Leaves die and berries develop very poorly but remain attached to the branch, or even fail to develop. Examination of the base of the dead branch will reveal a canker surrounding an old pruning wound. Dieback branch symptoms are mostly accompanied

by a canker, which often appears V-shaped in a cross-section of the perennial wood (Fig. 1). Cankers have a definite margin between the living and dead wood, and are also characterized by typical splitting and cracking of bark. These symptoms refer to the classical symptoms of Eutypa dieback, as has been described by Carter (1991).

The *Diatrypaceae* (Ascomycetes, Xylariales) includes several species that were found to be plant pathogenic (Trouillas et al. 2010). For many years it was thought that dieback of grapevines was primarily due to *E. lata*. However, in more recent years other species have been identified that are involved in canker diseases, e.g. *E. leptoplaca* (Trouillas and Gubler 2004), *Diatrype stigma* (Rolshausen et al. 2006), *Cryptovalsa ampelina* (Luque et al. 2006) and *Eutypella vitis* (Catal et al. 2007). Another species in this family, *Eutypella parasitica*, has shown to cause canker of maple (*Acer* spp.) in northern USA (Davidson and Lorenz 1938), and was recently reported in Slovenia (Jurc et al. 2006) and Croatia (Ogris et al. 2008).

Eutypa lata is a pathogen of woody plants worldwide and occurs on at least 88 species of woody dicotyledons in 52 genera including *Prunus* spp. (peaches, plums, cherries), *Malus* spp. (apples), *Pyrus* spp. (pears), *Ribes* spp. (berries) and *Juglans* spp. (walnuts) (Carter 1991). It is known as one of the most destructive diseases of *Vitis vinifera* (grapevine) and is responsible for significant economic damage in grape production (Wicks and Hall 1997; Munkvold et al. 1994). The pathogen is disseminated by ascospores, which infect (pruning) wounds. The fungus invades the vascular system of the trunk and shoots, eventually leading to a characteristic dark and wedge-shape necrosis of woody tissues. Due to a slow disease progress, fungal infection is hardly noticeable during the first years. After an incubation period of three or more years the host is slowly killed (Carter 1991). The anamorph and teleomorph states of the fungus are produced on dead wood.

Fig. 1 Typical V-shaped area in a cross-section of a canker due to Eutypa dieback in perennial red currant wood

Disease diagnosis of *Eutypa lata* is commonly done by isolating the fungus from wood cankers on Potato Dextrose Agar (PDA) medium. On this medium *E. lata* grows as a white cottony mycelium which can produce pycnidia with conidia of the anamorph sp., *Libertella*. Perithecia are not produced in culture and some isolates even fail to produce pycnidia in culture, making it difficult to distinguish *E. lata* from other diatrypaceous fungi. Some of the fungi from the family *Diatrypaceae* have also been recorded in association with *Eutypa lata* or *Eutypella* spp. in infected host tissue, e.g., *Diatrypella* spp., *Diatrype* spp. and *Cryptovalsa* spp. (Rolshausen et al. 2004; Trouillas et al. 2001). These related species are difficult to separate based on morphological features, leading to possible misidentification (Rolshausen et al. 2004).

In this study we identified *Eutypa lata* as the causal agent of dieback of red currant and gooseberry in the Netherlands. To get insight into the genetic variability of *Eutypa lata* we sequenced regions of rDNA-ITS (internal transcribed spacer) and compared them with rDNA-ITS sequences available in the National Center for Biotechnology Information (NCBI) DNA database GenBank.

Material and methods

Field surveys and isolations

Eleven commercial red currant and four gooseberry plantations in the Netherlands were inspected for the presence of plants showing dieback symptoms in 2006–2007. Infected branches with visible symptoms (i.e., cankers) were transported to the laboratory where isolations were made. Dead branches and stems were also inspected for the presence of the sexual stage (perithecia and ascospores) of the fungus.

In a gooseberry plantation the disease incidence was recorded by evaluating individual plants.

Isolates were obtained from several red currant cultivars with disease symptoms, e.g. cultivars 'Roodneus' and 'Rovada', and also from the gooseberry (*R. uva-crispa*) cultivars 'Achilles', 'May Duke' and 'Pax'. The isolations were made from wood chips, up to 1 cm^2, which were taken from the area along the margin of living and dead tissue. These wood chips, approximately 1–3 mm thick, were cut out and surface disinfected by soaking in 1.0% NaOCl (v/v) for 1 min. The chips were blotted dry and placed onto PDA (Crous et al. 2009). The PDA plates were incubated at 20°C in the dark. Colonies on the agar media showed an irregular margin and a whitish cottony appearance. These colonies were transferred onto fresh PDA plates and incubated for at least 4 weeks. The strains produced black pycnidia which contained the typical conidia of the *Libertella* anamorph, i.e. filiform, straight or curved, and very numerous, 20–45×0.5–1.5 μm.

Fourteen isolates were recovered from wood canker by classical isolation techniques, and 2 isolates were cultured from ascospores obtained from perithecia found in dead wood and germinated on PDA plates.

Pathogenicity test

A pathogenicity test was conducted on *R. rubrum* (cultivar 'Rovada'). Inoculations (March 2007) were made at cutting ends of two-year old shoots by placing an agar plug with fresh mycelium of 14-day-old colonies (isolate CBS 124243). Shoots were pruned back to 10–15 cm length, ending on a bud. Several canes (4 to 5) per red currant plant (four plants in total) were inoculated. Control shoots (of four different plants) were inoculated in the same way using sterile agar plugs. Inoculated plant materials were then covered and wrapped with Parafilm. Shoots were then collected after 21 months for assessment. Canes were surface disinfected. Fragments of necrotic tissue outside the area of inoculation were cultured according to the procedure as described above. The *Eutypa lata* isolates obtained from reisolation were identified based on colony shape and color, and PCR.

Fungal strains

A collection of fungal isolates of *Eutypella parasitica* and *Eutypa lata* was established at the culture collection of the National Reference Centre, Plant Protection Service, Wageningen, the Netherlands (Table 1). In total 16 assumed *Eutypa lata* isolates were collected from red currant and gooseberry plants from 11 different locations in the Netherlands. Two isolates (i.e., PD 07 03692171, PD 07 03692188) originated from ascospores plated onto PDA.

An additional 9 *Eutypa lata* isolates were kindly received from other culture collections within the European Union which had been isolated from hosts other than red currant and gooseberry (Table 1). Two out-groups were used in the analysis, the first group consisted of the *Eutypella vitis* strain EL 57C and the second group consisted of four *Eutypella parasitica* strains which included the type strain *Eutypella parasitica*, CBS 210.39. The neotype strain CBS 208.87 ex *Eutypa lata*, was used as a reference and the related isolates previously types as *Eutypa lata* var. *aceri*, and *Eutypa petrakii* var. *petrakii* (Rolshausen et al. 2006) were included in the phylogenetic analysis.

DNA extraction

A plug of mycelium from pure fungal cultures was transferred to a 1.5 ml micro centrifuge tube with a secure fitting flattop cap (Superlock tubes, BIOzym TC) containing 300 μl of extraction buffer (0.02 M PBS, 0.05% Tween T25, 2% polyvinylpolypyrrolidone, 0.2% bovine serum albumine) and 1 stainless steel bead (3.97 mm in diameter). The tube was placed in a bead beater (Mixer Mill MM 300, Retsch, Haan, Germany) for 80 s at 1,800 beats per min. The mixture was centrifuged for 5 s at maximum speed in a micro centrifuge (16,100 g) and 75 μl of the resulting supernatant was used for DNA isolation.

DNA isolation was performed with the KingFisher 96 magnetic particle processor (Thermo Electron Corporation, Breda, The Netherlands), following the protocol developed by the manufacturer (K. Kontu, personal communication). Briefly, 5 μl of proteinase K and 50 μl of Lysis Buffer were added to 75 μl of the supernatant described above. After 30 min incubation at 65°C, 5 μl of MagaZorb Magnetic Particles and 125 μl of Binding Buffer were added. The particle-bound DNA was washed twice with 200 μl of Wash Buffer and DNA was eluted in 130 μl of Elution Buffer.

Table 1 Isolates of *Eutypa lata*, *Eutypella parasitica* and *Eutypella vitis* used for sequencing of the internal transcribed spacer (ITS) region

Fungal species	Host	Origin	Source[a]	Isolate ID	GenBank accession number[b]
Eutypa lata[c]	*Crataegus* sp.	France	CBS	CBS289.87	DQ006928
Eutypa lata[c]	*Fraxinus excelsior*	Lithuania	SLU	olrim254	AY787699
Eutypa lata	*Malus sylvestris*	Austria	CBS	CBS 323.82, PD 07 03698088	–
Eutypa lata[c]	*Prunus armeniaca*	USA	UCD	E454	DQ006931
Eutypa lata[c]	*Prunus armeniaca*	Australia	UCD	WRPD001	DQ006938
Eutypa lata	*Pyrus malus*	Austria	CBS	CBS 324.82, PD 07 03698096	–
Eutypa lata	*Ribes rubrum*	The Netherlands	PPO	PD 06 03121553	–
Eutypa lata	*Ribes rubrum* 'Roodneus'	The Netherlands	PPO	PD 06 03205394	–
Eutypa lata	*Ribes rubrum* 'Roodneus'	The Netherlands	PD	CBS 124244, PD 07 03492354	GU071109
Eutypa lata	*Ribes rubrum* 'Rovada'	The Netherlands	PPO	CBS 124243, PD 06 03205386	GU071110
Eutypa lata	*Ribes rubrum* 'Rovada'	The Netherlands	PD	CBS 124245, PD 07 03499636	GU071111
Eutypa lata	*Ribes rubrum* 'Rovada'	The Netherlands	PPO	PD 06 03205361	–
Eutypa lata	*Ribes rubrum* 'Rovada'	The Netherlands	PPO	PD 06 03205378	–
Eutypa lata	*Ribes rubrum* 'Rovada'	The Netherlands	PD	PD 07 03205546	–
Eutypa lata	*Ribes rubrum* 'Rovada'	The Netherlands	PPO	PD 06 03205407	–
Eutypa lata	*Ribes rubrum*	The Netherlands	PPO	PD 07 03692171	–
Eutypa lata	*Ribes rubrum*	The Netherlands	PPO	PD 07 03692188	–
Eutypa lata	*Ribes uva-crispa*	The Netherlands	PPO	PD 06 03205351	–
Eutypa lata	*Ribes uva-crispa* 'Achilles'	The Netherlands	PD	CBS 124246, PD 07 03492178	GU071112
Eutypa lata	*Ribes uva-crispa* 'May Duke'	The Netherlands	PD	CBS 124248, PD 07 03205570	GU071114
Eutypa lata	*Ribes uva-crispa* 'Pax'	The Netherlands	PPO	PD 06 03122345	–
Eutypa lata	*Ribes uva-crispa* 'Pax'	The Netherlands	PD	CBS 124247, PD 07 03492186	GU071113
Eutypa lata[c]	*Tilia* sp.	Switzerland	CBS	CBS 208.87[e]	DQ006927
Eutypa lata	*Vitis* sp.	United Kingdom	CSL	CC489, PD 06 03487088	–
Eutypa lata	*Vitis* sp.	United Kingdom	CSL	CC490, PD 06 03487096	–
Eutypa lata[c]	*Vitis vinifera*	France	UCD	INRA8D	DQ006930
Eutypa lata	*Vitis vinifera*	Italy	DLR	DLR 8227, PD 07 03815686	–
Eutypa lata[c]	*Vitis vinifera*	Switzerland	UCD	ABESw	DQ006941
Eutypa lata	*Vitis vinifera* 'Ehrenfelser'	Germany	DLR	DLR 7312/50, PD 07 03815678	–
Eutypa lata	*Vitis vinifera* 'Ehrenfelser'	Germany	DLR	7291, PD 07 03815661	–
Eutypa lata	*Vitis vinifera* 'Huxelrebe'	Germany	DLR	DLR 8232–2, PD 06 03815707	–

Table 1 (continued)

Fungal species	Host	Origin	Source[a]	Isolate ID	GenBank accession number[b]
Eutypa lata	*Vitis vinifera* 'Huxelrebe'	Germany	DLR	DLR 8232–1, PD 07 03815694	–
Eutypa lata	*Vitis vinifera* 'Muller-Thurgau'	Germany	DLR	DLR 7261, PD 07 03815651	–
Eutypa lata var. acerі[c]	*Acer pseudoplatanus*	Switzerland	CBS	CBS 290.87	DQ006948
Eutypa petrakii var. petrakii[c]	*Prunus spinosa*	Switzerland	CBS	CBS 244.87	AJ302455
Eutypa petrakii var. petrakii[c]	*Salix borealis*	Norway	CBS	CBS 245.87	AJ302456
Eutypella parasitica	*Acer pseudoplatanus*	Slovenia	SFI	ZN1/1, PD 06 03202193	–
Eutypella parasitica	*Acer campestre*	Slovenia	SFI	D26/1, PD 06 03202185	–
Eutypella parasitica	*Acer saccharum*	USA	CBS	CBS 210.398, PD 07 03698061[d]	–
Eutypella parasitica[c]	*Acer pseudoplatanus*	Slovenia	SFI	DJ 2/3	DQ118965
Eutypella vitis[c]	*Vitis labrusca*	USA	CNB	EL57C	AJ302466

[a] *CBS* Fungal Biodiversity Center, Utrecht, The Netherlands; *PPO* Applied Plant Research, Fruit Research Unit, Randwijk, The Netherlands; *PD* Plant Protection Service, Wageningen, The Netherlands; *DLR* Dienstleistungszentrum Ländlicher Raum Rheinpflaz, Neustadt, Germany; *CSL* Central Science Laboratory, York, United Kingdom; *SFI* Slovenian Forest Institute, Ljubljana, Slovenia; *SLU* Swedish University of Agricultural Sciences, Uppsala, Sweden; *CNB* Centro Nacional de Biotecnologa, Madrid, Spain; *UCD* University of California, Davis, USA

[b] GenBank accession numbers of ITS sequences

[c] Sequences of isolates in the GenBank used in the phylogenetic analysis of the ITS region

[d] Type strain

[e] Neotype strain

PCR amplification

The PCR was performed with the primers ITS1 and ITS 4 (White et al. 1990) resulting in approximately 500-bp fragment depending on the species. The reactions were performed in 25-μl reaction mixes containing Taq polymerase buffer (Roche Diagnostics Nederland B.V., Almere The Netherlands), 1.6 U Taq polymerase (Roche), 80 μM of each dNTP, 0.12 μM of each primer, and 5 μl of template DNA. The PCR reactions were performed in a 96-well Peltier-type thermocycler with heated lid (PTC-200, MJ-Research: BIOzymTC, Landgraaf, The Netherlands) with the following parameters: 30 s at 94°C, 40 cycles of 15 s at 94°C, 60 s at 55°C, 30 at 72°C and a final step of 5 min at 72°C.

DNA sequencing

PCR products were purified with the QiaQuick PCR purification kit from Qiagen (Westburg, Leusden, The Netherlands) to remove excess primers and nucleotides and sequenced with the initial amplification primers using the BigDye sequencing kit (Applied Biosystems, Nieuwerkerk aan de IJssel, The Netherlands) on an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

The trace files were transferred to the Editseq module of the Lasergene software (DNAStar Inc., Madison, U.S.A.). Contig assembly was performed using the SeqMan module of the Lasergene software.

Phylogenetic analysis

The sequences generated in our study were aligned with published sequences from GenBank using a Clustal W analysis with Mega 5.0 software (Molecular Evolutionary Genetic Analysis, Tamura et al. (2007)). Phylogenetic trees were constructed by the maximum parsimony method also using Mega 5.0. The tree was obtained using the Close-Neighbor-Interchange algorithm (Nei and Kumar 2000) with a search level 1 in which the initial trees were obtained with the random addition of sequences (10 replicates). The analysis involved 25 nucleotide sequences and all sites were treated in the analysis. To test reliability of groupings bootstrapping was applied using 1000 randomisations (Felsenstein 1985). The tree was rooted with *Eutypella vitis* as the out-group, and *Eutypella parasitica* (4 strains) was also used as an out group taxon. Sequences generated in this study and used in the tree have been deposited in GenBank under accession numbers GU071109–GU071114.

Results

Field survey and isolations

Eutypa lata was isolated from diseased plants sampled on seven commercial red currant plantations and four gooseberry plantations in the Netherlands, out of 11 inspected red currant and four inspected gooseberry plantations, respectively. Disease incidences were difficult to estimate, as affected plants are usually removed by the growers. In some older (6–10 year) red currant plantings all plants were visibly affected, indicated by the removed main stems, possibly due to Eutypa dieback. Inspection of a 6-year old gooseberry plantation revealed that 130 out of 244 (=53%) plants (cultivar 'Pax') were affected, most likely due to Eutypa dieback.

A survey in heavily affected plantations revealed fruiting structures (stroma), asci and ascospores on dead infected red currant wood (cultivar 'Rovada'). This is the first record of sexual fruiting bodies observed in red currant. Perithecia were about 0.5 mm in diameter, and irregularly distributed in one layer (honeycomb-like appearance). Asci were 30–60×5–8 μm, very numerous, spindle-shaped, and eight-spored. Ascospores were allantoid, subhyaline, and 7–10×2 μm. Based on these morphological features, the fungus was identified as *Eutypa* sp. (Ascomycetes, Diatrypaceae). Plating of ascosporic suspensions on PDA resulted in the growth of mycelium that eventually produced pycnidia and conidia and were included in the phylogenetic analysis.

Pathogenicity test

After 21 months, vascular discolouration induced by *Eutypa lata* was visible in a cross section of most of the shoots inoculated with *E. lata* mycelium. In certain cases the discoloration progressed into the main branch, and showed the typical V-shape in the cross section. *Eutypa lata* was re-isolated from pieces of wood near the point of inoculation from all inoculated plants. The identity was confirmed based on colony shape and color, and PCR. The control shoots inoculated with sterile agar were without symptoms.

Phylogenetic analysis

The ITS sequences of 26 isolates of *Eutypa lata* analysed in our lab and the ITS sequences of seven other *E. lata* isolates that were present in GenBank at the beginning of this study showed little variability. Similarity among these sequences was at least 97%. A number of *E. lata* isolates had identical sequences. Where this was the case, one representative strain was deposited at the Fungal Biodiversity Centre (CBS) and the corresponding ITS sequence posted in GenBank (i.e. five GU entries representing 15 strains).

A maximum parsimony analysis of the ITS region yielded a total of 261 equally most-parsimonious trees (Consistency Index = 0.857, Retention Index = 0.944, Composite Index = 0.808). The phylogenetic tree (Fig. 2) shows two clades, i.e., for *Eutypa lata* and *Eutypella parasitica*. The three *Eutypella parasitica* isolates showed 100% identity with the *Eutypella parasitica* sequence in Genbank (accession DQ118965).Within the *E. lata* clade little variability in the ITS sequences was observed. There were no apparent subdivisions that could be related to the origin or region of these strains. There were two groups of two strains that were separated from the *Eutypa lata* taxon; (1) *Eutypa lata* CBS 289.87 (*Crataegus* sp.) and *Eutypa petrakii* var. *petrakii* CBS 244.87 (*Prunus spinosa*); (2) *Eutypa lata* CBS 124244 (*Ribes rubrum*) and *Eutypa lata* var. *aceri* CBS 290.87 (*Acer pseudoplatanus*).

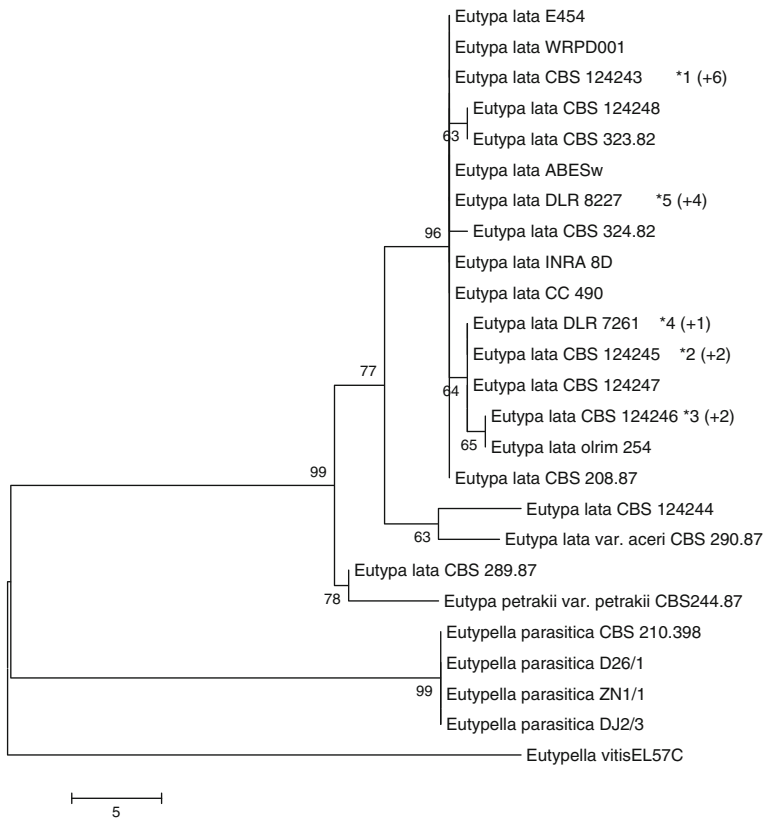

Fig. 2 One of the 261 most parsimonious phylogenetic trees based on sequence differences in the internal transcribed spacer (ITS) region. Clades that are supported by bootstrap analysis (1,000 randomisations) are indicated by numbers next to the clade (when more than 50%). The tree is drawn to scale; branch lengths calculated using the average pathway method and are in units of the number of changes over the whole sequence. Tree length = 98, consistency index = 0.857, retention index = 0.944, and composite index=0.808. * Isolates with identical ITS sequences: (*1) Six isolates: PD 06 03205394, PD 07 03205546, PD 06 03122345, PD 07 03692171, PD 07 03692188 and PD 06 03205407); (*2) Two isolates: PD 06 03205361 and PD 06 03205378; (*3) Two isolates: PD 06 03205351 and PD 06 03121553; (*4) One isolate: 7312/50; (*5) Four isolates: CC489, 8232–2, 8232–1 and 7291

Discussion

We have shown that *Eutypa lata* is the causal agent of dieback in red currant and gooseberry in the Netherlands. The phylogenetic analysis of the *E. lata* isolates found in red currant and gooseberry plantations showed that there is little genetic variation within the species. The position of *Eutypa lata* CBS 298.87 from *Crataegus* spp. was outside the *E. lata* clade, confirming the conclusion of Rolshausen et al. (2006) that this isolate should not be considered as *Eutypa lata* sensu stricto. This was also supported by Rappaz (1987) who noted distinctive morphological features of this isolate. The *Eutypa lata* isolate CBS 124244 (*R. rubrum*) was closely related to *Eutypa lata* var. *aceri* CBS 290.87 (*Acer pseudoplatanus*).

The results from our study show that there is very little variability in the ITS sequences of the *Eutypa lata* isolates from several cultivated and wild host species in different European countries. This supports the general opinion that although *E. lata* has a broad host range, there are no specific pathotypes yet. Our study confirms the observations that *E. lata* is a randomly mating species with a high degree of genetic diversity (Peros et al. 1997; Pilotti et al. 2005; Catal et al. 2007). Therefore, we expect that cross infections between different host plants can easily occur, especially with highly susceptible species as grapevine and, apparently red currants. This also implies that native–non-currant–hosts may act as inoculum sources for currant and grapevine plantations in the Netherlands.

In our research *Eutypa lata* was isolated from popular commercially used red currant cultivars, e.g., 'Junifer', 'Rovada' and 'Roodneus', and gooseberry cultivars, e.g., 'Achilles', 'May Duke' and 'Pax'. Most red currant and gooseberry cultivars are thus susceptible to *E. lata*. Differences in disease severity among plantations are more likely related to management practices, i.e., pruning intensity or strategy, than to cultivar resistance or tolerance. In grapevine cultivars differences have been found in their susceptibility to *E. lata* and subsequent symptom development (Peros and Berger 1994). However, no grape cultivar is known to be immune to infection.

Dieback of currants is not restricted to the Netherlands and is most likely widespread in Europe. Recently, seriously infected red currant plantations have been report in Italy (Prodorutti et al. 2008). They could unambiguously establish Koch's postulates for *Eutypa lata* on *R. rubrum*. Also in Belgium dieback of red currant and gooseberry is recognized as a serious disease (Pitsioudis, F. and Stevens, K., pers. com.). However, due to the generally small plantation sizes and often home growing practices of currants the disease remains often unnoticed. Wicks and Hall (1997) suggest that in Australia up to 60% of the grapevines might be affected in certain old vineyards. Duthie et al. (1991) estimate that in California over 90% of the grapevines may be affected by the time they reach 20 years. From our observations we conclude that in more than 6 years old red currants and gooseberry plantations disease incidences are also very high. Information from questionnaires and interviews with growers, extensionists and from our own surveys revealed that Eutypa dieback in red currant is present in all growing regions of the Netherlands.

The costs of the Eutypa dieback in red currants and gooseberries are due to decreased yields, increased management and reduced longevity of the plants. Increasingly more Dutch growers tend to replace old stocks with new material within 5 to 6 years, not awaiting severe disease development. In order to improve management of Eutypa dieback of currants in Dutch or European plantations it is necessary to identify potential inoculum sources in native and cultivated plant species and implement sanitation practices by removing diseased plant parts. Epidemiology and control measures are currently investigated in the Netherlands. Also the spread of the disease via propagation material (nurseries) cannot be excluded, as high disease incidences were observed in young plantations (2–3 years old) in a region without old currant or grapevine plantations in the neighbourhood. This mode of spreading of this disease was also suggested for olive trees in Italy (Tosi and Natalini 2008). For disease diagnosis *in planta*, the use of specific primers would be helpful, in order to avoid the laborious isolation. Recently, PCR primers have been developed (Lecomte et al. 2000) that are useful in the identification of *Eutypa lata in planta* or in vitro, and a nested multiplex PCR by Catal et al. (2007) that might be suitable for detecting latent infections in propagation material.

In conclusion, our study revealed that *Eutypa lata* is the causal agent of dieback in red currant and gooseberry cultivation in the Netherlands. The pathogenicity of *E. lata* on red currant was proven in this study, and also by Prodorutti et al. (2008). The isolated *E. lata* strains from the different hosts were

closely related, indicating that there is no direct evidence for an *E. lata* adapted to specific host species. To our knowledge this is the first report of *Eutypa lata* as a serious disease in commercial red currant and gooseberry plantations.

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