Final Report

Project title (Acronym)
Identification and early detection of *Cryphonectria parasitica* and *Ceratocystis platani* occurring on trees in Europe (CERACRY)

Project duration:

Start date:	2016-09-01
End date:	2018-08-01

Contents

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2. Short project report

2.1 Short executive summary

The project resulted in an excellent collaboration between specialists of Cryphonectria-disease of sweet chestnut, but also specialists on *Ceratocystis platani*. The project contributed to:

a) improve knowledge on the distribution of *Cryphonectria parasitica* in European countries. It was shown that *C. parasitica* is present in Belgium and United Kingdom, but not in Ireland. In the Netherlands, the fungus was found several times on imported *Castanea* stakes/fences in 2018, but not in nurseries or public green.

b) Develop a collection of *C. parasitica* isolates. The isolates were used to determine the population structure of *C. parasitica* in Europe. Evidence was found that in countries where the pathogen was relatively recently described (e.g. UK), the pathogen was introduced several times but has not spread widely, possibly due to suboptimal climatic conditions.

c) organise a test performance study (TPS) for the real-time PCR test from Pilotti et al. (2012) or the detection of *C. platani* in wood. Specifically, EvaGreen, SYBR Green and Taqman tests were validated. Standard curves confirmed that the tests have high amplification efficiency. For analytical sensitivity, all laboratories detected 15 and 3 fg *C. platani* gDNA, thus reproducing the previously reported detection threshold of the method. Testing blind samples yielded a 100% scoring sensitivity, accuracy and specificity in all the experiments, with an exception in the SYBR Green test for which results from one laboratory were nonconforming for the criterion of accuracy.

2.2 Project aims (1 page)

The overall goal of the project was to bring together scientists, practitioners and official national bodies (NPPOs) to share knowledge on two important quarantine diseases of trees in Europe: *Ceratocystis platani* and *Cryphonectria parasitica*. The threats posed by *Ceratocystis platani* are often underestimated and even in countries where the disease already occurs, not much measures are taken to limit the spread of this devastating disease. More specifically, the project objectives were:

- to collect isolates of *C. parasitica* from Europe and maintain a collection (WP2)
- to determine the current distribution of *Cryphonectria parasitica* in North-Western Europe (WP3)
- to study the genetic diversity of *Cryphonectria parasitica* by genotyping by sequencing in order to track and trace the new findings (WP4)
- to determine the current distribution of *Ceratocystis platani* in Europe (WP5)
- to organise a Test Performance Study (TPS) of the real-time PCR tests for *Ceratocystis platani* (WP6)

2.3 Description of the main activities, Material and methods

Isolate collection

In 2017, import permits were sent to the project partners who accepted to share their isolates of *Cryphonectria parasitica* within the consortium. A total of 103 isolates were received from nine countries; Belgium (29), Czech Republic (5), Croatia (15), Hungary (1), Italy (15), Portugal (16), Slovakia (1), The Netherlands (1), United Kingdom (20).

Upon reception by partner CRA-W, the isolates were subcultured on potato dextrose agar medium (PDA). They were stored at 2-4°C under paraffin oil in two separate tubes. Total DNA was extracted from all isolates, to be used in the genetic analysis carried-out in WP4. To increase the DNA yield, mycelium was grown on PDA overlaid with cellophane membrane as described by Chandelier *et al*. (2019). DNA was extracted with a commercial kit (High Pure Template Preparation kit, Roche Diagnostic, Germany) following manufacturer's instructions.

Current distribution of *Cryphonectria parasitica* in Europe

Cryphonectria parasitica infects mainly *Castanea* species, and to a lesser extent *Quercus* species, generally in areas with chestnut in close proximity. For this reason, the analysis was conducted in the distribution area of sweet chestnut in Europe. According to Conedera *et al*. (2016), the tree species has temperature and precipitation requirements that limit its northward extension to Ireland, England, Belgium, and to a lesser extent the Netherlands and western Germany (Fig. 1).

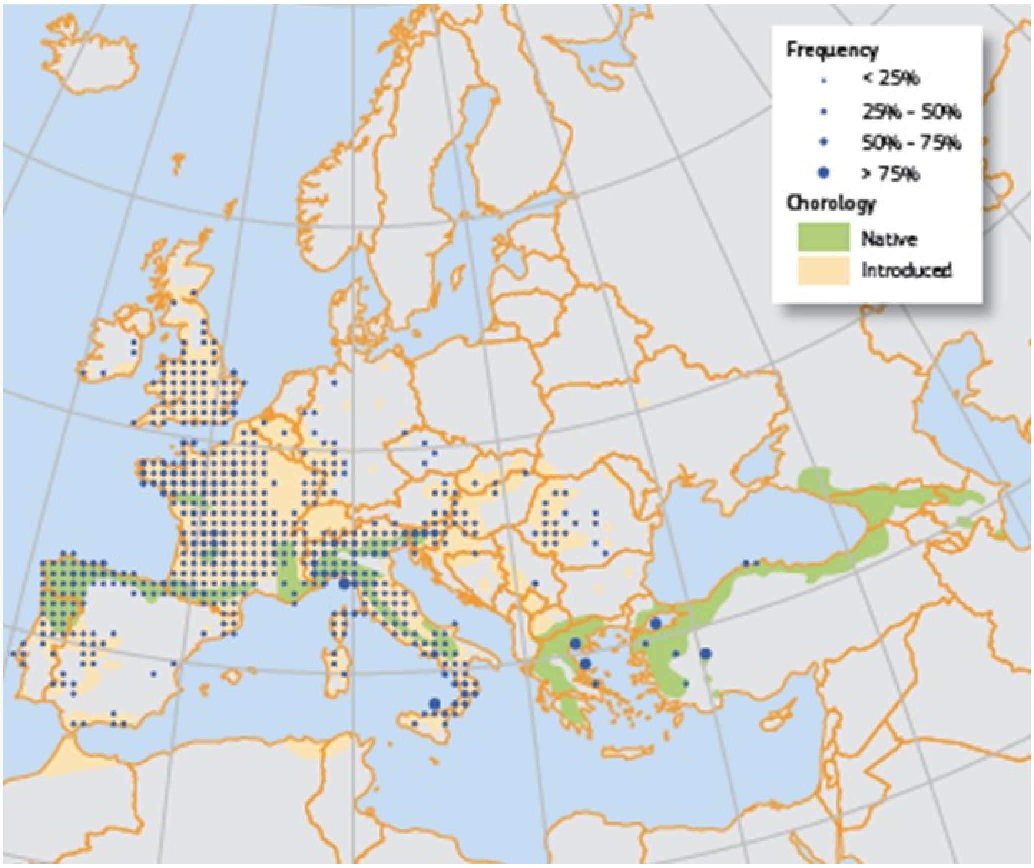

Fig. 1 Chorology map for *Castanea sativa* in Europe. Native and introduced spatial ranges are indicated in green and yellow, respectively. Dots represent the frequency of occurrences within the field observations as reported by the National Forest Inventories [Source: Conedera *et al*. (2016)].

Four partners of the project (Ireland, United Kingdom, Belgium and The Netherlands) participated in a survey to map the occurrence of *Cryphonectria parasitica* from 2015 to 2017.

Genetic diversity of *Cryphonectria parasitica*

The data generated should allow to establish the relationship between isolates and depending on the results, may help in determining the origin of new introductions in countries. The level of genetic diversity observed will indicate if single or multiple introductions occurred, and if there are indications of local establishment or not.
Partner CRA-W prepared DNA-extracts of the isolates collected and sent it to partner ILVO for analysis using Genotyping by Sequencing (GBS) and to partner WSL for analysis using SSR markers. A total of 139 samples were processed. The data obtained with the two genotyping techniques were compared and used to determine the population structure of *C. parasitica* in Europe.

Current distribution of *Ceratocystis platani* in Europe
To determine the current distribution of *Ceratocystis platani* in Europe, partner TFL contacted professionals working in Slovenia, Croatia, Serbia, Bulgaria, Macedonia, Montenegro and Romania.

Test Performance Study
The real-time PCR test for *Ceratocystis platani,* described in the EPPO Diagnostic Protocol PM 7/014(2) (EPPO, 2014) for in-wood detection of the pathogen was tested in an interlaboratory evaluation involving nine European laboratories. Validation data were collected. One of the objectives was to compare the results obtained with BIO-RAD mastermixes with these obtained from other companies, e.g. Eurogentec

2.4 Main results (knowledge, tools, etc.)

Isolate collection
An overview of the 103 *Cryphonectria parasitica* isolates collected from different countries and now stored at CRA-W is given in Appendix 1.

Current distribution of *Cryphonectria parasitica* in Europe
Based on the data collected from 2015 to 2017, the current distribution of *C. parasitica* in North-Western Europe (Ireland, United Kingdom, Belgium and The Netherlands) has been updated (Appendix 2).
In Belgium, the disease was first identified in December 2014 on several trees located along a road in Brussels (official report at the beginning of 2015). All infected trees were cut in spring 2015. In 2015, the fungus was isolated from 2 chestnut stakes in 2 different parks in Brussels. These posts were part of recently installed chestnut fences. In 2016 and 2017, in the framework of a national project (FUNGIFOR), survey units were selected in chestnut plantations, public parks and along roads. The pathogen was identified in two chestnut plantations in 2016 (2 infected trees), and in two additional chestnut plantations in 2017 (three infected trees). In 2017, it was also detected on numerous trees from a recreation area in the northern part of the country. All the infected trees were cut. The Belgian Plant Protection Service also conducted surveys in nurseries. The pathogen was detected in one nursery plant in 2017. All plants with symptoms of the disease were analysed by isolations and real-time PCR for the early detection of the fungus (Chandelier *et al*., 2019).
In Ireland, surveys were conducted in forests and nurseries. To date, symptoms of the disease have not yet been identified.

In the Netherlands, surveys were conducted in forests, parks and nurseries. The pathogen was not detected. However, in 2018, the pathogen was detected on several imported *Castanea* stakes.
In the United Kingdom, there was no survey in 2015. In 2016, the pathogen was first reported on an urban tree. In 2017, the pathogen was identified in forests, parks, nurseries and on trees along roads.

Genetic diversity of *Cryphonectria parasitica*
The data from the SSR and GBS analysis of the *C. parasitica* samples brought to similar conclusions. Using the highly reproducible GBS technique, a total of 1057 informative single nucleotide polymorphisms (SNPs) were identified, which provided an even higher level of resolution in the population structure than SSR. The diversity in this population was relatively large because it contained representatives from different original introductions. However, as could be expected, the population also contained several sets of clonal or very closely related isolates. Combination of the genotypic information with the information on geographic source and year of isolation demonstrated examples of (international) movement of the pathogen. This was not only the case for the original European population that was introduced from Asia via the US and Italy, but also for the later population that was introduced from Asia via the South of France. On the other hand, there were no indications that the Asian isolates that were introduced in Georgia have been introduced into Europe (Prospero et al., 2013).
In Europe, the pathogen was quite recently described in countries such as the Czech Republic, Belgium and the UK. In all those cases there was evidence of introductions of several different genotypes that are still restricted in distribution within those countries. This would suggest the pathogen was introduced several times in those countries but has not spread widely within them, possibly due to suboptimal climatic conditions.

Current distribution of *Ceratocystis platani* in Europe
The occurrence of *Ceratocystis platani* (CSP) has been confirmed in Turkey (on the European side of the Bosporus), Greece, Albania, Italy, Switzerland and France (and was eradicated from Spain). In order to try to gain a better picture of the distribution of this species, partner TFL was in contact with professionals working in Slovenia, Croatia, Serbia, Bulgaria, Macedonia, Montenegro and Romania.
Slovenia believes that CSP is not present in the country, although it is known to be in Trieste (Italy), just a few km from the border, so it is a concern that it could arrive soon. The Administration for the Republic of Slovenia National Plant Protection Organisation commissioned official licensed inspectors to undertake random surveys in parks, cities and nurseries, during which material is gathered for laboratory testing for CSP.
Croatia does not hold any information to confirm or deny that CSP is in the country. According to the Department for Forest Protection and Game Management at the Croatian Forest Institute, no surveys to look for the disease are being done. CSP is a concern in Croatia because London plane makes up a significant proportion of the urban forest and because of the proximity of Croatia to Italy.
Serbia has been looking for signs of CSP for the last 8-10 years. Planes in Belgrade are inspected for symptoms and information about the disease has been disseminated to the southern regions of the country where the warmer climate might be more conducive to CSP

establishment. Reports of suspected CSP are sent to the University of Belgrade and inspected. CSP has not yet been found.
In Bulgaria, the Bulgarian Forest Research Institute do not believe that CSP is currently present, but Bulgaria is very close to Turkey and Greece and therefore the disease is of concern. The health of planes in the central and south-eastern part of the country is being monitored; surveys are undertaken and samples collected for testing.
Macedonia: the proximity of Macedonia to Greece means that there are concerns around possible spread of CSP, but the disease is not known to be in the country yet. Of particular concern are the natural stands of *Platanus orientalis,* many of which are very close to the Greek border. The Faculty of Forestry at the university of Skopje is home to the official body for recognition and identification of tree pests and diseases.

In Montenegro, CSP has not yet been recorded, although no coordinated surveys are taking place at the current time. Planes are a common species in the country in both urban and rural areas, so CSP would be of considerable concern were it to arrive in the country.
In Romania, CSP is not thought to be present. Coordinated surveys are not taking place but a forest pathologist from the National Research & Development Institute in Forestry has looked at some of the plane trees in cities across Romania and has not yet seen any symptoms.

Test Performance Study
Generating standard curves
Standard curves were made in order to assess the reproducibility of the amplification efficiency on wood extracts. The PLs and the OL generated standard curves with a DNA-aliquot derived from a *C. platani*-infected wood primary-sample. In addition to the DNA extract "as it was", six five-fold serial dilutions were used (thus 1:15.625 was the lowest dilution) and each was tested in triplicate, see Figure 2.

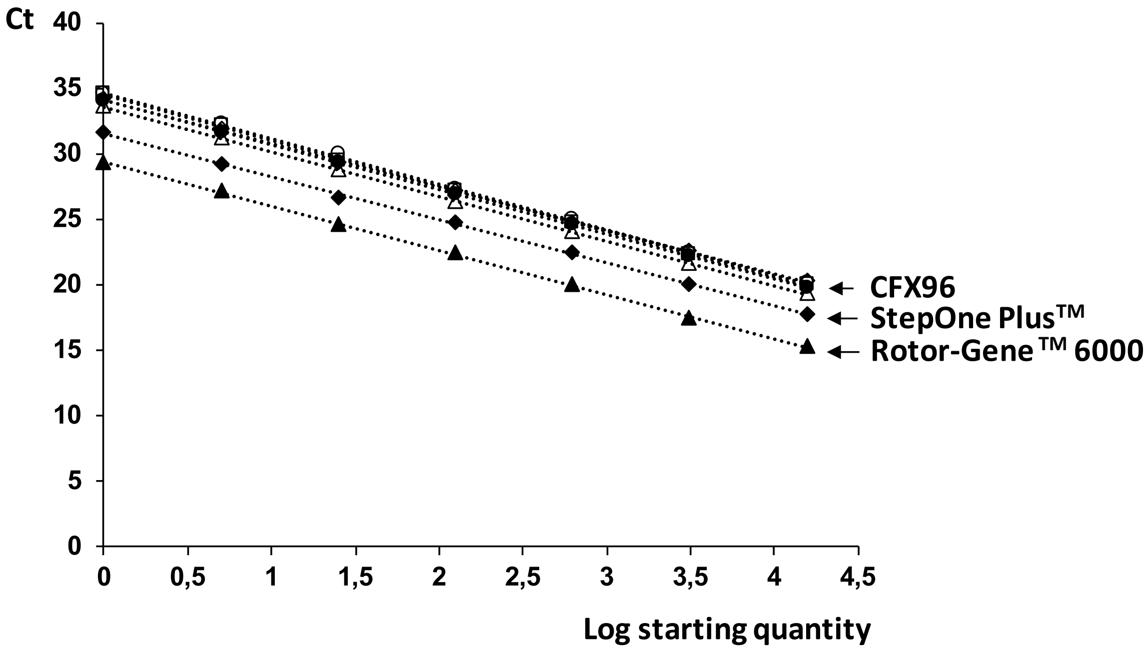

Fig. 2. Effect of PCR systems. Standard curves performed by different PLs using the same Bio-Rad master mix and three different real-time PCR systems

A total of 25 standard curves were generated. The performance parameters of all the standard curve experiments were in line with a maximum or very high amplification efficiency and data linearity (R^2 = 0.99-1). Thus, this TPS phase reproduced the same performance previously reported by the OL (Pilotti *et al*., 2012; Lumia *et al*., 2018).
Most master mixes did not differ statistically in term of detection Ct and performance parameters, and this gives an added value to this real-rime PCR test as different reagents from different commercial companies can be used to perform diagnosis without altering the diagnostic potential.
The same can be stated for the PCR systems, although the real-time PCR test, when performed in Rotor-Gene TM 6000, StepOne PlusTM and CFX96TM, detected the same samples at different Ct, the earliest in Rotor-Gene TM 6000 and the latest in CFX96. However, without prejudice to the amplification efficiency.

Testing blind samples
The EvaGreen test yielded a total of 7 false positives out of 90 negative samples tested. However, as Ct values were higher than 37 and not confirmed by their technical replicates, detection was considered negative.
Similar results were obtained with Taqman, 15 false positive signals were scored (out of 198 negative samples), which however were considered negative as Ct values were higher than 37. In SYBR Green a total of 14 false positive signals out of 72 (negative) were found, of which six samples had a Ct value lower than 37. These six samples were thus considered positive, and not in agreement with the assigned value for those samples.
In all three assays all positive samples, including all the replicates, were found positive.

Analytical sensitivity
Eight laboratories (the OL and 7 PLs) performed the analytical sensitivity tests. All were able to detect 15 and 3 fg *C. platani* gDNA per PCR reaction with a detection Ct < 37 and irrespective of the PCR system used (six replicates).
Based on the results regarding detection of blind samples, some performance parameters were calculated for each experiment (test-laboratory-supermix): Sensitivity (SE), Accuracy (AC), Specificity (SP). All parameters resulted in a score of 100%. Only the results of one PL, performing SYBR Green test, were nonconforming for the global criterion of accuracy (SP = 66.7, AC = 87.5) because of false positive results (see above).
Reproducibility (concordance) of the Real-Time PCR method inferred for each assay was 100% (EvaGreen), 93.75% (SYBR Green) and 100% (Taqman).

References

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- Prospero S, Lutz A, Tavadze B, Supatashvili A & Rigling D (2013). Discovery of a new gene pool and a high genetic diversity of the chestnut blight fungus *Cryphonectria parasitica* in Caucasian Georgia. Infection, Genetics and Evolution 20, 131-139

2.5 Conclusions and recommendations to policy makers

In the past, efforts have been made to restrict further dispersal of *Cryphonectria parasitica* into areas in Europe where the disease did not occur yet. Our study shows that not only planting material but also stakes/poles made from wood of Castanea can be a source of inoculum of *C. parasitica.* Sweet Chestnut fencing is very popular, because it is resistant to decay for longer than many other types of timber. Debarking of poles/stakes is recommended to prevent the introduction of *C. parasitica* in areas still free from *C. parasitica.*
Extra attention is certainly needed to prevent plane canker from spreading further into northern Europe. Just recently, *C. platani* was detected in the municipality of Nantes (France), and as far north as in the suburbs of Paris (EPPO, 2019). Surveys for *Ceratocystis platani* in countries as Germany, Belgium, the Netherlands should be intensified. This is to avoid the introduction of this pathogen.

As indicated aboved, there is a real danger that *Ceratocystis platani* spreads to northern areas in Europe. In the last years there has been considerable discussion already among policy makers on the regulation of plants, plant products and other objects associated with *C. platani.* As a result, the pathogen was transferred from Annex IIAII to Annex IAII (= Union Quarantine pest in December 2019). Also, special requirements are put on movement within the Union territory of machinery and vehicles which have been operated for agricultural or forestry purpose (Annex VIII). It is stated that “The machinery or vehicles have been:

a) moved from an area free from *Ceratocystis platani* (J.M. Walter) Engelbr. & T.C. Harr., established by the competent authorities in accordance with the relevant International Standards for Phytosanitary Measures, or
b) cleaned and made free from soil and plant debris prior to movement out of the infected area”.

2.6 Benefits from trans-national cooperation

Because of a mix of the right set of partners, genotyping of *Cryphonectria parasitica* populations was possible using a large set of isolates coming from the personal collections of the persons/countries involved (e.g. France, Belgium, Portugal, Switzerland, United Kingdom). Synergies clearly showed up as different partners were familiar with different techniques (for example SSR vs GBS). In the starting up meeting in October 2016, the EPPO Diagnostic Protocols for both organisms were mentioned and discussed. Materials and knowledge developed within CERACRY will give a boost in updating both protocols. For example, the validation data obtained in the TPS on *C. platani* can be incorporated in a new version of the EPPO DP on this pathogen, and also added to the EPPO Validation database.

3. Publications

3.1. Article(s) for publication in the EPPO Bulletin

None.

3.2. Article for publication in the EPPO Reporting Service

None.

3.3. Article(s) for publication in other journals

A manuscript on the results of the genotyping of isolates of the international collection of *Cryphonectria parasitica* using SSR and GBS techniques is currently in preparation for submission in a peer-reviewed journal such as Molecular Ecology. This will probably be in the second half of 2019 or 2020.

Publication of results regarding the Test Performing Study on *C. platani* detection has been planned. Writing of an article is in progress and submission is expected in the first half of 2020.

4. Open Euphresco data

Validation data will be published in the EPPO Database for diagnostic expertise.

Appendix 1. List of *Cryphonectria parasitica* isolates in the CERACRY-collection at CRAW-Belgium and their characteristics

N°	CRAW Code	Partner Code	Origin	Place of collection	Contact person	Environment	Year
1	4714	4714	Belgium	Jette	Chandelier A.	urban tree	2014
2	4774	4774	Belgium	Jette	Chandelier A.	urban tree	2015
3	4775	4775	Belgium	Jette	Chandelier A.	urban tree	2015
4	4776	4776	Belgium	Jette	Chandelier A.	urban tree	2015
5	4777	4777	Belgium	Jette	Chandelier A.	urban tree	2015
6	4778	4778	Belgium	Jette	Chandelier A.	urban tree	2015
7	4779	4779	Belgium	Jette	Chandelier A.	urban tree	2015
8	4780	4780	Belgium	Jette	Chandelier A.	urban tree	2015
9	4781	4781	Belgium	Jette	Chandelier A.	urban tree	2015
10	4782	4782	Belgium	Jette	Chandelier A.	urban tree	2015
11	4783	4783	Belgium	Jette	Chandelier A.	urban tree	2015
12	4784	4784	Belgium	Jette	Chandelier A.	urban tree	2015
13	4785	4785	Belgium	Jette	Chandelier A.	urban tree	2015
14	4786	4786	Belgium	Jette	Chandelier A.	urban tree	2015
15	4787	4787	Belgium	Jette	Chandelier A.	urban tree	2015
16	4789	4789	Belgium	Jette	Chandelier A.	urban tree	2015
17	4790	4790	Belgium	Jette	Chandelier A.	urban tree	2015
18	4792	4792	Belgium	Jette	Chandelier A.	urban tree	2014
19	4793	4793	Belgium	Jette	Chandelier A.	urban tree	2014
20	4794	4794	Belgium	Auderghem	Chandelier A.	chestnut stake	2015
21	4801	4801	Belgium	West Flanders	Chandelier A.	isolated tree	2015
22	4839	DCP2015003323	Belgium	Moen	Heungens K.	isolated tree	2015
23	4840	DCP2015004144	Belgium	Wichelen	Heungens K.	isolated tree	2015
24	4932	DCP201600701	Belgium	Maldegem	Heungens K.	isolated tree	2016

25	4933	INBO De Haeck	Belgium	na	Heungens K.	isolated tree	2016
26	4940	4940	Belgium	Stambruges	Chandelier A.	Plantation	2016
27	4943	4943	Belgium	Colfontaine site 1	Chandelier A.	Plantation	2016
28	5024	5024	Belgium	Colfontaine site 1	Chandelier A.	Plantation	2017
29	5025	5025	Belgium	Colfontaine site 2	Chandelier A.	Plantation	2017
30	5069	819	Cech Rep.	Moravský	Svobodová I	ornamental nursery	2004
31	5070	851	Cech Rep.	Rohovec	Svobodová I	private garden	2006
32	5071	852	Cech Rep.	Kuřim	Svobodová I	public park	2006
33	5072	876	Cech Rep.	Žatčany	Svobodová I	forest nursery	2006
34	5073	1201	Cech Rep.	Hovorčovice	Svobodová I	import from Italy	2009
35	5105	DOT. T1	Croatia	Zagreb	Diminic D, Kranjec J	forest	2017
36	5106	DOT. T2	Croatia	Zagreb	Diminic D, Kranjec J	forest	2017
37	5107	DOT. T3	Croatia	Zagreb	Diminic D, Kranjec J	forest	2017
38	5108	DOT. T4	Croatia	Zagreb	Diminic D, Kranjec J	forest	2017
39	5109	DOT. T5	Croatia	Zagreb	Diminic D, Kranjec J	forest	2017
40	5110	PET. T1	Croatia	Petrinja	Diminic D, Kranjec J	forest	2017
41	5111	PET. T3	Croatia	Petrinja	Diminic D, Kranjec J	forest	2017
42	5112	PET. T4	Croatia	Petrinja	Diminic D, Kranjec J	forest	2017
43	5113	PET. T5	Croatia	Petrinja	Diminic D, Kranjec J	forest	2017
44	5114	PET. T6	Croatia	Petrinja	Diminic D, Kranjec J	forest	2017
45	5115	PAZ. T1	Croatia	Pazin	Diminic D, Kranjec J	forest	2017
46	5116	PAZ. T2	Croatia	Pazin	Diminic D, Kranjec J	forest	2017

47	5117	PAZ. T3	Croatia	Pazin	Diminic D, Kranjec J	forest	2017
48	5118	PAZ. T5	Croatia	Pazin	Diminic D, Kranjec J	forest	2017
49	5119	PAZ. T6	Croatia	Pazin	Diminic D, Kranjec J	forest	2017
50	5026	PA'LHA'ZA	Hungary	Filekháza	Halász A.	plantation	2012
51	5049	C8 [14PA1N13]	Italy	San Martini al Cimino	Vettraino AM	forest	2017
52	5050	C16 [1PB2N5]	Italy	San Martini al Cimino	Vettraino AM	forest	2017
53	5051	C17 [1PB2N7]	Italy	San Martini al Cimino	Vettraino AM	forest	2017
54	5052	C24 [3FP1BG24]	Italy	Ronciglione	Vettraino AM	forest	2017
55	5053	C25 [3FP1BG25]	Italy	Ronciglione	Vettraino AM	forest	2017
56	5054	C34 [3PD1B5]	Italy	Soriano al Cimino	Vettraino AM	forest	2017
57	5055	C63 [C1B]	Italy	Monte Fogliano	Vettraino AM	forest	2017
58	5056	C68 [GMC1G]	Italy	Monte Fogliano	Vettraino AM	forest	2017
59	5057	C69 [GMC2A]	Italy	Monte Fogliano	Vettraino AM	forest	2017
60	5058	C81 [C18GMC]	Italy	Monte Fogliano	Vettraino AM	forest	2017
61	5059	C84 [C49CRA]	Italy	Monte Fogliano	Vettraino AM	forest	2017
62	5060	C86 [C19CRE]	Italy	Monte Fogliano	Vettraino AM	forest	2017
63	5061	C87 [C20DB]	Italy	Monte Fogliano	Vettraino AM	forest	2017
64	5062	C97 [C155]	Italy	Monte Fogliano	Vettraino AM	forest	2017
65	5063	C103 [C161]	Italy	Monte Fogliano	Vettraino AM	forest	2017
66	5027	95/6040	Netherlands	Geleen , Limburg	De Gruyter J.	urban tree	1995
67	5074	C0001	Portugal	Trás-os-Montes	Bragança H	Plantation/Orchard/farm	2001
68	5075	C0002	Portugal	Trás-os-Montes	Bragança H	Plantation/Orchard/farm	2001
69	5076	C0003	Portugal	Trás-os-Montes	Bragança H	Plantation/Orchard/farm	2003
70	5077	C0004	Portugal	Beira Interior	Bragança H	Plantation/Orchard/farm	2001
71	5078	C0006	Portugal	Alentejo	Bragança H	Plantation/Orchard/farm	2002
72	5079	C0007	Portugal	Trás-os-Montes	Bragança H	Plantation/Orchard/farm	2003

73	5080	C0008	Portugal	Terceira, Azores	Bragança H	Plantation/Orchard/farm	2003
74	5081	C0009	Portugal	Madeira	Bragança H	Forest	2003
75	5082	C0014	Portugal	Terceira, Azores	Bragança H	Plantation/Orchard/farm	2003
76	5083	C0016	Portugal	Entre Douro e Minho	Bragança H	Plantation/Orchard/farm	2003
77	5084	C0645	Portugal	Beira Interior	Bragança H	Plantation/Orchard/farm	2001
78	5085	C0652	Portugal	Beira Interior	Bragança H	Forest	2001
79	5086	C0665	Portugal	Alentejo	Bragança H	Plantation/Orchard/farm	2001
80	5087	C0671	Portugal	Beira Interior	Bragança H	Plantation/Orchard/farm	2001
81	5088	C0720	Portugal	Trás-os-Montes	Bragança H	Plantation/Orchard/farm	2002
82	5089	C0722	Portugal	Terceira, Azores	Bragança H	Plantation/Orchard/farm	2003
83	5068	766	Slovakia	Prašice	Svobodová I	na	na
84	5029	UK1	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
85	5030	UK2	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
86	5031	UK3	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
87	5032	UK4	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
88	5033	UK5	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
89	5034	UK6	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
90	5035	UK7	Unted Kingdom	Devon	Perez Sierra A.	Car park	2017
91	5036	UK8	Unted Kingdom	Devon	Perez Sierra A.	Car park	2017
92	5037	UK9	Unted Kingdom	Devon	Perez Sierra A.	Car park	2017
93	5038	UK10	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
94	5039	UK11	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
95	5040	UK12	Unted Kingdom	Dorset	Perez Sierra A.	Plantation	2017
96	5041	UK13	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
97	5042	UK14	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
98	5043	UK15	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
99	5044	UK16	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
100	5045	UK17	Unted Kingdom	Devon	Perez Sierra A.	Plantation	2017
101	5046	UK18	Unted Kingdom	Warwickshire	Perez Sierra A.	Farm	2011

102	5047	UK19	Unted Kingdom	Warwickshire	Perez Sierra A.	Farm	2011
103	5048	UK20	Unted Kingdom	East Sussex	Perez Sierra A.	Farm	2011

Appendix 2. Survey results, occurrence of *Cryphonectria parasitica* in four Northwestern countries in Europe

		2015			2016			2017			
Country	Environment	Vol.	Method	Results	Vol.	Method	Results	Vol.	Method	Results	status
Belgium	Forest	1	visual, isolation & qPCR[1]	negative	22	visual, isolation & qPCR[1]	**positive (2/22)**	35	visual, isolation & qPCR[1]	**positive (2/35)**	present in limited areas
	Nurseries	134	visual	negative	127	visual	negative	123	visual, isolation & qPCR[1]	**positive (1/123)**	
	Urban trees	1	visual, isolation & qPCR[1]	**positive**	3	visual, isolation & qPCR[1]	negative	4	visual, isolation & qPCR[1]	negative	
	Parks	-	-	-	8	visual, isolation & qPCR[1]	negative	5	visual, isolation & qPCR[1]	**positive (1/5)**	
	Chestnut stakes	2	visual, isolation & qPCR[1]	**positive (2/2)**	-	-	-	-	-	-	
Ireland	Forest	14	visual	negative	18	visual	negative	15	visual, isolation	negative	absent
	Nurseries	3	visual, isolation	negative	3	visual	negative	3	visual	negative	
	Urban trees	-	-	-	-	-	-	-	-	-	
	Parks	-	-	-	-	-	-	-	-	-	
	Chestnut stakes	-	-	-	-	-	-	-	-	-	
The Netherlands	Forest, urban trees/ park	10	damp chamber & isolation	negative	10	damp chamber & isolation	negative	10	damp chamber & isolation	negative	absent[2]
	Nurseries	115	damp chamber & isolation	negative	115	damp chamber & isolation	negative	115	damp chamber & isolation	negative	
	Chestnut stakes	-	-	-	-	-	-	-	-	-	
United Kingdom	Forest	-	-	-	-	-	-	14	visual, isolation & qPCR[1]	**positive**	present in limited areas, under eradication
	Nurseries	-	-	-	-	-	-	1	visual, isolation & qPCR[1]	**positive**	
	Urban trees	-	-	-	1	isolation & sequencing	**positive**	1	visual, isolation & qPCR[1]	**positive**	
	Parks	-	-	-	-	-	-	5	visual, isolation & qPCR[1]	**positive**	
	Chestnut stakes	-	-	-	-	-	-	-	-	-	

[1]Chandelier et al. (2019)

[2]In 2018, several findings in the Netherlands on imported *Castanea* stakes/fences