Supplementary Material

Fig. S1 - Repeatability of the detection limit of the three Real-Time PCR assays. Experiments were performed by two different operators. Detection limit refers to the lowest quantity of *Ceratocystis platani* gDNA that is detected per PCR reaction (i.e. in-mycelium DQP-ASe). gDNA extracted from a pure culture directly grown on PDA. Each bar represents the mean of eight values. Letters indicate homogeneous groups (99.0% confidence level). Numbers indicate the independent statistical analyses. Vertical lines indicate standard error of the mean.

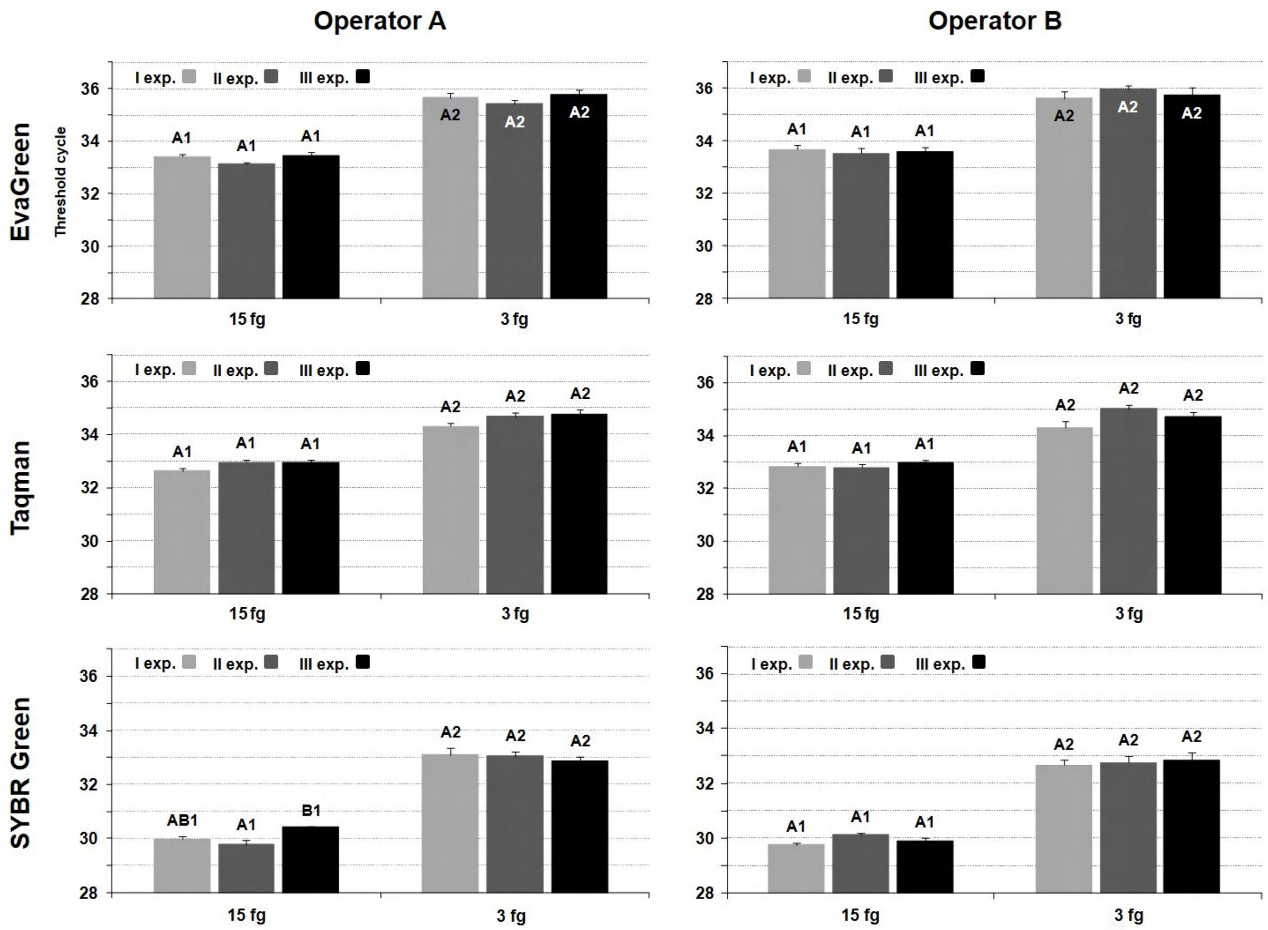


Fig. S2 - Further validation for using the cycling protocol in conventional PCR. Agarose gel analysis of Real-Time PCR amplicons obtained from reactions charged with different volumes of wood extracts obtained from *Ceratocystis platani* naturally-infected trees. Amplicons are compared with a GeneRuler 50 bp DNA ladder (Thermo Fisher Scientific). We marked the 100 bp molecule with a white line where it was too faint to be visualized.

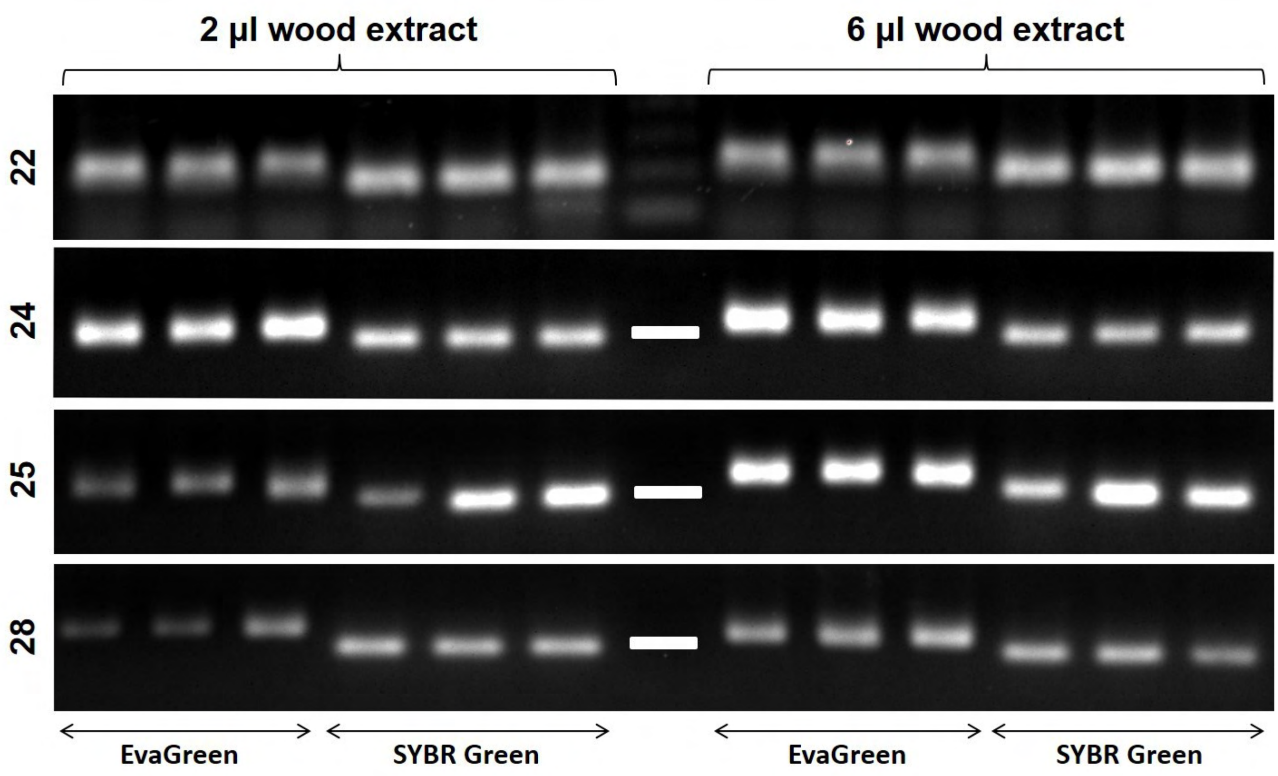


Fig. S3 - The validation and implementation of Real-Time PCR carried out in this work.

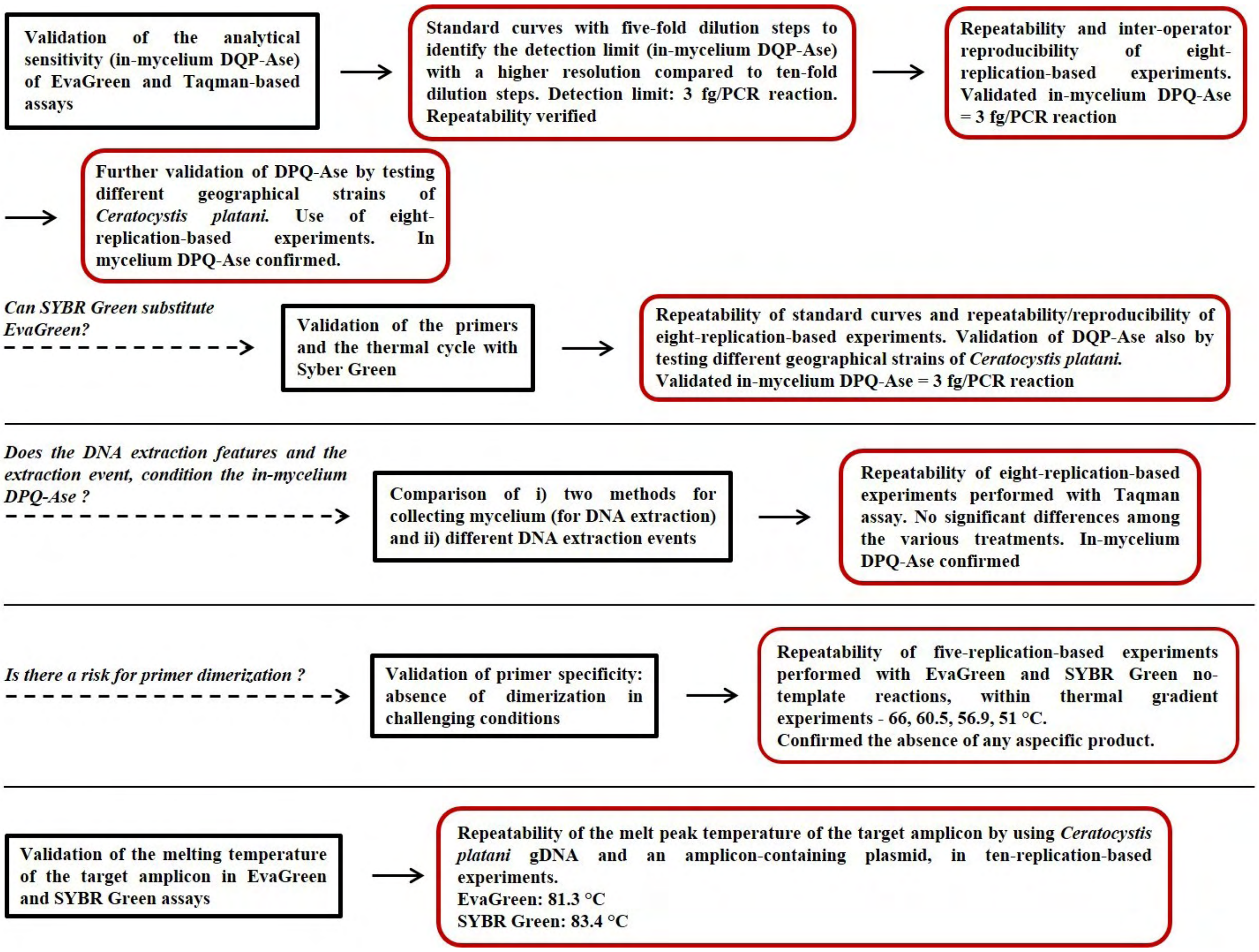
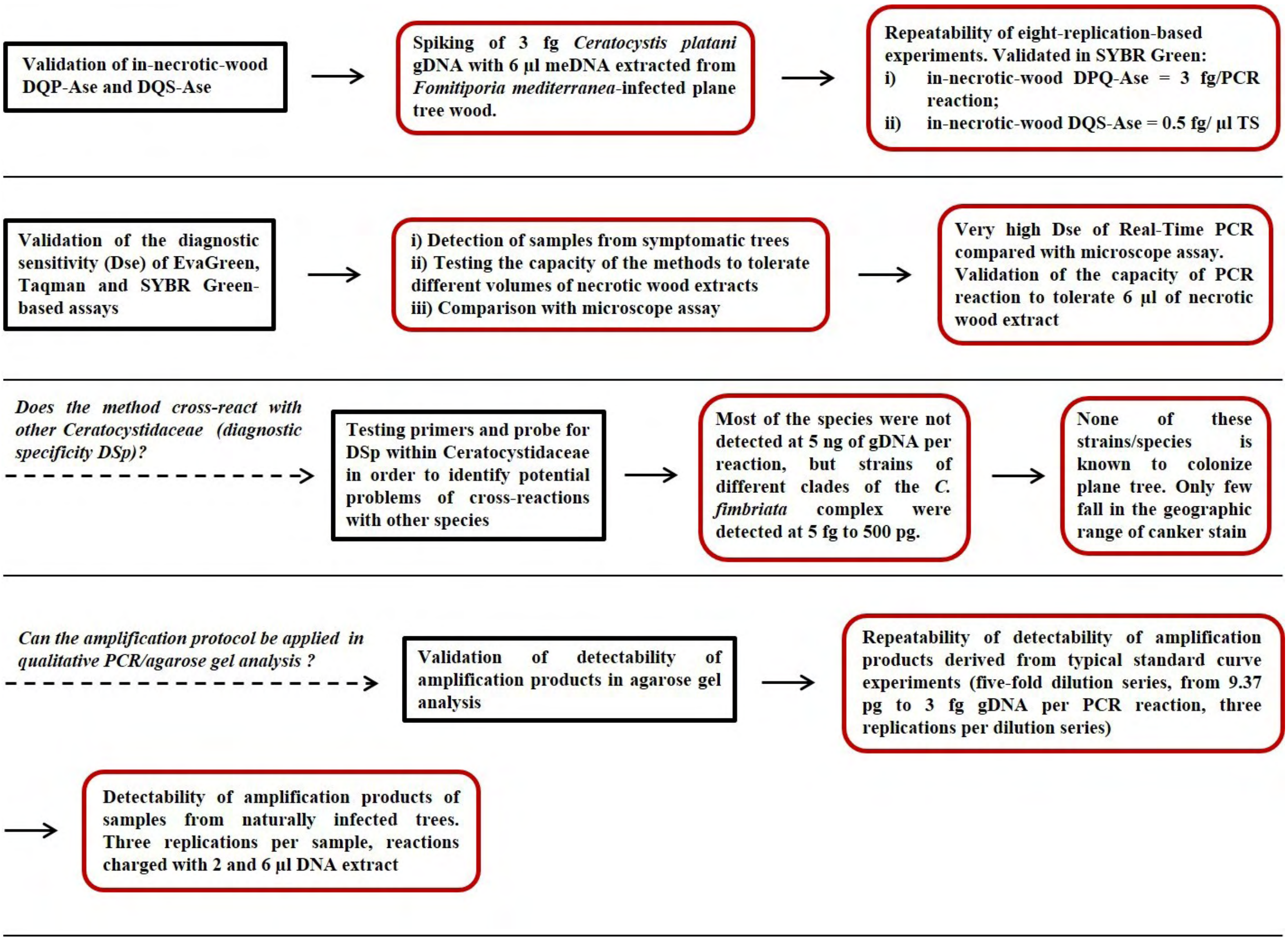


Fig. S3 (continued) - The validation and implementation of Real-Time PCR carried out in this work.



Appendix 1 - Details on methods of Real-Time PCR for detection of *Ceratocystis platani*. DNA extraction, primer and probe sequences, reaction composition, thermal cycling conditions, fluorescence capture features, and cloning the PCR target into a plasmid.

Nucleic acid extraction and quantification

Regarding DNA extraction from pure fungal cultures, mycelium (100 mg) was collected with a sterile pipette tip by scraping from an actively growing culture directly cultured on the surface of a PDA medium (agar modality) or on sterile cellophane discs layered on PDA surface (cellophane modality).

The extraction from wood, either healthy or *C. platani*-infected, started from a 100 mg aliquot. Fungal and wood tissue aliquots were grinded to powder with liquid nitrogen using an autoclaved pestle and mortar and DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

DNA was quantified using a Thermo Scientific NanoDrop™ 1000 Spectrophotometer.

Molecular target of the Real-Time PCR

Internal Transcribed Spacer 1 region (ITS1).

Amplicon location: from position 89 to 183 of the *C. platani* reference sequence DQ399853.

Expected size of the amplicon: 95bp (including primer sequences).

Primers and probe

Forward primer: C.P.Sn.For.I 5'-CGTACCTATCTTGTAGTGAGATGAATGC-3' (from position 89 to 116 of the *C. platani* reference sequence DQ399853).

Reverse primer: C.P.Sn.Rev.I 5'-GAGTTTACAGTGGCGAGACTATACTG-3' (from position 158 to 183).

Taqman probe: C.P.TM.Pr. 5'-CGGTGCCCTTCAGAAGGGCCCTACCACC-3', (from position 123 to 150).

The probe was labeled with FAM (6- carboxy-fluorescein) at 5' end, and contained Black Hole Quencher™ 1 (BHQ-1) at 3' end.

Supermixes (BIO-RAD)

SsoFast™ EvaGreen® Supermix (EvaGreen assay)

SsoAdvanced™ Universal Probes Supermix (Taqman assay)

SsoAdvanced™ Universal SYBR® Green Supermix (SYBR Green assay)

All three supermixes contain dNTPs, antibody-mediated hot-start Sso7d-fusion polymerase, $MgCl_2$, and stabilizers). In addition a blend of passive reference dyes, including ROX and fluorescein, is contained in the supermix for use in Taqman assay.

Reaction assembly

Reagent	Working concentration	Volume per reaction (µl)	Final concentration
Molecular grade water [1]	N.A.	to make up to 20	N.A.
Supermix	2 x	10	1x
Forward Primer (C.P.Sn.For.I)	10 µmol l^{-1}	1	0.5 µmol l^{-1}
Reverse Primer (C.P.Sn.Rev.I)	10 µmol l^{-1}	1	0.5 µmol l^{-1}
Probe 1 (C.P.TM.Pr.)	20 µmol l^{-1}	0.3	0.3 µmol l^{-1}
Subtotal		variable	
Diluted DNA sample		It is possible up to a maximum of 8 or 7.7 (if the probe is used)	variable
Total		20	

[1] Molecular grade water should be preferably used or prepared purified (deionised or distilled), sterile (autoclaved or 0.45 µm filtered) and nuclease-free.

Fluorescence capture

EvaGreen has a maximum absorbance at 500 nm and a maximum emission at 530 nm. SYBR Green has a maximum absorbance at 497 nm, with a secondary excitation peak near 254 nm. Maximum emission is at 520 nm. 6-FAM (6-carboxy-fluorescein) has a maximum absorbance at 495 nm and a maximum emission at 518 nm.

For all three fluorophores the dedicated excitation led in CFX 96 was channel 1 (450-490 nm) and the photo-body detector was channel 1 (515-530nm).

Cloning

The complete ITS region of *C. platani* was amplified using ITS5/ITS4 primers. The PCR product was analyzed by agarose gel electrophoresis, excised from gel, eluted and cloned into pCR®2.1 plasmid (Invitrogen) and sequenced, as previously described (Pilotti et al. 2010)

References

Pilotti M, Tizzani L, Brunetti A, Gervasi F, Di Lernia G, Lumia V (2010). Molecular identification of *Fomitiporia mediterranea* on declining and decayed hazelnut. Journal of Plant Pathology 92: 115-129.

Tab. S1 - Statistical analysis to compare the different groups of Ct values. The groups represent different experiments (repeatability), different operators (reproducibility), extraction modalities and extraction events, as well as the different Real-Time PCR assays. Null hypotheses were the normal distribution of data, the homogeneity of variances (homoscedasticity), the lack of statistically significant difference among mean/median of data group. Acceptance of null hypothesis was at P-value > 0.05. In the post-hoc analysis, acceptance of null hypothesis was at a P-value > 0.01. Results of post-hoc analyses are depicted in figures 2, 3, 5, 6 and S1. [1] EG = EvaGreen, Tq = Taqman, SG = SYBR Green. [2] The column indicates which group data have a normal or non-normal distribution (+ and - respectively) for each comparison.

Statistical comparisons [1]		**Normality test (Shapiro-Wilk)** [2]	**Levene omogeneity of variance p-value**	**Test statistic (Mean/median comparison)**	**Statistic value**	**P-value**	**Post-hoc test (pairwise comparison)**
Repeatability, comparison of three experiments per operator and per DNA quantity (3 group data per comparison, each group with 8 replications) Fig. 2	Operator A EG 15fg	2+, 1-	0.7394	Kruskal-Wallis	H = 7.099	0.02857	Mann-Whitney Bonferroni corrected
	Operator A EG 3fg	3+	0.6741	ANOVA	F = 1.44	0,2584	Student-Newman-Keuls
	Operator A Tq 15fg	3+	0.9646	ANOVA	F = 4.192	0.02939	Student-Newman-Keuls
	Operator A Tq 3fg	2+, 1-	0.2288	Kruskal-Wallis	H = 4.999	0.08169	Mann-Whitney Bonferroni corrected
	Operator A SG 15fg	3+	3.634E-09	Welch	F = 15.23	0.0006169	Tukey
	Operator A SG 3fg	3+	0.2307	ANOVA	F = 0.3028	0.7419	Student-Newman-Keuls
	Operator B EG 15fg	3+	0.6708	ANOVA	F = 0.2024	0.8184	Student-Newman-Keuls

Statistical comparisons [1]		**Normality test (Shapiro-Wilk)** [2]	**Levene omogeneity of variance p-value**	**Test statistic (Mean/median comparison)**	**Statistic value**	**P-value**	**Post-hoc test (pairwise comparison)**
	Operator B EG 3fg	3+	0.1071	ANOVA	F = 0.5973	0.5594	Student-Newman-Keuls
	Operator B Tq 15fg	3+	0.5196	ANOVA	F = 0.8965	0.423	Student-Newman-Keuls
	Operator B Tq 3fg	3+	0.05973	ANOVA	F = 3.349	0.05464	Student-Newman-Keuls
	Operator B SG 15fg	2+, 1-	0.04136	Kruskal-Wallis	H = 6.045	0.04862	Mann-Whitney Bonferroni corrected
	Operator B SG 3fg	3+	0.5722	ANOVA	F = 0.1725	0.8427	Student-Newman-Keuls
inter-operator reproducibility, comparison between PCR-performer-operator A and B (2 group data per comparison, each group with 24 replications) Fig. 3	EG 15fg	2+	0.007303	Welch	F = 4.887	0.03291	Tukey
	EG 3fg	2+	0.1576	ANOVA	F = 0.97	0.3298	Student-Newman-Keuls
	Tq 15fg	2+	0.8316	ANOVA	F = 0.04117	0.8401	Student-Newman-Keuls
	Tq 3fg	1+, 1-	0.4054	Kruskal-Wallis	H = 0.5821	0.4452	Mann-Whitney Bonferroni corrected
	SG 15fg	1+, 1-	0.4458	Kruskal-Wallis	H = 2.894	0.08888	Mann-Whitney Bonferroni corrected

Statistical comparisons [1]		Normality test (Shapiro-Wilk) [2]	Levene omogeneity of variance p-value	Test statistic (Mean/median comparison)	Statistic value	P-value	Post-hoc test (pairwise comparison)
	SG 3fg	2+	0.366	ANOVA	F = 2.08	0.156	Student-Newman-Keuls
Comparison of the three assays (3 group data per comparison, each group with 48 replications) Fig. 4a	EG vs Tq vs SG 15 fg	2+, 1-	0.00793	Friedman	Chi^2 = 83.344	6.6042-19 Asymptotic	Wilcoxon Bonferroni corrected
	EG vs Tq vs SG 3 fg	3+	0.5966	ANOVA	F = 304.9	6.272E-52	Student-Newman-Keuls
Comparison of the three assays with additional *C. platani* strains, one experiment per assay and per fungal strain (3 group data per comparison, each group with 8 replications) Fig. 4b	CP 25	3+	0.5025	ANOVA	F = 14.44	0,00011	Student-Newman-Keuls
	CP 33	3+	0.12	ANOVA	F = 60.42	1.948E-09	Student-Newman-Keuls
	CP 1176	2+, 1-	0.01554	Friedman	Chi^2 = 14.25	6.1E-05 (exact)	Wilcoxon Bonferroni corrected
	CP 11292	3+	0.1524	ANOVA	F = 93.58	3.468E-11	Student-Newman-Keuls
	CP 11259	2+, 1-	0.8794	Kruskal-Wallis	H = 17.78	0.0001378	Mann-Whitney Bonferroni corrected

Statistical comparisons [1]		**Normality test (Shapiro-Wilk)** [2]	**Levene omogeneity of variance p-value**	**Test statistic (Mean/median comparison)**	**Statistic value**	**P-value**	**Post-hoc test (pairwise comparison)**
Repeatability, comparison of three experiments per DNA-purifier-operator and per DNA quantity (3 group data per comparison, each group with 8 replications) Fig. 6a	Operator A agar 15 fg	3+	0.04837	Welch	F = 1.486	0.2643	Tukey
	Operator A agar 3fg	2+, 1-	0.6387	Kruskal-Wallis	H = 3.571	0.1673	Mann-Whitney Bonferroni corrected
	Operator C agar 15 fg	3+	0.3289	ANOVA	F = 0.4134	0.6667	Student-Newman-Keuls
	Operator C agar 3fg	3+	0.2583	ANOVA	F = 1.604	0.2247	Student-Newman-Keuls
	Operator A cellophane 15 fg	2+, 1-	0.5623	Kruskal-Wallis	H = 2.176	0.3364	Mann-Whitney Bonferroni corrected
	Operator A cellophane 3 fg	2+, 1-	0.5487	Kruskal-Wallis	H = 2.889	0.2353	Mann-Whitney Bonferroni corrected
	Operator C cellophane, 15 fg	3+	0.6519	ANOVA	F = 3.789	0.03935	Student-Newman-Keuls
	Operator C cellophane 3 fg	3+	0.1988	ANOVA	F = 0.1319	0.8771	Student-Newman-Keuls
Inter-operator reproducibility, comparison between DNA-purifier-operator A and C (2 group data per comparison, each group with 24 replications) Fig. 6b	Agar A vs C 15 fg	2+	0.4865	ANOVA	F = 0.1376	0.7124	Student-Newman-Keuls
	Agar A vs C 3 fg	2+	0.4089	ANOVA	F = 0.2345	0.6305	Student-Newman-Keuls
	Cellophane A vs C 15 fg	2+	0.9054	ANOVA	F = 1.928	0.1717	Student-Newman-Keuls

	Cellophane A vs C 3 fg	1+, 1-	0.8446	Welch	F = 6.161	0.01679	Tukey
Comparison of the DNA extraction modalities (2 group data per comparison each group with 48 replications) Fig. 6c	Agar vs Cell. 15 fg	2+	0.1042	ANOVA	F = 5.077	0.02658	Student-Newman-Keuls
	Agar vs Cell. 3 fg	2+	0.6198	ANOVA	F = 12.47	0.000643	Student-Newman-Keuls
Repeatability, comparison of three experiments (3 group data each group with 8 replications) Fig. 8	Spiking experiments	3+	0.8191	ANOVA	F = 1.752	0.1979	Student-Newman-Keuls
Repeatability, comparison of the melting temperature of two DNA types, in EG [2 group data, each group with 20 replications (sum of 2 experiments)] see the text	gDNA vs pDNA	2-	0.3236	Kruskal-Wallis	H = 0.01829	0.8774	Mann-Whitney Bonferroni corrected
Repeatability, comparison of the melting temperature of two DNA types, in SG [2 group data, each group with 20 replications (sum of 2 experiments)] see the text	gDNA vs pDNA	2-	0.3867	Kruskal-Wallis	H = 0.08854	0.723	Mann-Whitney Bonferroni corrected
Comparison between the melting temperature of EG and SG assays (2 group data, each group with 40 replications) see the text	EG vs SG	2-	0.09582	Kruskal-Wallis	H = 59.26	1.658E-15	Mann-Whitney Bonferroni corrected

Tab. S2 - Data groups used for the statistical analysis depicted in Tab. S1, in the figures 2, 3, 5, 6 and S1 and in the text.

Section 1 - Detection of 3 fg *Ceratocystis platani* gDNA. Repeatability: comparison among three experiment (Exp.) performed by Operator (Op) A and B. See Fig. S1. Inter-operator reproducibility. See Fig. 2. EvaGreen (EG) vs Taqman (Tq) vs SYBR Green (SG): See Fig. 3a. In bold (the third column of each dataset) mean, standard deviation and standard error of the replication series are reported.

1. Repeatability, PCR-performer-operator A

EvaGreen			**Taqman**			**SYBR Green**		
I Exp.	35,16	**35,66**	I Exp.	34,02	**34,31**	I Exp.	34,2	**33,10**
I Exp.	35,95	**0,51789**	I Exp.	34,62	**0,37133**	I Exp.	32,71	**0,78691**
I Exp.	35,32	**0,1831**	I Exp.	34,39	**0,13128**	I Exp.	33,53	**0,27821**
I Exp.	36,45		I Exp.	34,13		I Exp.	32,53	
I Exp.	36,10		I Exp.	34,02		I Exp.	33,21	
I Exp.	35,76		I Exp.	34,06		I Exp.	31,66	
I Exp.	34,89		I Exp.	34,14		I Exp.	33,27	
I Exp.	35,64		I Exp.	35,07		I Exp.	33,69	
II Exp.	35,45	**35,41**	II Exp.	34,30	**34,68**	II Exp.	33,28	**33,05**
II Exp.	35,17	**0,3854**	II Exp.	34,62	**0,40377**	II Exp.	32,57	**0,42725**
II Exp.	35,11	**0,13626**	II Exp.	34,43	**0,14275**	II Exp.	33,46	**0,15106**
II Exp.	35,68		II Exp.	35,12		II Exp.	32,83	
II Exp.	35,51		II Exp.	35,18		II Exp.	33,01	
II Exp.	35,19		II Exp.	35,14		II Exp.	33,03	
II Exp.	36,17		II Exp.	34,21		II Exp.	32,49	
II Exp.	34,98		II Exp.	34,43		II Exp.	33,73	
III Exp.	35,48	**35,79**	III Exp.	35,25	**34,76**	III Exp.	32,7	**32,88**
III Exp.	36,00	**0,43911**	III Exp.	34,99	**0,5468**	III Exp.	32,7	**0,4626**
III Exp.	35,24	**0,15525**	III Exp.	35,34	**0,19332**	III Exp.	33,56	**0,16355**
III Exp.	35,63		III Exp.	35,36		III Exp.	33,12	
III Exp.	35,92		III Exp.	34,57		III Exp.	32,37	
III Exp.	35,60		III Exp.	34,39		III Exp.	32,22	
III Exp.	36,70		III Exp.	34,06		III Exp.	33,28	
III Exp.	35,70		III Exp.	34,10		III Exp.	33,12	

2. Repeatability, PCR-performer-operator B

EvaGreen			**Taqman**			**SYBR Green**		
I Exp.	34,42	**35,61**	I Exp.	35,10	**34,31**	I Exp.	32,57	**32,67**
I Exp.	35,74	**0,71393**	I Exp.	33,65	**0,70892**	I Exp.	32,15	**0,55904**
I Exp.	34,88	**0,25241**	I Exp.	33,74	**0,25064**	I Exp.	32,23	**0,19765**
I Exp.	35,51		I Exp.	33,34		I Exp.	33,04	
I Exp.	36,47		I Exp.	34,61		I Exp.	33,29	
I Exp.	36,51		I Exp.	34,00		I Exp.	32,75	
I Exp.	35,78		I Exp.	34,81		I Exp.	33,43	
I Exp.	35,61		I Exp.	35,19		I Exp.	31,89	
II Exp.	35,31	**35,96**	II Exp.	34,59	**35,01**	II Exp.	33,62	**32,77**
II Exp.	36,01	**0,39981**	II Exp.	34,23	**0,46479**	II Exp.	32,35	**0,58426**
II Exp.	35,74	**0,14135**	II Exp.	35,35	**0,16433**	II Exp.	32,92	**0,20657**
II Exp.	36,67		II Exp.	35,76		II Exp.	33,23	
II Exp.	35,84		II Exp.	35,07		II Exp.	31,95	
II Exp.	35,84		II Exp.	35,01		II Exp.	32,32	
II Exp.	35,99		II Exp.	34,89		II Exp.	33,31	
II Exp.	36,28		II Exp.	35,19		II Exp.	32,47	
III Exp.	36,47	**35,74**	III Exp.	35,12	**34,73**	III Exp.	31,68	**32,86**
III Exp.	35,42	**0,74634**	III Exp.	34,78	**0,42787**	III Exp.	33,74	**0,75661**
III Exp.	36,72	**0,26387**	III Exp.	35,19	**0,15128**	III Exp.	32,26	**0,2675**
III Exp.	35,10		III Exp.	34,05		III Exp.	33,7	
III Exp.	34,78		III Exp.	34,62		III Exp.	32,8	
III Exp.	36,39		III Exp.	34,59		III Exp.	32,98	
III Exp.	35,06		III Exp.	34,27		III Exp.	33,46	
III Exp.	36,01		III Exp.	35,19		III Exp.	32,23	

3. Inter-operator reproducibility

EvaGreen			**Taqman**			**SYBR Green**		
Op. A	35,16	**35,62**	Op. A	34,02	**34,58**	Op. A	34,2	**33,01**
Op. A	35,95	**0,45943**	Op. A	34,62	**0,47253**	Op. A	32,71	**0,56397**
Op. A	35,32	**0,09378**	Op. A	34,39	**0,09646**	Op. A	33,53	**0,11512**
Op. A	36,45		Op. A	34,13		Op. A	32,53	
Op. A	36,10		Op. A	34,02		Op. A	33,21	
Op. A	35,76		Op. A	34,06		Op. A	31,66	
Op. A	34,89		Op. A	34,14		Op. A	33,27	
Op. A	35,64		Op. A	35,07		Op. A	33,69	
Op. A	35,45		Op. A	34,30		Op. A	33,28	
Op. A	35,17		Op. A	34,62		Op. A	32,57	
Op. A	35,11		Op. A	34,43		Op. A	33,46	
Op. A	35,68		Op. A	35,12		Op. A	32,83	
Op. A	35,51		Op. A	35,18		Op. A	33,01	
Op. A	35,19		Op. A	35,14		Op. A	33,03	
Op. A	36,17		Op. A	34,21		Op. A	32,49	
Op. A	34,98		Op. A	34,43		Op. A	33,73	
Op. A	35,48		Op. A	35,25		Op. A	32,7	
Op. A	36,00		Op. A	34,99		Op. A	32,7	
Op. A	35,24		Op. A	35,34		Op. A	33,56	
Op. A	35,63		Op. A	35,36		Op. A	33,12	
Op. A	35,92		Op. A	34,57		Op. A	32,37	
Op. A	35,60		Op. A	34,39		Op. A	32,22	
Op. A	36,70		Op. A	34,06		Op. A	33,28	
Op. A	35,70		Op. A	34,10		Op. A	33,12	
Op. B	34,42	**35,77**	Op. B	35,10	**34,68**	Op. B	32,57	**32,7654**
Op. B	35,74	**0,62795**	Op. B	33,65	**0,60166**	Op. B	32,15	**0,61593**
Op. B	34,88	**0,12818**	Op. B	33,74	**0,12281**	Op. B	32,23	**0,12573**
Op. B	35,51		Op. B	33,34		Op. B	33,04	
Op. B	36,47		Op. B	34,61		Op. B	33,29	
Op. B	36,51		Op. B	34,00		Op. B	32,75	
Op. B	35,78		Op. B	34,81		Op. B	33,43	
Op. B	35,61		Op. B	35,19		Op. B	31,89	
Op. B	35,31		Op. B	34,59		Op. B	33,62	
Op. B	36,01		Op. B	34,23		Op. B	32,35	
Op. B	35,74		Op. B	35,35		Op. B	32,92	
Op. B	36,67		Op. B	35,76		Op. B	33,23	
Op. B	35,84		Op. B	35,07		Op. B	31,95	
Op. B	35,84		Op. B	35,01		Op. B	32,32	
Op. B	35,99		Op. B	34,89		Op. B	33,31	
Op. B	36,28		Op. B	35,19		Op. B	32,47	
Op. B	36,47		Op. B	35,12		Op. B	31,68	

3. Inter-operator reproducibility

EvaGreen		**Taqman**		**SYBR Green**	
Op. B	35,42	Op. B	34,78	Op. B	33,74
Op. B	36,72	Op. B	35,19	Op. B	32,26
Op. B	35,10	Op. B	34,05	Op. B	33,7
Op. B	34,78	Op. B	34,62	Op. B	32,8
Op. B	36,39	Op. B	34,59	Op. B	32,98
Op. B	35,06	Op. B	34,27	Op. B	33,46
Op. B	36,01	Op. B	35,19	Op. B	32,23

4. EvaGreen vs Taqman vs SYBR Green

EG	35,16	**35,69**
EG	35,95	**0,54993**
EG	35,32	**0,07938**
EG	36,45	
EG	36,10	
EG	35,76	
EG	34,89	
EG	35,64	
EG	35,45	
EG	35,17	
EG	35,11	
EG	35,68	
EG	35,51	
EG	35,19	
EG	36,17	
EG	34,98	
EG	35,48	
EG	36,00	
EG	35,24	
EG	35,63	
EG	35,92	
EG	35,60	
EG	36,70	
EG	35,70	
EG	34,42	
EG	35,74	
EG	34,88	
EG	35,51	
EG	36,47	
EG	36,51	
EG	35,78	
EG	35,61	
EG	35,31	
EG	36,01	
EG	35,74	
EG	36,67	
EG	35,84	
EG	35,84	
EG	35,99	
EG	36,28	
EG	36,47	
EG	35,42	

4. EvaGreen vs Taqman vs SYBR Green

EG	36,72	
EG	35,10	
EG	34,78	
EG	36,39	
EG	35,06	
EG	36,01	
Tq	34,02	**34,63**
Tq	34,62	**0,53751**
Tq	34,39	**0,07758**
Tq	34,13	
Tq	34,02	
Tq	34,06	
Tq	34,14	
Tq	35,07	
Tq	34,30	
Tq	34,62	
Tq	34,43	
Tq	35,12	
Tq	35,18	
Tq	35,14	
Tq	34,21	
Tq	34,43	
Tq	35,25	
Tq	34,99	
Tq	35,34	
Tq	35,36	
Tq	34,57	
Tq	34,39	
Tq	34,06	
Tq	34,10	
Tq	35,10	
Tq	33,65	
Tq	33,74	
Tq	33,34	
Tq	34,61	
Tq	34,00	
Tq	34,81	
Tq	35,19	
Tq	34,59	
Tq	34,23	
Tq	35,35	
Tq	35,76	

4. EvaGreen vs Taqman vs SYBR Green		
Tq	35,07	
Tq	35,01	
Tq	34,89	
Tq	35,19	
Tq	35,12	
Tq	34,78	
Tq	35,19	
Tq	34,05	
Tq	34,62	
Tq	34,59	
Tq	34,27	
Tq	35,19	
SG	34,2	**32,89**
SG	32,71	**0,59726**
SG	33,53	**0,08621**
SG	32,53	
SG	33,21	
SG	31,66	
SG	33,27	
SG	33,69	
SG	33,28	
SG	32,57	
SG	33,46	
SG	32,83	
SG	33,01	
SG	33,03	
SG	32,49	
SG	33,73	
SG	32,7	
SG	32,7	
SG	33,56	
SG	33,12	
SG	32,37	
SG	32,22	
SG	33,28	
SG	33,12	
SG	32,57	
SG	32,15	
SG	32,23	
SG	33,04	
SG	33,29	
SG	32,75	

4. EvaGreen vs Taqman vs SYBR Green	
SG	33,43
SG	31,89
SG	33,62
SG	32,35
SG	32,92
SG	33,23
SG	31,95
SG	32,32
SG	33,31
SG	32,47
SG	31,68
SG	33,74
SG	32,26
SG	33,7
SG	32,8
SG	32,98
SG	33,46
SG	32,23

Section 2 - Detection of 15 fg Ceratocystis platani gDNA. Repeatability: comparison among three experiment (Exp.) performed by Operator (Op) A and B. See Fig. S1. Inter-operator reproducibility. See Fig. 2. EvaGreen (EG) vs Taqman (Tq) vs SYBR Green (SG). See Fig. 3a. In bold (the third column of each dataset) mean, standard deviation and standard error of the replication series are reported.

1. Repeatability, PCR-performer-operator A

EvaGreen			**Taqman**			**SYBR Green**		
I Exp.	33,19	**33,42**	I Exp.	32,82	**32,64**	I Exp.	30,25	**30,00**
I Exp.	33,69	**0,25393**	I Exp.	32,52	**0,26679**	I Exp.	30,16	**0,22558**
I Exp.	33,24	**0,08978**	I Exp.	32,79	**0,09432**	I Exp.	29,65	**0,07975**
I Exp.	33,14		I Exp.	32,82		I Exp.	30,06	
I Exp.	33,55		I Exp.	32,23		I Exp.	29,91	
I Exp.	33,33		I Exp.	32,69		I Exp.	30,11	
I Exp.	33,35		I Exp.	32,28		I Exp.	30,14	
I Exp.	33,85		I Exp.	32,96		I Exp.	29,68	
II Exp.	33,07	**33,12**	II Exp.	33,28	**32,97**	II Exp.	29,4	**29,77**
II Exp.	33,10	**0,26569**	II Exp.	33,18	**0,25518**	II Exp.	29,31	**0,50782**
II Exp.	33,08	**0,09394**	II Exp.	33,07	**0,09022**	II Exp.	30,25	**0,17954**
II Exp.	33,22		II Exp.	33,13		II Exp.	30,26	
II Exp.	33,07		II Exp.	33,01		II Exp.	30,26	
II Exp.	33,22		II Exp.	32,82		II Exp.	29,27	
II Exp.	33,59		II Exp.	32,74		II Exp.	29,22	
II Exp.	32,62		II Exp.	32,52		II Exp.	30,21	
III Exp.	33,38	**33,44**	III Exp.	33,40	**32,96**	III Exp.	30,37	**30,42**
III Exp.	33,98	**0,35292**	III Exp.	33,18	**0,2532**	III Exp.	30,35	**0,10796**
III Exp.	34,01	**0,12478**	III Exp.	32,71	**0,08952**	III Exp.	30,6	**0,03817**
III Exp.	33,17		III Exp.	32,86		III Exp.	30,45	
III Exp.	33,39		III Exp.	32,72		III Exp.	30,3	
III Exp.	33,10		III Exp.	33,04		III Exp.	30,53	
III Exp.	33,26		III Exp.	33,04		III Exp.	30,3	
III Exp.	33,26		III Exp.	32,71		III Exp.	30,43	

2. Repeatability, PCR-performer-operator B

EvaGreen			Taqman			SYBR Green		
I Exp.	33,87	**33,68**	I Exp.	33,03	**32,83**	I Exp.	29,73	**29,76**
I Exp.	34,61	**0,48255**	I Exp.	32,91	**0,33638**	I Exp.	29,63	**0,15267**
I Exp.	33,76	**0,17061**	I Exp.	32,81	**0,11893**	I Exp.	29,77	**0,05398**
I Exp.	33,78		I Exp.	32,49		I Exp.	29,75	
I Exp.	33,28		I Exp.	33,34		I Exp.	29,6	
I Exp.	33,76		I Exp.	32,25		I Exp.	30,1	
I Exp.	33,35		I Exp.	32,82		I Exp.	29,78	
I Exp.	33,02		I Exp.	33,02		I Exp.	29,7	
II Exp.	33,08	**33,52**	II Exp.	32,93	**32,79**	II Exp.	30,47	**30,12**
II Exp.	32,99	**0,51969**	II Exp.	33,19	**0,33628**	II Exp.	29,86	**0,26126**
II Exp.	33,66	**0,18374**	II Exp.	32,65	**0,11889**	II Exp.	30,09	**0,09237**
II Exp.	33,98		II Exp.	32,64		II Exp.	30,33	
II Exp.	34,26		II Exp.	32,32		II Exp.	29,69	
II Exp.	32,88		II Exp.	33,28		II Exp.	30,05	
II Exp.	33,96		II Exp.	32,88		II Exp.	30,12	
II Exp.	33,34		II Exp.	32,47		II Exp.	30,35	
III Exp.	34,15	**33,58**	III Exp.	33,00	**32,99**	III Exp.	30,03	**29,92**
III Exp.	33,72	**0,52471**	III Exp.	32,86	**0,23429**	III Exp.	30,22	**0,30649**
III Exp.	33,37	**0,18551**	III Exp.	33,12	**0,08284**	III Exp.	29,64	**0,10836**
III Exp.	32,75		III Exp.	33,41		III Exp.	30,22	
III Exp.	34,05		III Exp.	33,07		III Exp.	29,62	
III Exp.	34,16		III Exp.	33,04		III Exp.	30,29	
III Exp.	33,29		III Exp.	32,71		III Exp.	29,53	
III Exp.	33,13		III Exp.	32,70		III Exp.	29,83	

3. Inter-operator reproducibility

EvaGreen			Taqman			SYBR Green		
Op. A	33,19	**33,33**	Op. A	32,82	**32,86**	Op. A	30,25	**30,06**
Op. A	33,69	**0,31838**	Op. A	32,52	**0,29208**	Op. A	30,16	**0,41459**
Op. A	33,24	**0,06499**	Op. A	32,79	**0,05962**	Op. A	29,65	**0,08463**
Op. A	33,14		Op. A	32,82		Op. A	30,06	
Op. A	33,55		Op. A	32,23		Op. A	29,91	
Op. A	33,33		Op. A	32,69		Op. A	30,11	
Op. A	33,35		Op. A	32,28		Op. A	30,14	
Op. A	33,85		Op. A	32,96		Op. A	29,68	
Op. A	33,07		Op. A	33,28		Op. A	29,4	
Op. A	33,10		Op. A	33,18		Op. A	29,31	
Op. A	33,08		Op. A	33,07		Op. A	30,25	
Op. A	33,22		Op. A	33,13		Op. A	30,26	
Op. A	33,07		Op. A	33,01		Op. A	30,26	
Op. A	33,22		Op. A	32,82		Op. A	29,27	
Op. A	33,59		Op. A	32,74		Op. A	29,22	
Op. A	32,62		Op. A	32,52		Op. A	30,21	
Op. A	33,38		Op. A	33,40		Op. A	30,37	
Op. A	33,98		Op. A	33,18		Op. A	30,35	
Op. A	34,01		Op. A	32,71		Op. A	30,6	
Op. A	33,17		Op. A	32,86		Op. A	30,45	
Op. A	33,39		Op. A	32,72		Op. A	30,3	
Op. A	33,10		Op. A	33,04		Op. A	30,53	
Op. A	33,26		Op. A	33,04		Op. A	30,3	
Op. A	33,26		Op. A	32,71		Op. A	30,43	
Op. B	33,87	**33,59**	Op. B	33,03	**32,87**	Op. B	29,73	**29,9333**
Op. B	34,61	**0,49136**	Op. B	32,91	**0,30449**	Op. B	29,63	**0,28173**
Op. B	33,76	**0,1003**	Op. B	32,81	**0,06215**	Op. B	29,77	**0,05751**
Op. B	33,78		Op. B	32,49		Op. B	29,75	
Op. B	33,28		Op. B	33,34		Op. B	29,6	
Op. B	33,76		Op. B	32,25		Op. B	30,1	
Op. B	33,35		Op. B	32,82		Op. B	29,78	
Op. B	33,02		Op. B	33,02		Op. B	29,7	
Op. B	33,08		Op. B	32,93		Op. B	30,47	
Op. B	32,99		Op. B	33,19		Op. B	29,86	
Op. B	33,66		Op. B	32,65		Op. B	30,09	
Op. B	33,98		Op. B	32,64		Op. B	30,33	
Op. B	34,26		Op. B	32,32		Op. B	29,69	
Op. B	32,88		Op. B	33,28		Op. B	30,05	
Op. B	33,96		Op. B	32,88		Op. B	30,12	
Op. B	33,34		Op. B	32,47		Op. B	30,35	
Op. B	34,15		Op. B	33,00		Op. B	30,03	

3. Inter-operator reproducibility

EvaGreen		Taqman		SYBR Green	
Op. B	33,72	Op. B	32,86	Op. B	30,22
Op. B	33,37	Op. B	33,12	Op. B	29,64
Op. B	32,75	Op. B	33,41	Op. B	30,22
Op. B	34,05	Op. B	33,07	Op. B	29,62
Op. B	34,16	Op. B	33,04	Op. B	30,29
Op. B	33,29	Op. B	32,71	Op. B	29,53
Op. B	33,13	Op. B	32,70	Op. B	29,83

4. EvaGreen vs Taqman vs SYBR Green

EG	33,19	**33,46**
EG	33,69	**0,4308**
EG	33,24	**0,06218**
EG	33,14	
EG	33,55	
EG	33,33	
EG	33,35	
EG	33,85	
EG	33,07	
EG	33,10	
EG	33,08	
EG	33,22	
EG	33,07	
EG	33,22	
EG	33,59	
EG	32,62	
EG	33,38	
EG	33,98	
EG	34,01	
EG	33,17	
EG	33,39	
EG	33,10	
EG	33,26	
EG	33,26	
EG	33,87	
EG	34,61	
EG	33,76	
EG	33,78	
EG	33,28	
EG	33,76	
EG	33,35	
EG	33,02	
EG	33,08	
EG	32,99	
EG	33,66	
EG	33,98	
EG	34,26	
EG	32,88	
EG	33,96	
EG	33,34	
EG	34,15	
EG	33,72	

EG	33,37	
EG	32,75	
EG	34,05	
EG	34,16	
EG	33,29	
EG	33,13	
Tq	32,82	**32,86**
Tq	32,52	**0,29526**
Tq	32,79	**0,04262**
Tq	32,82	
Tq	32,23	
Tq	32,69	
Tq	32,28	
Tq	32,96	
Tq	33,28	
Tq	33,18	
Tq	33,07	
Tq	33,13	
Tq	33,01	
Tq	32,82	
Tq	32,74	
Tq	32,52	
Tq	33,40	
Tq	33,18	
Tq	32,71	
Tq	32,86	
Tq	32,72	
Tq	33,04	
Tq	33,04	
Tq	32,71	
Tq	33,03	
Tq	32,91	
Tq	32,81	
Tq	32,49	
Tq	33,34	
Tq	32,25	
Tq	32,82	
Tq	33,02	
Tq	32,93	
Tq	33,19	
Tq	32,65	
Tq	32,64	
Tq	32,32	

Tq	33,28	
Tq	32,88	
Tq	32,47	
Tq	33,00	
Tq	32,86	
Tq	33,12	
Tq	33,41	
Tq	33,07	
Tq	33,04	
Tq	32,71	
Tq	32,70	
SG	30,25	**30,00**
SG	30,16	**0,35655**
SG	29,65	**0,05146**
SG	30,06	
SG	29,91	
SG	30,11	
SG	30,14	
SG	29,68	
SG	29,4	
SG	29,31	
SG	30,25	
SG	30,26	
SG	30,26	
SG	29,27	
SG	29,22	
SG	30,21	
SG	30,37	
SG	30,35	
SG	30,6	
SG	30,45	
SG	30,3	
SG	30,53	
SG	30,3	
SG	30,43	
SG	29,73	
SG	29,63	
SG	29,77	
SG	29,75	
SG	29,6	
SG	30,1	
SG	29,78	
SG	29,7	

SG	30,47
SG	29,86
SG	30,09
SG	30,33
SG	29,69
SG	30,05
SG	30,12
SG	30,35
SG	30,03
SG	30,22
SG	29,64
SG	30,22
SG	29,62
SG	30,29
SG	29,53
SG	29,83

Section 3 - Detection of 3 fg gDNA in different *Ceratocystis platani* (CP) strains: EvaGreen vs Taqman vs SYBR Green. Strains: 25, 33, 1176, 11292, 11259: See Fig. 3b. EG = EvaGreen; Tq= Taqman; SG = SYBR Green. In bold (the third column of each dataset) mean, standard deviation and standard error of the replication series are reported.

25 EG	34,46	**34,75**	33 EG	36,60	**35,87**	1176 EG	36,13	**35,76**
25 EG	34,51	**0,4568**	33 EG	35,31	**0,4925**	1176 EG	34,79	**0,5759**
25 EG	35,07	**0,1615**	33 EG	35,72	**0,1741**	1176 EG	36,15	**0,2036**
25 EG	35,05		33 EG	35,58		1176 EG	36,17	
25 EG	35,00		33 EG	35,82		1176 EG	36,47	
25 EG	34,32		33 EG	35,32		1176 EG	35,64	
25 EG	34,11		33 EG	36,17		1176 EG	35,15	
25 EG	35,44		33 EG	36,46		1176 EG	35,58	
25 Tq	34,37	**34,05**	33 Tq	33,38	**33,50**	1176 Tq	33,95	**33,99**
25 Tq	34,75	**0,4985**	33 Tq	33,29	**0,2976**	1176 Tq	33,48	**0,2196**
25 Tq	34,04	**0,1763**	33 Tq	33,69	**0,1052**	1176 Tq	34,08	**0,0776**
25 Tq	33,65		33 Tq	33,86		1176 Tq	33,97	
25 Tq	34,13		33 Tq	33,97		1176 Tq	34,04	
25 Tq	34,30		33 Tq	33,31		1176 Tq	34,08	
25 Tq	33,10		33 Tq	33,28		1176 Tq	34,15	
25 Tq	34,07		33 Tq	33,20		1176 Tq	34,15	
25 SG	32,69	**33,27**	33 SG	32,55	**32,56**	1176 SG	33,31	**32,70**
25 SG	34,2	**0,67**	33 SG	32,11	**0,91**	1176 SG	32,36	**0,8716**
25 SG	32,16	**0,2369**	33 SG	33,49	**0,3217**	1176 SG	34,1	**0,3082**
25 SG	33,45		33 SG	34,24		1176 SG	32,63	
25 SG	34,01		33 SG	32,45		1176 SG	31,56	
25 SG	33,5		33 SG	32,3		1176 SG	33,54	
25 SG	33,08		33 SG	31,33		1176 SG	32,08	
25 SG	33,07		33 SG	32		1176 SG	32,01	

11292 EG	34,87	**35,36**	11259 EG	36,01	**35,53**
11292 EG	35,74	**0,4225**	11259 EG	35,22	**0,6044**
11292 EG	36,11	**0,1494**	11259 EG	35,60	**0,2137**
11292 EG	35,49		11259 EG	35,55	
11292 EG	35,45		11259 EG	35,94	
11292 EG	35,20		11259 EG	36,40	
11292 EG	35,01		11259 EG	34,58	
11292 EG	35,00		11259 EG	34,91	
11292 Tq	34,17	**34,20**	11259 Tq	34,42	**33,58**
11292 Tq	33,82	**0,2599**	11259 Tq	33,26	**0,5282**
11292 Tq	33,95	**0,0919**	11259 Tq	33,53	**0,1867**
11292 Tq	34,41		11259 Tq	33,20	
11292 Tq	34,13		11259 Tq	33,50	
11292 Tq	34,66		11259 Tq	33,24	

11292 Tq	34,26		11259 Tq	34,38	
11292 Tq	34,17		11259 Tq	33,10	
11292 SG	33,36	**33,20**	11259 SG	33,71	**32,84**
11292 SG	33,17	**0,2324**	11259 SG	33,46	**0,704**
11292 SG	33,38	**0,0822**	11259 SG	33,08	**0,2489**
11292 SG	32,96		11259 SG	32,69	
11292 SG	32,9		11259 SG	31,4	
11292 SG	32,99		11259 SG	32,46	
11292 SG	33,27		11259 SG	33,01	
11292 SG	33,55		11259 SG	32,88	

Section 4 - Detection limit in relation to: (i) repeatability: comparison among three experiments (for each gDNA sample); (ii) reproducibility: different gDNA extraction events performed bydifferent DNA-purifier-operators; (iii) different modalities of collecting the fungal mycelium for DNA extraction - the agar modality (agar) and the cellophane modality (cell.). Performed with Taqman assay. In bold (the third column of each dataset) mean, standard deviation and standard error of the replication series are reported.

1. Repeatability

15 fg gDNA Agar Op. A			**3fg gDNA Agar Op. A**		
I Esp.	32,55	**32,6225**	I Esp.	34,58	**34,54**
I Esp.	32,42	**0,21578759**	I Esp.	34,19	**0,4407603**
I Esp.	32,88	**0,07629244**	I Esp.	34,66	**0,1558323**
I Esp.	32,35		I Esp.	34,42	
I Esp.	32,91		I Esp.	35	
I Esp.	32,66		I Esp.	34,17	
I Esp.	32,44		I Esp.	34,01	
I Esp.	32,77		I Esp.	35,3	
II Esp.	32,26	**32,40125**	II Esp.	34,06	**34,14875**
II Esp.	32,68	**0,32511262**	II Esp.	33,56	**0,5670585**
II Esp.	32,27	**0,11494467**	II Esp.	35,03	**0,2004855**
II Esp.	32,32		II Esp.	33,32	
II Esp.	32,03		II Esp.	34,2	
II Esp.	32,1		II Esp.	34,73	
II Esp.	32,54		II Esp.	33,93	
II Esp.	33,01		II Esp.	34,36	
III Esp.	32,49	**32,61**	III Esp.	34,22	**34,65**
III Esp.	32,55	**0,12244532**	III Esp.	34,73	**0,369418**
III Esp.	32,61	**0,04329096**	III Esp.	35	**0,130609**
III Esp.	32,72		III Esp.	34,96	
III Esp.	32,76		III Esp.	34,19	
III Esp.	32,59		III Esp.	34,24	
III Esp.	32,75		III Esp.	35,01	
III Esp.	32,43		III Esp.	34,84	

15 fg gDNA Agar Op. C			**3 fg gDNA Agar Op. C**		
I Esp.	32,5	**32,545**	I Esp.	34,49	**34,7425**
I Esp.	32,98	**0,23114621**	I Esp.	34,08	**0,4863787**
I Esp.	32,49	**0,08172253**	I Esp.	34,8	**0,1719609**
I Esp.	32,55		I Esp.	35,18	
I Esp.	32,31		I Esp.	34,37	
I Esp.	32,33		I Esp.	35,36	
I Esp.	32,79		I Esp.	35,29	
I Esp.	32,41		I Esp.	34,37	
II Esp.	32,48	**32,53**	II Esp.	34,35	**34,38625**
II Esp.	32,46	**0,23886637**	II Esp.	33,87	**0,5357755**
II Esp.	32,2	**0,08445202**	II Esp.	34,47	**0,1894252**
II Esp.	32,89		II Esp.	34,68	
II Esp.	32,32		II Esp.	35,31	
II Esp.	32,74		II Esp.	33,5	
II Esp.	32,75		II Esp.	34,45	
II Esp.	32,4		II Esp.	34,46	
III Esp.	33	**32,64**	III Esp.	34,12	**34,41**
III Esp.	32,08	**0,33354963**	III Esp.	34,25	**0,264251**
III Esp.	32,85	**0,1179276**	III Esp.	34,74	**0,0934268**
III Esp.	33		III Esp.	34,24	
III Esp.	32,6		III Esp.	34,43	
III Esp.	32,82		III Esp.	34,39	
III Esp.	32,32		III Esp.	34,87	
III Esp.	32,48		III Esp.	34,24	

15 fg gDNA Cell. Op. A			**3 fg gDNA Cell. Op. A**		
I Esp.	32,58	**32,73125**	I Esp.	34,38	**34,82375**
I Esp.	32,16	**0,3244088**	I Esp.	35,37	**0,424363**
I Esp.	33,16	**0,11469583**	I Esp.	34,39	**0,150035**
I Esp.	32,55		I Esp.	34,43	
I Esp.	32,65		I Esp.	35,22	
I Esp.	32,77		I Esp.	35,22	
I Esp.	32,87		I Esp.	34,56	
I Esp.	33,11		I Esp.	35,02	
II Esp.	32,95	**32,4925**	II Esp.	35,5	**35,21375**
II Esp.	32,94	**0,36861711**	II Esp.	34,87	**0,4607428**
II Esp.	32,89	**0,13032583**	II Esp.	34,98	**0,1628972**
II Esp.	32,34		II Esp.	35,16	
II Esp.	32,12		II Esp.	35,04	
II Esp.	32,12		II Esp.	34,87	
II Esp.	32,27		II Esp.	35,05	
II Esp.	32,31		II Esp.	36,24	
III Esp.	32,43	**32,66**	III Esp.	34,42	**34,82**
III Esp.	32,99	**0,20704986**	III Esp.	35,04	**0,2948607**
III Esp.	32,49	**0,07320318**	III Esp.	34,51	**0,104249**
III Esp.	32,77		III Esp.	35,03	
III Esp.	32,85		III Esp.	34,52	
III Esp.	32,5		III Esp.	35,02	
III Esp.	32,5		III Esp.	34,84	
III Esp.	32,76		III Esp.	35,18	

15 fg gDNA Cell. Op. C			**3 fg gDNA Cell. Op. C**		
I Esp.	32,67	**32,58**	I Esp.	35,51	**34,59875**
I Esp.	32,89	**0,30060653**	I Esp.	33,96	**0,535362**
I Esp.	32,36	**0,10628046**	I Esp.	35,12	**0,1892791**
I Esp.	32,55		I Esp.	34,84	
I Esp.	32,49		I Esp.	34,44	
I Esp.	33,05		I Esp.	34,59	
I Esp.	32,57		I Esp.	34,3	
I Esp.	32,08		I Esp.	34,03	
II Esp.	32,91	**32,715**	II Esp.	34,84	**34,6875**
II Esp.	32,46	**0,21705825**	II Esp.	34,67	**0,2920739**
II Esp.	32,48	**0,07674168**	II Esp.	34,27	**0,1032637**
II Esp.	32,89		II Esp.	34,99	
II Esp.	32,7		II Esp.	35,12	
II Esp.	32,97		II Esp.	34,62	
II Esp.	32,84		II Esp.	34,64	
II Esp.	32,47		II Esp.	34,35	
III Esp.	33,25	**32,96**	III Esp.	35,07	**34,69**
III Esp.	33,02	**0,3133118**	III Esp.	34,17	**0,3559871**
III Esp.	32,71	**0,11077245**	III Esp.	35	**0,1258604**
III Esp.	33,32		III Esp.	34,41	
III Esp.	32,51		III Esp.	34,42	
III Esp.	33,28		III Esp.	34,53	
III Esp.	32,97		III Esp.	34,81	
III Esp.	32,64		III Esp.	35,12	

2. Inter-operator reproducibility of the extraction event

15 fg gDNA Agar Op. A vs C			3 fg gDNA Agar Op. A vs C		
Agar Op.A	32,55	**32,5454167**	Agar Op.A	34,58	**34,44625**
Agar Op.A	32,42	**0,24852573**	Agar Op.A	34,19	**0,4967005**
Agar Op.A	32,88	**0,0507301**	Agar Op.A	34,66	**0,1013886**
Agar Op.A	32,35		Agar Op.A	34,42	
Agar Op.A	32,91		Agar Op.A	35	
Agar Op.A	32,66		Agar Op.A	34,17	
Agar Op.A	32,44		Agar Op.A	34,01	
Agar Op.A	32,77		Agar Op.A	35,3	
Agar Op.A	32,26		Agar Op.A	34,06	
Agar Op.A	32,68		Agar Op.A	33,56	
Agar Op.A	32,27		Agar Op.A	35,03	
Agar Op.A	32,32		Agar Op.A	33,32	
Agar Op.A	32,03		Agar Op.A	34,2	
Agar Op.A	32,1		Agar Op.A	34,73	
Agar Op.A	32,54		Agar Op.A	33,93	
Agar Op.A	33,01		Agar Op.A	34,36	
Agar Op.A	32,49		Agar Op.A	34,22	
Agar Op.A	32,55		Agar Op.A	34,73	
Agar Op.A	32,61		Agar Op.A	35	
Agar Op.A	32,72		Agar Op.A	34,96	
Agar Op.A	32,76		Agar Op.A	34,19	
Agar Op.A	32,59		Agar Op.A	34,24	
Agar Op.A	32,75		Agar Op.A	35,01	
Agar Op.A	32,43		Agar Op.A	34,84	
Agar Op.C	32,5	**32,5729167**	Agar Op.C	34,49	**34,512917**
Agar Op.C	32,98	**0,2648458**	Agar Op.C	34,08	**0,4563035**
Agar Op.C	32,49	**0,05406142**	Agar Op.C	34,8	**0,0931426**
Agar Op.C	32,55		Agar Op.C	35,18	
Agar Op.C	32,31		Agar Op.C	34,37	
Agar Op.C	32,33		Agar Op.C	35,36	
Agar Op.C	32,79		Agar Op.C	35,29	
Agar Op.C	32,41		Agar Op.C	34,37	
Agar Op.C	32,48		Agar Op.C	34,35	
Agar Op.C	32,46		Agar Op.C	33,87	
Agar Op.C	32,2		Agar Op.C	34,47	
Agar Op.C	32,89		Agar Op.C	34,68	
Agar Op.C	32,32		Agar Op.C	35,31	
Agar Op.C	32,74		Agar Op.C	33,5	
Agar Op.C	32,75		Agar Op.C	34,45	
Agar Op.C	32,4		Agar Op.C	34,46	
Agar Op.C	33		Agar Op.C	34,12	

Agar Op.C	32,08	Agar Op.C	34,25
Agar Op.C	32,85	Agar Op.C	34,74
Agar Op.C	33	Agar Op.C	34,24
Agar Op.C	32,6	Agar Op.C	34,43
Agar Op.C	32,82	Agar Op.C	34,39
Agar Op.C	32,32	Agar Op.C	34,87
Agar Op.C	32,48	Agar Op.C	34,24

15 fg gDNA Cell. Op. A vs C			**3 fg gDNA Cell. Op. A vs C**		
Cell. Op.A	32,58	**32,6283333**	Cell. Op.A	34,38	**34,9525**
Cell. Op.A	32,16	**0,31130394**	Cell. Op.A	35,37	**0,4260154**
Cell. Op.A	33,16	**0,06354465**	Cell. Op.A	34,39	**0,08696**
Cell. Op.A	32,55		Cell. Op.A	34,43	
Cell. Op.A	32,65		Cell. Op.A	35,22	
Cell. Op.A	32,77		Cell. Op.A	35,22	
Cell. Op.A	32,87		Cell. Op.A	34,56	
Cell. Op.A	33,11		Cell. Op.A	35,02	
Cell. Op.A	32,95		Cell. Op.A	35,5	
Cell. Op.A	32,94		Cell. Op.A	34,87	
Cell. Op.A	32,89		Cell. Op.A	34,98	
Cell. Op.A	32,34		Cell. Op.A	35,16	
Cell. Op.A	32,12		Cell. Op.A	35,04	
Cell. Op.A	32,12		Cell. Op.A	34,87	
Cell. Op.A	32,27		Cell. Op.A	35,05	
Cell. Op.A	32,31		Cell. Op.A	36,24	
Cell. Op.A	32,43		Cell. Op.A	34,42	
Cell. Op.A	32,99		Cell. Op.A	35,04	
Cell. Op.A	32,49		Cell. Op.A	34,51	
Cell. Op.A	32,77		Cell. Op.A	35,03	
Cell. Op.A	32,85		Cell. Op.A	34,52	
Cell. Op.A	32,5		Cell. Op.A	35,02	
Cell. Op.A	32,5		Cell. Op.A	34,84	
Cell. Op.A	32,76		Cell. Op.A	35,18	
Cell. Op.C	32,67	**32,7533333**	Cell. Op.C	35,51	**34,659167**
Cell. Op.C	32,89	**0,31240535**	Cell. Op.C	33,96	**0,3920062**
Cell. Op.C	32,36	**0,06376947**	Cell. Op.C	35,12	**0,0800179**
Cell. Op.C	32,55		Cell. Op.C	34,84	
Cell. Op.C	32,49		Cell. Op.C	34,44	
Cell. Op.C	33,05		Cell. Op.C	34,59	
Cell. Op.C	32,57		Cell. Op.C	34,3	
Cell. Op.C	32,08		Cell. Op.C	34,03	
Cell. Op.C	32,91		Cell. Op.C	34,84	
Cell. Op.C	32,46		Cell. Op.C	34,67	
Cell. Op.C	32,48		Cell. Op.C	34,27	
Cell. Op.C	32,89		Cell. Op.C	34,99	
Cell. Op.C	32,7		Cell. Op.C	35,12	
Cell. Op.C	32,97		Cell. Op.C	34,62	
Cell. Op.C	32,84		Cell. Op.C	34,64	
Cell. Op.C	32,47		Cell. Op.C	34,35	
Cell. Op.C	33,25		Cell. Op.C	35,07	
Cell. Op.C	33,02		Cell. Op.C	34,17	

Cell. Op.C	32,71	Cell. Op.C	35
Cell. Op.C	33,32	Cell. Op.C	34,41
Cell. Op.C	32,51	Cell. Op.C	34,42
Cell. Op.C	33,28	Cell. Op.C	34,53
Cell. Op.C	32,97	Cell. Op.C	34,81
Cell. Op.C	32,64	Cell. Op.C	35,12

3. Agar-grown vs Cellophane-grown mycelium collection

15 fg Agar vs Cellophane			3 fg Agar vs Cellophane		
Agar	32,55	**32,56**	Agar	34,58	**34,48**
Agar	32,42	**0,25444837**	Agar	34,19	**0,4730299**
Agar	32,88	**0,03672646**	Agar	34,66	**0,068276**
Agar	32,35		Agar	34,42	
Agar	32,91		Agar	35	
Agar	32,66		Agar	34,17	
Agar	32,44		Agar	34,01	
Agar	32,77		Agar	35,3	
Agar	32,26		Agar	34,06	
Agar	32,68		Agar	33,56	
Agar	32,27		Agar	35,03	
Agar	32,32		Agar	33,32	
Agar	32,03		Agar	34,2	
Agar	32,1		Agar	34,73	
Agar	32,54		Agar	33,93	
Agar	33,01		Agar	34,36	
Agar	32,49		Agar	34,22	
Agar	32,55		Agar	34,73	
Agar	32,61		Agar	35	
Agar	32,72		Agar	34,96	
Agar	32,76		Agar	34,19	
Agar	32,59		Agar	34,24	
Agar	32,75		Agar	35,01	
Agar	32,43		Agar	34,84	
Agar	32,5		Agar	34,49	
Agar	32,98		Agar	34,08	
Agar	32,49		Agar	34,8	
Agar	32,55		Agar	35,18	
Agar	32,31		Agar	34,37	
Agar	32,33		Agar	35,36	
Agar	32,79		Agar	35,29	
Agar	32,41		Agar	34,37	
Agar	32,48		Agar	34,35	
Agar	32,46		Agar	33,87	
Agar	32,2		Agar	34,47	
Agar	32,89		Agar	34,68	
Agar	32,32		Agar	35,31	
Agar	32,74		Agar	33,5	
Agar	32,75		Agar	34,45	
Agar	32,4		Agar	34,46	
Agar	33		Agar	34,12	

Agar	32,08		Agar	34,25	
Agar	32,85		Agar	34,74	
Agar	33		Agar	34,24	
Agar	32,6		Agar	34,43	
Agar	32,82		Agar	34,39	
Agar	32,32		Agar	34,87	
Agar	32,48		Agar	34,24	
Cell.	32,58	**32,6908333**	Cell.	34,38	**34,805833**
Cell.	32,16	**0,31491865**	Cell.	35,37	**0,4312566**
Cell.	33,16	**0,04545459**	Cell.	34,39	**0,0622465**
Cell.	32,55		Cell.	34,43	
Cell.	32,65		Cell.	35,22	
Cell.	32,77		Cell.	35,22	
Cell.	32,87		Cell.	34,56	
Cell.	33,11		Cell.	35,02	
Cell.	32,95		Cell.	35,5	
Cell.	32,94		Cell.	34,87	
Cell.	32,89		Cell.	34,98	
Cell.	32,34		Cell.	35,16	
Cell.	32,12		Cell.	35,04	
Cell.	32,12		Cell.	34,87	
Cell.	32,27		Cell.	35,05	
Cell.	32,31		Cell.	36,24	
Cell.	32,43		Cell.	34,42	
Cell.	32,99		Cell.	35,04	
Cell.	32,49		Cell.	34,51	
Cell.	32,77		Cell.	35,03	
Cell.	32,85		Cell.	34,52	
Cell.	32,5		Cell.	35,02	
Cell.	32,5		Cell.	34,84	
Cell.	32,76		Cell.	35,18	
Cell.	32,67		Cell.	35,51	
Cell.	32,89		Cell.	33,96	
Cell.	32,36		Cell.	35,12	
Cell.	32,55		Cell.	34,84	
Cell.	32,49		Cell.	34,44	
Cell.	33,05		Cell.	34,59	
Cell.	32,57		Cell.	34,3	
Cell.	32,08		Cell.	34,03	
Cell.	32,91		Cell.	34,84	
Cell.	32,46		Cell.	34,67	
Cell.	32,48		Cell.	34,27	
Cell.	32,89		Cell.	34,99	

Cell.	32,7	Cell.	35,12
Cell.	32,97	Cell.	34,62
Cell.	32,84	Cell.	34,64
Cell.	32,47	Cell.	34,35
Cell.	33,25	Cell.	35,07
Cell.	33,02	Cell.	34,17
Cell.	32,71	Cell.	35
Cell.	33,32	Cell.	34,41
Cell.	32,51	Cell.	34,42
Cell.	33,28	Cell.	34,53
Cell.	32,97	Cell.	34,81
Cell.	32,64	Cell.	35,12

Section 5 - Detection of 3 fg *Ceratocystis platani* gDNA in PCR reactions spiked with 6 µl DNA extract obtained from a necrotic wood infected with *Fomitiporia mediterranea* and uninfected with C. platani (SYBR Green assay) : See Figure 6. In bold mean, standard deviation and standard error of the replication series (the third column of the dataset) and of all data (the fourth column of the dataset) are reported.

Repeatability: comparison among three experiment (Exp.).

I Exp.	33,08	**32,61375**	**32,46833**
I Exp.	32,53	**0,317577**	**0,321216**
I Exp.	32,22	**0,112281**	**0,065568**
I Exp.	32,38		
I Exp.	32,59		
I Exp.	32,43		
I Exp.	33,1		
I Exp.	32,58		
II Exp.	32,34	**32,46875**	
II Exp.	33	**0,338376**	
II Exp.	32,03	**0,119634**	
II Exp.	32,11		
II Exp.	32,59		
II Exp.	32,53		
II Exp.	32,31		
II Exp.	32,84		
III Exp.	32,51	**32,3225**	
III Exp.	32,19	**0,274213**	
III Exp.	31,98	**0,096949**	
III Exp.	32,34		
III Exp.	32,82		
III Exp.	32,49		
III Exp.	32,09		
III Exp.	32,16		

Section 6 - Melting temperature of the amplicon in EvaGreen (EG) and SYBR Green (SG) assays. Use of the *Ceratocystis platani* genomic DNA (gDNA) and the PCR-target-containing plasmid (pDNA) See the text. In bold (the third column of each dataset) mean, standard deviation and standard error of the replication series are reported.

1. gDNA vs pDNA in EvaGreen and SYBR Green

EG gDNA	81,5	**81,355**	SG gDNA	83,4	**83,43**
EG gDNA	81,4	**0,060481**	SG gDNA	83,4	**0,047016**
EG gDNA	81,4	**0,013524**	SG gDNA	83,4	**0,010513**
EG gDNA	81,4		SG gDNA	83,4	
EG gDNA	81,4		SG gDNA	83,4	
EG gDNA	81,4		SG gDNA	83,4	
EG gDNA	81,4		SG gDNA	83,4	
EG gDNA	81,4		SG gDNA	83,4	
EG gDNA	81,4		SG gDNA	83,5	
EG gDNA	81,4		SG gDNA	83,5	
EG gDNA	81,3		SG gDNA	83,5	
EG gDNA	81,3		SG gDNA	83,4	
EG gDNA	81,3		SG gDNA	83,5	
EG gDNA	81,3		SG gDNA	83,4	
EG gDNA	81,3		SG gDNA	83,4	
EG gDNA	81,3		SG gDNA	83,5	
EG gDNA	81,3		SG gDNA	83,4	
EG gDNA	81,3		SG gDNA	83,4	
EG gDNA	81,3		SG gDNA	83,5	
EG gDNA	81,3		SG gDNA	83,4	
EG pDNA	81,4	**81,35**	SG pDNA	83,4	**83,425**
EG pDNA	81,4	**0,051299**	SG pDNA	83,3	**0,071635**
EG pDNA	81,4	**0,011471**	SG pDNA	83,4	**0,016018**
EG pDNA	81,4		SG pDNA	83,4	
EG pDNA	81,4		SG pDNA	83,4	
EG pDNA	81,4		SG pDNA	83,4	
EG pDNA	81,4		SG pDNA	83,5	
EG pDNA	81,4		SG pDNA	83,4	
EG pDNA	81,4		SG pDNA	83,4	
EG pDNA	81,4		SG pDNA	83,4	
EG pDNA	81,3		SG pDNA	83,3	
EG pDNA	81,3		SG pDNA	83,4	
EG pDNA	81,3		SG pDNA	83,4	
EG pDNA	81,3		SG pDNA	83,5	
EG pDNA	81,3		SG pDNA	83,4	
EG pDNA	81,3		SG pDNA	83,4	
EG pDNA	81,3		SG pDNA	83,5	
EG pDNA	81,3		SG pDNA	83,5	
EG pDNA	81,3		SG pDNA	83,6	
EG pDNA	81,3		SG pDNA	83,5	

2. EvaGreen vs SYBR Green

EG	81,5	**81,3525**
EG	81,4	**0,055412**
EG	81,4	**0,008761**
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
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EG	81,3	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,4	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
EG	81,3	
SG	83,4	**83,4275**
SG	83,4	**0,059861**
SG	83,4	**0,009465**
SG	83,4	
SG	83,4	
SG	83,4	

SG	83,4
SG	83,4
SG	83,5
SG	83,5
SG	83,5
SG	83,4
SG	83,5
SG	83,4
SG	83,4
SG	83,5
SG	83,4
SG	83,4
SG	83,5
SG	83,4
SG	83,4
SG	83,3
SG	83,4
SG	83,4
SG	83,4
SG	83,4
SG	83,5
SG	83,4
SG	83,4
SG	83,4
SG	83,3
SG	83,4
SG	83,4
SG	83,5
SG	83,4
SG	83,4
SG	83,5
SG	83,5
SG	83,6
SG	83,5

Tab. S3 - Performance parameters of EvaGreen, Taqman and SYBR Green assays evaluated through the inference of standard curves. The curves were generated using 5-fold serial dilutions of *Ceratocystis platani* gDNA (eight serial dilutions at 4.685 pg μl^{-1} → 60 ag μl^{-1} corresponding to 9.37 pg → 120 ag *per* PCR reaction). As the two lowest dilutions were not detected repeatedly or were not detected, we excluded them in the analysis of the performance parameters. Thus, the lowest dilution included was 1.5 fg μl^{-1} (3 fg *per* PCR reaction). gDNA was extracted from a pure culture directly grown on PDA. [1] In bold are the experiments depicted in figure 1. [2] E = PCR efficiency, 100% is the maximum theoretical value, which means perfect doubling of molecules at each cycle; [3] R^2 is a measure of data linearity among technical replicates of the same and the different serial dilutions, 1 is the best fit; [4] the slope is the angular coefficient (m) of the equation for the standard curve (y = mx + b), 3.32 is the best fit.

Experiment [1]	Method	E [2] (%)	R^2 [3]	Slope [4]	Int.
1	EvaGreen	98.5	0.991	3.359	47.285
2	**EvaGreen**	**98.4**	**0.992**	**3.360**	**47.449**
3	EvaGreen	98.6	0.990	3.356	47.715
1	Taqman	107.8	0.996	3.149	46.056
2	**Taqman**	**100.9**	**0.995**	**3.300**	**46.186**
3	Taqman	102.9	0.975	3.253	46.359
1	SYBR Green	100.2	0.990	3.317	44.060
2	SYBR Green	99.5	0.994	3.335	44.618
3	**SYBR Green**	**97.3**	**0.997**	**3.388**	**44.956**

Tab. S4 - Mean (standard deviation and standard error) of C*t* values obtained in the validation experiments. Validation regarded: (a) repeatability and reproducibility of the detection limit meant as the lowest *C. platani* gDNA quantity detectable in a PCR reaction (referred to in the paper as: in-mycelium DQP-ASe). Genomic DNA was extracted from a pure culture directly grown on PDA. [1] The upper values in the cells refer to the detection of 15 fg fungal gDNA, the lower values refer to 3 fg gDNA *per* PCR reaction. [2] Values refer to the detection of 3 fg fungal gDNA *per* PCR reaction. (b) repeatability of the detection limit meant as the lowest *C. platani* gDNA quantity (3 fg) spiked with 6 µl of extract from *C. platani*-uninfected necrotic wood, which is detectable in a PCR reaction (referred to in the paper as*:* in-necrotic-wood DQP-ASe). SYBR Green assay. Genomic DNA was extracted from a pure culture directly grown on PDA.

(a)

Methods	EvaGreen		Taqman		SYBR Green	
Operator Experiments	A	B	A	B	A	B
Exp. I (C.P. 32) [1]	33.4 (0.25 - 0.09) 35.7 (0.52 - 0.18)	33.7 (0.48 - 0.17) 35.6 (0.71 - 0.25)	32.6 (0.27 - 0.09) 34.3 (0.37 - 0.13)	32.8 (0.34 - 0.12) 34.3 (0,71 - 0.25)	30.0 (0.23 - 0.08) 33.1 (0.79 - 0.28)	29.8 (0.15 - 0.05) 32.7 (0.56 - 0.20)
Exp. II (C.P. 32) [1]	33.1 (0.27 - 0.09) 35.4 (0.38 - 0.14)	33.5 (0.52 - 0.18) 36.0 (0.40 - 0.14)	33.0 (0.25 - 0.09) 34.7 (0.40 - 0.14)	32.8 (0.34 - 0.12) 35.0 (0.46 - 0.16)	29.8 (0.51 - 0.18) 33.0 (0.43 - 0.15)	30.1 (0.26 - 0.09) 32.8 (0.58 - 0.21)
Exp. III (C.P. 32) [1]	33.4 (0.35 - 0.12) 35.8 (0.44 - 0.15)	33.6 (0.52 - 0.18) 35.7 (0.75 - 0.26)	33.0 (0.25 - 0.09) 34.8 (0.55 - 0.19)	33.0 (0.23 - 0.08) 34.7 (0.43 - 0.15)	30.4 (0.11 - 0.04) 32.9 (0.46 - 0.16)	29.9 (0.31 - 0.11) 32.9 (0.76 - 0.27)
C*t* mean of all the experiments for each operator [1]	33.3 (0.32 - 0.06) 35.6 (0.46 - 0.09)	33.6 (0.49 - 0.10) 35.8 (0.63 - 0.13)	32.9 (0.29 - 0.06) 34.6 (0.47 - 0.10)	32.9 (0.30 - 0.06) 34.7 (0.60 - 0.12)	30.1 (0.41 - 0.08) 33.0 (0.56 - 0.11)	29.9 (0.28 - 0.06) 32.8 (0.62 - 0.13)
C*t* mean of all the experiments for each method [1]	33.5 (0.43 - 0.06) 35.7 (0.55 - 0.08)		32.9 (0.29 - 0.04) 34.6 (0.54 - 0.08)		30.0 (0.36 - 0.05) 32.9 (0.60 - 0.09)	
C*t* (3fg) - C*t* (15fg)	2.2		1.7		2.9	

Other fungal strain [2]						
Exp. I (C.P. 25)	34.7 (0.46 - 0.16)	-	34.0 (0.50 - 0.18)	-	33.3 (0.67 - 0.24)	-
Exp. I (C.P. 33)	35.9 (0.49 - 0.17)	-	33.5 (0.30 - 0.10)	-	32.6 (0.91 - 0.32)	-
Exp. I (C.P. 1176)	35.8 (0.58 - 0.20)	-	34.0 (0.22 - 0.08)	-	32.7 (0.87 - 0.31)	-
Exp. I (C.P. 11259)	35.5 (0.60 - 0.21)	-	33.6 (0.53 - 0.19)	-	32.8 (0.70 - 0.25)	-
Exp. I (C.P. 11292)	35.4 (0.42 - 0.15)	-	34.2 (0.26 - 0.09)	-	33.2 (0.23 - 0.08)	-

(b)

Assay: SYBR Green Detection of 3 fg *C. platani* gDNA spiked with 6 µl *C. platani*-uninfected necrotic wood extract			
Exp. I	Exp. II	Exp.III	C*t* mean of all three experiments
32.6 (0.32 - 0.11)	32.5 (0.34 - 0.12)	32.3 (0.27 - 0.10)	32.5 (0.32 - 0.07)

Tab. S5 - Detection of samples from naturally and artificially-infected trees by Real-Time PCR and microscope observation. [1] Samples from naturally-infected trees are from 1 to 28. Samples from artificially-infected trees are marked with T (time-point). The DNA samples corresponding to artificially-infected trees were those previously tested for diagnostic sensitivity in Pilotti et al. (2012) (wood sampling after plant death, at different time-points). In the code of T samples, the first number refers to the time-point in term of months passed after death at the moment of sampling; the second number refers to the sample. All samples with asterisks were tested in triplicate, the others in duplicate. [2] N.D. = negative detection. [3] The data regarding the presence of aleuroconidia in T samples are from Pilotti et al. (2012).

Sample [1]	EvaGreen [2]		SYBR Green [2]		Taqman [2]		Presence of aleurioconidia [3]
	2 µl	6 µl	2 µl	6 µl	2 µl	6 µl	
1	14.2 (0.08)	13.6 (0.80)	12.3 (0.11)	10.3 (0.08)	14.3 (0.31)	15.9 (1.0)	-
2	18.7 (0.04)	17.7 (0.05)	17.3 (0.06)	15.6 (0.05)	18.9 (0.15)	17.5 (0.29)	-
3	17.6 (0.06)	16.3 (0.18)	15.9 (0.13)	13.9 (0.28)	17.6 (0.01)	16.0 (0.15)	-
4	18.2 (0.04)	16.6 (0.24)	16.6 (0.08)	14.8 (0.03)	18.1 (0.00)	16.5 (0.21)	-
5	36.3 (0.08)	36.2 (0.21)	37.2 (0.46)	33.4 (0.09)	37.8 (0.32)	35.8 (0.08)	-
6	36.0 (0.03)	34.7 (0.20)	33.2 (0.53)	33.6 (0.39)	35.9 (0.72)	33.7 (0.52)	-
7	36.0 (0.02)	35.3 (0.05)	35.0 (0.39)	34.0 (0.45)	37.1 (0.00)	35.0 (0.43)	-
8	35.5 (0.14)	33.4 (0.02)	33.3 (1.41)	32.1 (0.03)	36.1 (1.0)	33.6 (0.47)	-
9	23.4 (0.12)	22.3 (0.13)	21.4 (0.06)	19.6 (0.05)	23.5 (0.07)	22.2 (0.29)	-
10	37.0 (0.10)	35.6 (0.08)	35.3 (0.36)	33.8 (1.30)	36.7 (0.09)	35.9 (0.10)	-
11	34.3 (0.03)	34.1 (0.13)	32.7 (0.34)	30.9 (0.11)	34.1 (0.44)	32.7 (0.33)	-
12	36.2 (0.35)	35.9 (0.04)	34.2 (0.04)	32.2 (0.09)	35.2 (1.4)	33.9 (0.54)	-
13	34.7 (0.17)	32.7 (0.02)	32.2 (0.43)	30.8 (0.01)	33.3 (0.50)	32.2 (0.75)	-
14	35.2 (0.08)	33.0 (0.08)	32.0 (0.05)	30.0 (0.07)	32.8 (0.04)	31.3 (0.04)	-
15	33.1 (0.03)	31.8 (0.42)	31.3 (0.35)	29.0 (0.03)	32.1 (0.08)	N.A.	-
16	35.8 (0.13)	33.7 (0.08)	33.0 (0.18)	30.8 (0.23)	34.1 (0.17)	33.7 (0.08)	-
17	18.3 (0.07)	16.7 (0.29)	16.8 (0.11)	14.9 (0.15)	17.6 (0.32)	16.1 (0.17)	-
18	36.6 (0.08)	36.5 (0.46)	34.9 (0.22)	32.8 (1.50)	37.6 (0.98)	36.0 (1.42)	-
19	20.2 (0.13)	20.7 (0.26)	18.0 (0.01)	16.1 (0.02)	19.8 (0.1)	19.8 (1.1)	+
20	37.2 (1.51)	36.2 (0.38)	33.7 (0.10)	31.6 (0.42)	28.0 (0.84)	31.5 (0.18)	-
21	36.0 (1.08)	35.5 (0.45)	34.4 (0.23)	33.0 (0.65)	34.3 (0.39)	33.7 (0.37)	-
22	34.2 (0.66)	32.8 (0.12)	32.1 (0.56)	30.3 (0.15)	31.9 (0.32)	31.3 (0.12)	-
23	18.4 (0.04)	17.0 (0.03)	16.5 (0.02)	14.9 (0.12)	16.5 (0.09)	15.7 (0.14)	-
24	31.6 (0.08)	30.4 (0.12)	29.6 (0.26)	27.9 (0.23)	29.5 (0.18)	28.8 (0.13)	-
25	35.1 (0.48)	34.3 (0.15)	33.2 (0.81)	32.4 (0.21)	35.4 (1.71)	33.2 (0.69)	-
26	33.9 (0.66)	33.2 (0.27)	31.7 (0.24)	30.4 (0.39)	32.6 (0.37)	31.2 (0.19)	-
27	35.1 (0.24)	34.9 (0.55)	33.0 (0.88)	32.0 (0.59)	34.9 (0.28)	33.6 (0.39)	-
28	36.4 (0.44)	32.7 (0.12)	31.8 (0.86)	30.6 (0.27)	31.7 (0.59)	31.3 (0.23)	-
T0.1	15.1 (0.11)	13.5 (0.10)			15.0 (0.41)	13.2 (0.11)	+
T0.2	15.6 (0.22)	14.3 (0.27)			16.5 (0.17)	15.0 (0.18)	+

Sample [1]	EvaGreen [2]		SYBR Green [2]		Taqman [2]		Presence of aleurioconidia [3]
	2 µl	6 µl	2 µl	6 µl	2 µl	6 µl	
T0.3	14.3 (0.21)	13.0 (0.28)			13.8 (0.17)	13.2 (0.10)	+
T0.4	14.9 (0.14)	14.0 (0.28)			15.2 (0.14)	14.1 (0.12)	+
T0.5	17.4 (0.15)	17.1 (0.24)			16.3 (0.10)	16.1 (0.13)	+
T5.1	10.3 (0.14)	N.D.			22.2 (0.73)	N.D.	+
T5.2	19.1 (0.05)	23.5 (0.75)			19.9 (0.09)	N.D.	+
T5.3	18.5 (0.14)	18.9 (0.34)			18.9 (0.18)	N.D.	+
T5.4	22.5 (0.16)	N.D.			21.0 (0.38)	N.D.	+
T5.5	20.1 (0.21)	19.6 (0.13)			19.4 (0.08)	20.3 (0.30)	+
T27.1	24.5 (0.20)	N.D.			23.7 (0.26)	N.D.	-
T27.2	21.1 (0.11)	35.7 (0.27)			21.8 (0.20)	N.D.	-
T27.3	24.5 (0.04)	N.D.			22.3 (0.11)	N.D.	-
T27.4	24.4 (0.12)	24.8 (1.16)			27.1 (0.19)	N.D.	-
T27.5	24.4 (0.22)	N.D.			22.9 (0.33)	N.D.	-