SUPPLEMENTARY MATERIALS

Hidden in plain sight: a molecular field survey of three wheat leaf blotch fungal diseases in North-Western Europe shows co-infection is widespread

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Supplementary Table 1. Validation of *Ptr*-specific primers for quantitative polymerase chain reaction (qPCR) with DNA extracted from *Pyrenophora tritici-repentis* (*Ptr*), *Parastagonospora nodorum* (*Pn*) and *Zymoseptoria tritici* (*Zt*) fungal isolates. For cross comparison, qPCR using previously published primers specific to *P. nodorum* and *Z. tritici* were also undertaken (Bearchell et al. 2005). qPCR reactions were also tested using wheat genomic DNA as a template. *P. tritici-repentis* qPCR primer sequences are listed in the Methods. Amplification is designated as the mean C_t value of two qPCR reactions. No amplification is designated 'ud – undetermined'. DE = Germany, DK = Denmark, LV = Latvia, NO = Norway, NZ = , New Zeland, SE = Sweeden.

Isolate name	**Country of origin**	**Pathogen**	**Year of origin**	***Ptr* qPCR assay (C_t)**	***Pn* qPCR assay (C_t)**	***Zt* qPCR assay (C_t)**
15306-1 2	DK	*P. tritici-repentis*	2015	21.9	ud	ud
15306-1 5	DK	*P. tritici-repentis*	2015	21.0	ud	ud
15Efecta7M1 1	DK	*P. tritici-repentis*	2015	21.9	ud	ud
15302-1 7-I	DK	*P. tritici-repentis*	2015	22.0	ud	ud
15302-1 8-1	DK	*P. tritici-repentis*	2015	18.8	ud	ud
15058 Efecta04	DK	*P. tritici-repentis*	2015	24.3	ud	ud
PTR-SN-001 A	DE	*P. tritici-repentis*	2015	25.9	ud	ud
PTR-SN-001 C	DE	*P. tritici-repentis*	2015	25.9	ud	ud
PTR-SN-002 A	DE	*P. tritici-repentis*	2015	25.2	ud	ud
PTR-SN-003 A	DE	*P. tritici-repentis*	2015	25.5	ud	ud
PTR-BB-001 B	DE	*P. tritici-repentis*	2015	27.0	ud	ud
16-DTR-DK-01-01	DK	*P. tritici-repentis*	2016	24.2	ud	ud
16-DTR-DK-01-03	DK	*P. tritici-repentis*	2016	25.9	ud	ud

16-DTR-DK-01-11	DK	*P. tritici-repentis*	2016	22.9	ud	ud
16-DTR-DK-01-16	DK	*P. tritici-repentis*	2016	23.4	ud	ud
16-DTR-DK-03-13	DK	*P. tritici-repentis*	2016	25.9	ud	ud
BB-001/1	DE	*P. tritici-repentis*	2016	24.0	ud	ud
BB-002/5	DE	*P. tritici-repentis*	2016	22.3	ud	ud
BW-001/1	DE	*P. tritici-repentis*	2016	23.1	ud	ud
BY-001/2	DE	*P. tritici-repentis*	2016	23.6	ud	ud
SN-003/1	DE	*P. tritici-repentis*	2016	23.5	ud	ud
201618	NO	*P. nodorum*	2012	ud	22.3	ud
201593	NO	*P. nodorum*	2014	ud	22.9	ud
NZ13-11	NZ	*Z. tritici*	2013	ud	ud	26.5
NZ13-13	NZ	*Z. tritici*	2013	ud	ud	24.9
LV1	LV	*Z. tritici*	2014	ud	ud	24.6
LV2	LV	*Z. tritici*	2014	ud	ud	23.3
53-5	DK	*Z. tritici*	2013	ud	ud	28.9
144 DK	DK	*Z. tritici*	2014	ud	ud	24.3
14-FlMa-25	DK	*Z. tritici*	2014	ud	ud	28.3
14-LMOHe-20	DK	*Z. tritici*	2014	ud	ud	25.3
FlHer46	DK	*Z. tritici*	2014	ud	ud	23.4
SV216 Vär-16	SE	*Z. tritici*	2016	ud	ud	26.6
MAC	DK	*Triticum aestivum*	2015	ud	ud	ud

Supplementary Table 2. The number of field trials conducted during 2016 2017 and 2018 for the assessment of 40 winter wheat genotypes for susceptibility to the necrotrophic diseases tan spot (TS), septoria tritici blotch (STB), and septoria nodorum blotch (SNB).

2016	**TS**	**STB**	**SNB**
Denmark	1	-	-
Germany	1	1	-
Norway	-	-	1
United Kingdom	-	-	-
2017	**TS**	**STB**	**SNB**
Denmark	1	-	-
Germany	1	1	-
Norway	-	-	1
United Kingdom	1	-	-
2018	**TS**	**STB**	**SNB**
Denmark	-	-	-
Germany	-	-	-
Norway	-	-	1
United Kingdom	1	-	-

Supplementary Table 3. Field trial inoculation methods for the assessment of tan spot (TS), septoria tritici blotch (STB), and stagonospora nodorum blotch (SNB) severity.

		Denmark	**Germany**	**Norway**	**United Kingdom**
TS	2016	Naturally *P. tritici-repentis* infected straw	Natural infection	-	-
	2017	Naturally *P. tritici-repentis* infected straw	Naturally *P. tritici-repentis* infected straw	-	Spore suspension
	2018	-	-	-	Spore suspension
STB	2016	-	Spore suspension	-	-
	2017	-	Spore suspension	-	-
SNB	2016	-	-	Naturally *P. nodorum* infected straw	-
	2017	-	-	Naturally *P. nodorum* infected straw	Spore suspension
	2018	-	-	Naturally *P. nodorum* infected straw	

- no trial was carried out

Supplementary Table 4. Results of qPCR for *Pyrenophora tritici-repentis* (*Ptr*), *Parastagonospora nodorum* (*Pn*) and *Zymoseptoria tritici* (*Zt*) from wheat flag leaf samples collected from 330 sites in Denmark (DK), Finland (FI), Germany (DE), Latvia (LV), Norway (NO), Sweden (SE) and the United Kingdom (UK) in 2015, 2016 and 2017. Spring wheat (sw) cultivars are indicated. All other cultivars were winter wheat. * indicates samples that originated from fields with reduced tillage and pre-crop winter wheat.

Sample Name	Year	Country	*Ptr* (pg fungal DNA/ng plant DNA)	*Pn* (pg fungal DNA/ng plant DNA)	*Zt* (pg fungal DNA/ng plant DNA)
31	2015	DK*	15.3	0.0	14.2
32	2015	DK*	4.5	0.0	9.6
33	2015	DK*	2.7	0.0	2.7
34	2015	DK*	5.7	0.3	0.4
35	2015	DK*	4.6	0.1	0.5
36	2015	DK*	11.5	0.1	1.1
37	2015	DK*	7.8	0.0	5.6
38	2015	DK*	3.4	0.0	0.2
39	2015	DK*	25.9	0.4	1.2
40	2015	DK*	1.7	0.0	8.9
41	2015	DK*	6.6	0.0	2.3
42	2015	DK	0.0	0.0	16.5
43	2015	DK	0.0	0.0	4.3
44	2015	DK*	2.7	0.0	110.5
45	2015	DK	0.1	0.0	32.8
46	2015	DK	0.8	0.0	50.1
47	2015	DK	0.3	0.0	12.4
48	2015	DK*	7.0	0.0	1.6
49	2015	DK	0.5	0.0	353.7
50	2015	DK	0.0	0.0	89.9
51	2015	DK	0.0	0.0	10.4
52	2015	DK	0.0	0.0	62.9

53	2015	DK	0.0	0.0	22.5
54	2015	DK	0.0	0.0	17.7
55	2015	DK	0.0	0.0	12.6
56	2015	DK	0.0	0.0	4.5
57	2015	DK	0.5	0.0	9.6
58	2015	DK	0.0	3.2	17.3
59	2015	DK	0.3	0.0	26.2
60	2015	DK	0.0	0.0	9.9
61	2015	DK	1.4	0.0	45.1
62	2015	DK	0.0	0.0	18.2
63	2015	DK	0.1	0.0	9.3
100	2015	DK	0.0	5.6	75.7
69	2015	DE	3.3	0.0	0.9
70	2015	DE	0.4	0.0	0.0
71	2015	DE	28.7	10.2	62.8
72	2015	DE	5.4	0.2	18.0
73	2015	DE	11.3	0.1	4.6
74	2015	DE	110.2	0.2	7.9
75	2015	DE	3.0	1.0	0.1
76	2015	DE	128.9	0.7	0.8
77	2015	DE	1.2	0.0	54.1
78	2015	DE	0.2	0.0	58.7
79	2015	DE	10.2	0.0	32.7
80	2015	NO	1.3	15.4	37.4
81	2015	NO	23.8	47.2	56.6
82	2015	NO	8.2	40.7	27.6
83	2015	NO	2.3	135.6	48.5
84	2015	NO	38.3	24.2	4.2
85	2015	NO	0.3	22.3	10.9
86	2015	NO	1.0	161.3	40.9
87	2015	NO	11.5	119.0	301.1
88	2015	NO	6.5	96.8	460.6

89	2015	NO	4.8	3.3	471.6
90	2015	NO	0.0	95.1	0.1
91	2015	NO	0.0	124.9	5.9
92	2015	NO	0.0	25.0	0.0
93	2015	NO	0.0	73.5	0.9
94	2015	NO	0.0	18.5	2.5
95	2015	NO	0.0	118.7	4.2
96	2015	NO	0.0	318.6	1.3
97	2015	NO	0.0	0.3	1.2
98	2015	NO	0.0	4.2	0.0
99	2015	NO	0.0	0.6	9.2
101	2015	SE	42.9	2.8	2.6
65	2015	FI	15.9	29.5	0.0
66	2015	FI	16.3	1.9	0.2
67	2015	FI	0.0	104.8	0.0
68	2015	FI	0.7	8.9	0.0
102	2015	FI	25.7	0.5	0.4
103	2015	FI	7.1	11.6	33.7
64	2015	LV	0.0	0.9	4.2
1	2015	UK	0.0	0.0	0.0
9	2015	UK	0.0	0.0	0.0
17	2015	UK	0.0	0.0	5.0
19	2015	UK	0.0	0.0	0.1
40	2015	UK	0.0	0.0	0.0
56	2015	UK	0.0	0.0	0.0
60	2015	UK	0.0	0.0	0.0
64	2015	UK	0.0	0.0	1.4
69	2015	UK	0.0	0.0	0.0
70	2015	UK	0.0	0.0	0.0
71	2015	UK	0.0	0.0	24.8
92	2015	UK	0.0	0.0	0.6
96	2015	UK	0.0	0.0	0.0

106	2015	UK	0.0	0.0	0.0
109	2015	UK	0.0	0.0	1.0
110	2015	UK	0.0	0.0	0.1
122	2015	UK	0.0	0.0	9.7
130	2015	UK	0.0	0.0	4.4
136	2015	UK	0.0	0.0	0.0
138	2015	UK	0.0	0.0	0.1
140	2015	UK	0.0	0.0	0.0
147	2015	UK	0.0	0.0	7.3
158	2015	UK	0.0	0.0	0.1
159	2015	UK	0.0	0.0	1.3
160	2015	UK	0.0	0.0	446.9
162	2015	UK	0.0	0.0	545.2
163	2015	UK	0.9	0.0	10.2
165	2015	UK	0.2	0.0	369.4
183	2015	UK	0.0	0.0	0.5
196	2015	UK	0.0	0.0	111.5
199	2015	UK	0.0	0.0	29.0
201	2015	UK	0.0	0.0	49.2
206	2015	UK	0.0	0.0	12.3
219	2015	UK	0.0	0.0	0.0
221	2015	UK	0.0	0.0	6.5
229	2015	UK	0.0	0.0	0.1
241	2015	UK	0.0	0.0	0.0
245	2015	UK	0.0	0.0	2.6
256	2015	UK	0.0	0.0	0.0
291	2015	UK	0.0	0.0	0.0
292	2015	UK	0.0	0.0	0.1
228	2016	DK*	96.8	0.0	2.9
229	2016	DK*	5.1	0.0	0.6
230	2016	DK*	141.8	0.1	0.2
231	2016	DK	1.7	0.0	199.3

232	2016	DK	0.1	3.1	98.9
233	2016	DK	0.2	4.7	103.6
234	2016	DK*	13.9	0.1	154.3
235	2016	DK	1.7	0.0	84.6
236	2016	DK	0.0	0.0	89.9
237	2016	DK	1.3	0.0	1.5
238	2016	DK	3.1	2.5	30.5
239	2016	DK*	10.5	0.0	31.7
240	2016	DK	0.7	1.0	79.8
241	2016	DK	2.8	0.0	13.4
242	2016	DK*	40.1	0.0	0.7
243	2016	DK	0.8	0.1	172.6
244	2016	DK	6.0	0.6	98.5
245	2016	DK	0.1	0.0	25.4
246	2016	DK	0.0	0.6	28.2
247	2016	DK	0.1	7.0	113.0
248	2016	DK	2.2	3.7	0.2
249	2016	DK*	16.3	0.8	0.1
250	2016	DE	79.2	17.5	0.9
251	2016	DE	58.0	5.0	38.8
252	2016	DE	41.0	1.7	13.2
253	2016	DE	12.7	1.1	4.1
254	2016	DE	0.2	1.0	9.1
255	2016	DE	0.3	8.9	89.1
256	2016	DE	12.0	1.2	95.3
257	2016	DE	4.0	6.0	28.6
258	2016	DE	13.0	7.0	21.5
259	2016	DE	2.0	0.0	1.7
260	2016	DE	0.3	0.5	5.6
261	2016	DE	0.0	0.4	41.3
262	2016	DE	1.6	1.0	15.0
263	2016	DE	0.1	0.2	20.3

264	2016	DE	1.7	2.3	36.5
265	2016	DE	1.3	74.2	26.9
266	2016	DE	0.3	9.5	6.2
267	2016	DE	1.1	2.9	41.0
268	2016	DE	3.4	6.2	25.2
269	2016	DE	0.0	0.0	35.0
270	2016	DE	9.7	0.0	1.2
271	2016	DE	0.4	1.2	2.0
272	2016	DE	89.9	1.4	2.0
273	2016	NO	6.0	80.2	120.2
274	2016	NO	3.4	146.3	138.2
275	2016	NO	9.9	167.9	155.5
276	2016	NO sw	0.2	157.7	0.7
277	2016	NO sw	6.0	114.7	0.4
278	2016	NO sw	2.9	101.6	0.4
279	2016	NO sw	1.2	96.3	0.2
280	2016	NO	19.2	76.7	234.4
281	2016	NO sw	0.9	102.4	0.3
282	2016	NO sw	0.3	101.1	0.4
283	2016	NO	1.1	14.6	59.5
284	2016	NO	3.4	8.6	175.5
285	2016	NO	1.2	33.6	70.2
286	2016	NO	0.0	45.4	0.1
287	2016	NO	0.0	24.0	0.5
288	2016	NO	0.1	48.8	0.3
289	2016	NO	5.1	22.0	1.3
290	2016	NO	22.1	69.9	32.6
291	2016	NO	22.2	27.8	22.4
292	2016	NO sw	8.5	75.3	2.5
293	2016	NO sw	7.2	8.1	0.2
294	2016	NO sw	25.8	35.8	0.2
295	2016	NO	80.2	35.4	618.8

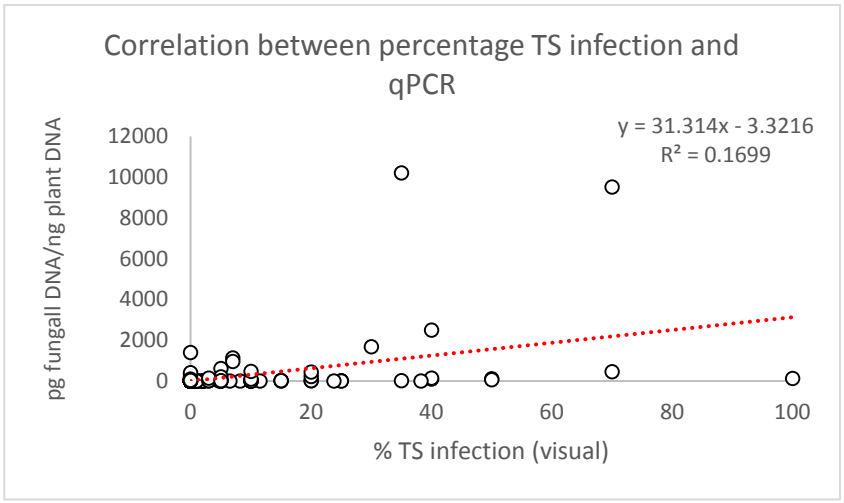
296	2016	NO	94.2	50.2	156.2
297	2016	NO	78.8	20.1	238.3
298	2016	NO sw	0.2	19.1	1.6
299	2016	NO sw	0.1	87.2	1.3
300	2016	NO sw	1.1	92.3	1.8
301	2016	NO sw	0.2	6.7	0.4
302	2016	NO sw	0.0	3.0	0.0
303	2016	NO	0.2	4.0	0.5
304	2016	NO	2.3	0.0	11.7
305	2016	NO	0.0	11.2	1.3
306	2016	NO sw	0.1	1.3	0.0
307	2016	NO	0.0	11.1	11.5
308	2016	NO sw	0.0	10.2	0.7
309	2016	NO sw	4.2	88.9	0.1
310	2016	NO sw	0.1	71.7	0.2
311	2016	NO sw	0.1	132.6	0.0
312	2016	NO sw	0.3	116.2	0.2
11	2016	UK	0.0	0.0	90.1
21	2016	UK	0.0	0.0	0.3
29	2016	UK	0.0	0.0	0.5
33	2016	UK	0.0	0.0	0.0
51	2016	UK	0.0	0.0	0.8
61	2016	UK	0.0	0.0	1.9
79	2016	UK	0.1	0.0	11.1
81	2016	UK	0.0	0.0	0.8
90	2016	UK	0.0	0.0	19.2
92-16	2016	UK	0.0	0.0	0.0
107	2016	UK	0.0	0.0	4.0
116	2016	UK	0.0	0.0	60.9
124	2016	UK	0.0	0.0	0.8
129	2016	UK	0.0	0.0	111.0
139	2016	UK	0.0	0.0	5.3

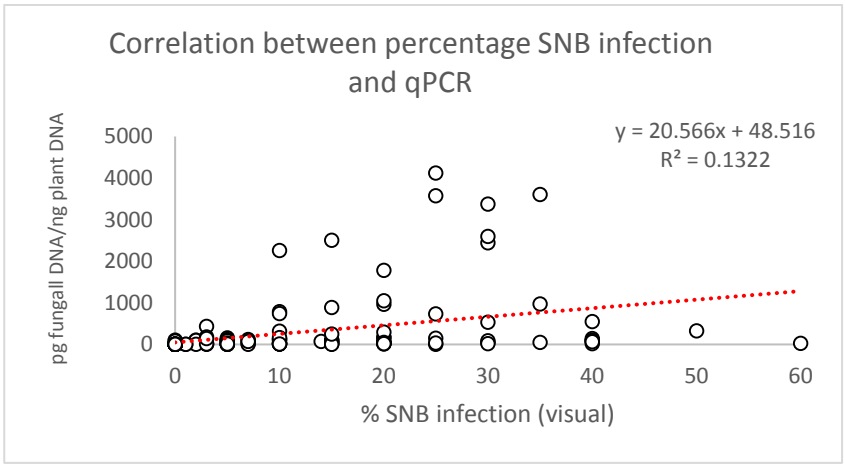
145	2016	UK	0.0	0.0	0.2
148	2016	UK	0.0	0.0	6.5
153	2016	UK	0.0	0.0	0.6
154	2016	UK	0.0	0.0	4.3
156	2016	UK	0.0	0.0	1.7
161	2016	UK	0.0	0.0	8.2
165-16	2016	UK	0.0	0.0	432.0
171	2016	UK	0.0	0.0	0.5
175	2016	UK	0.0	0.0	50.5
179	2016	UK	0.2	0.0	49.2
181	2016	UK	0.0	0.0	10.2
189	2016	UK	0.0	0.0	0.1
198	2016	UK	0.0	0.0	0.0
200	2016	UK	0.0	0.0	1254.6
201-16	2016	UK	0.0	0.0	33.9
205	2016	UK	0.4	0.0	101.0
208	2016	UK	0.0	0.0	3.8
215	2016	UK	0.0	0.0	0.2
216	2016	UK	0.0	0.0	0.2
222	2016	UK	0.0	0.0	0.0
226	2016	UK	0.0	0.0	2.3
234	2016	UK	0.0	0.0	12.6
235	2016	UK	0.0	0.0	0.3
239	2016	UK	0.0	0.0	0.2
242	2016	UK	0.0	0.0	0.1
515	2017	DK	30.9	2.0	181.8
516	2017	DK	1.4	0.0	125.0
517	2017	DK	5.1	0.0	1033.3
518	2017	DK	0.5	0.0	23.6
536	2017	DK	26.3	0.3	702.2
537	2017	DK	9.6	75.6	601.9
538	2017	DK	0.0	0.0	57.6

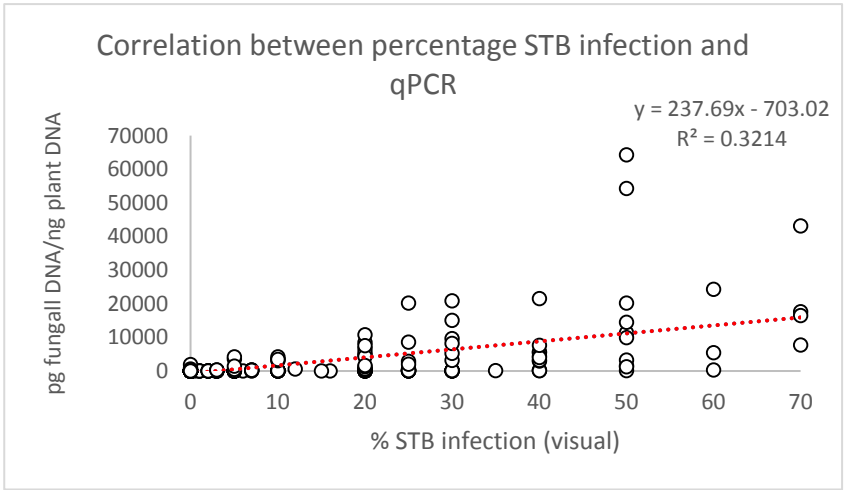
539	2017	DK	0.0	0.0	206.8
540	2017	DK	0.4	0.0	111.1
541	2017	DK	0.1	0.1	27.2
542	2017	DK	8.3	0.0	6.9
543	2017	DK	21.3	0.0	153.9
544	2017	DK	2.6	102.9	1851.3
545	2017	DK	0.0	16.0	160.3
546	2017	DK	0.7	0.0	349.4
547	2017	DK	0.1	0.0	689.1
519	2017	DE	737.2	3.7	0.5
520	2017	DE	79.6	29.9	297.6
521	2017	DE	615.4	0.9	4.1
522	2017	DE	48.5	0.9	28.7
523	2017	DE	93.8	0.2	16.3
524	2017	DE	2.4	0.1	11.0
525	2017	DE	0.0	0.1	0.2
526	2017	DE	0.9	0.0	0.0
527	2017	DE	1.0	0.5	592.9
528	2017	DE	146.8	0.1	0.6
529	2017	DE	5.9	0.1	93.9
530	2017	DE	10.2	0.0	0.8
531	2017	DE	1.2	3.5	21.0
532	2017	DE	23.0	0.8	173.3
533	2017	DE	2.3	0.3	7.5
534	2017	DE	11.6	2.2	593.3
535	2017	DE	21.2	31.7	478.2
548	2017	NO sw	0.0	119.7	0.8
549	2017	NO sw	0.0	42.5	0.1
550	2017	NO sw	0.1	56.6	105.7
551	2017	NO	6.4	3.9	363.7
552	2017	NO	22.7	37.1	1056.8
553	2017	NO	2.7	19.7	588.0

554	2017	NO	3.6	21.8	121.7
555	2017	NO sw	0.3	6.8	1.0
556	2017	NO	0.7	3.1	209.1
557	2017	NO	3.8	11.0	175.7
558	2017	NO sw	0.2	1.7	95.6
559	2017	NO sw	0.1	10.5	24.2
560	2017	NO sw	8.3	11.8	0.2
561	2017	NO	3.2	2.2	208.0
562	2017	NO	4.3	55.1	1516.0
563	2017	NO sw	108.9	57.8	0.5
564	2017	NO	1.3	129.9	6.0
565	2017	NO	0.3	281.6	280.0
566	2017	NO	0.1	190.8	1.6
567	2017	NO	0.1	146.7	6.0
568	2017	NO sw	0.1	17.4	0.1
569	2017	NO sw	1.1	51.2	0.1
570	2017	NO sw	0.0	0.7	0.0
571	2017	NO sw	0.0	236.1	0.1
572	2017	NO sw	0.0	44.8	0.0
573	2017	NO sw	0.0	42.9	0.0
574	2017	NO	0.1	797.7	0.0
575	2017	NO	0.0	169.3	39.3
576	2017	NO	0.0	0.7	1184.1
577	2017	NO	0.0	4.0	729.6
578	2017	NO sw	0.9	37.4	0.2
579	2017	NO sw	0.9	103.6	0.1
580	2017	NO sw	2.5	70.1	10.9
581	2017	NO	0.1	65.6	70.3
582	2017	NO	0.0	62.2	67.6
583	2017	NO	1.0	127.8	128.4
584	2017	NO	0.0	80.7	3.8
585	2017	NO	0.0	40.2	29.3

586	2017	UK	0.0	0.0	187.6
587	2017	UK	0.0	0.0	132.3
588	2017	UK	0.0	2.9	4140.7
589	2017	UK	0.0	0.0	54.2
590	2017	UK	0.0	0.0	952.9
591	2017	UK	0.0	0.0	10.7
592	2017	UK	0.0	0.0	777.2
593	2017	UK	0.0	0.0	135.9
594	2017	UK	0.4	67.7	49781.9
595	2017	UK	0.0	0.0	958.5
596	2017	UK	0.0	237.2	18391.1
597	2017	UK	0.0	0.0	311.5
598	2017	UK	0.0	0.0	320.6
599	2017	UK	0.0	0.0	112.3
600	2017	UK	0.0	0.1	181.7
601	2017	UK	0.0	0.0	247.1
602	2017	UK	10.7	19.5	1507.7
603	2017	UK	0.3	0.0	1759.5
604	2017	UK	0.1	0.3	1182.6
605	2017	UK	0.0	0.0	2830.8







Supplementary Fig 1 Correlations between visual assessments of disease for tan spot (TS), septoria nodorum blotch (SNB) and septoria tritici blotch (STB) versus quantification of the corresponding fungal DNA via quantitative polymerase chain reaction (qPCR) assay.