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1 **The emerging British *Verticillium longisporum* population consists of aggressive**
2 ***Brassica* pathogens**
3
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16 **ABSTRACT**

17 The impact of diseases depends on the dynamic interplay between host, pathogen and the
18 environment. Newly emerging diseases may be the consequence of novel pathogen
19 introductions that are typically associated with unpredictable outcomes, as their interaction
20 with the host in a novel environment is unprecedented. Alternatively, new diseases may
21 emerge from latent, previously established, pathogen populations that are triggered by
22 changes in environmental factors like weather, agricultural practices and ecosystem
23 management. Verticillium stem striping recently emerged in British oilseed rape (*Brassica*
24 *napus)* production from a latent *Verticillium longisporum* population. *V. longisporum* is a
25 hybrid fungal pathogen consisting of three lineages, each representing a separate
26 hybridization event. Despite its prevalence, little is known of the pathogenicity of the British
27 *V. longisporum* population. In this study, the pathogenicity of British isolates was tested on
28 four different cultivars of three different *Brassica* crop species as well as on the model plant
29 *Arabidopsis thaliana* and compared with previously characterized *V. longisporum* strains
30 from other regions of the world, including representatives of all three hybrid lineages.
31 Intriguingly, the British isolates appeared to be amongst the most pathogenic strains on
32 *Brassica* crops. In conclusion, Verticillium stem striping poses a genuine threat to oilseed
33 rape production as the British *V. longisporum* population consists of aggressive pathogens
34 that have the potential to significantly impact *Brassica* crops.

35

36 **KEYWORDS**

37 Verticillium stem striping, Verticillium wilt, oilseed rape, pathogenicity, hybrid, cauliflower,
38 cabbage, *Arabidopsis*

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39 **Introduction**

40 *Verticillium* fungi cause wilt diseases on hundreds of plant species of which many are
41 economically important crops. *Verticillium dahliae* is the most notorious member of these
42 fungi and can cause severe yield losses in crops like olive and cotton (Fradin and Thomma
43 2006; Levin et al. 2003; Melero-Vara et al. 1995). Despite a wide host range that comprises
44 hundreds of plant species, *V. dahliae* generally does not infect brassicaceous plants (Depotter
45 *et al.*, 2016a). In contrast, *V. longisporum* is specialized on Brassicaceae hosts, with oilseed
46 rape as its most economically important target (Depotter *et al.*, 2016a; Inderbitzin *et al.*,
47 2011). In contrast to all other *Verticillium* spp, including *V. dahliae*, *V. longisporum* is not a
48 haploid organism but rather an allopolyploid as a consequence of interspecific hybridization.
49 The species *V. longisporum* consists of three lineages, each representing a separate
50 hybridization event. Four parental lines, including two *V. dahliae* isolates (D2 and D3),
51 contributed to the different hybridization events. The two remaining parental lines represent
52 two previously uncharacterized *Verticillium* species that have been provisionally called
53 Species A1 and Species D1 (Inderbitzin et al. 2011). Species A1 participated in all three
54 hybridization events and hybridized with D1, D2 and D3 to form the lineages A1/D1, A1/D2
55 and A1/D3, respectively. Conceivably, the allopolyploid genome of *V. longisporum*
56 contributed to the host range shift such that *V. longisporum* gained the capacity to infect
57 Brassicaceae (Inderbitzin et al. 2011; Depotter et al. 2016b).

58 Interspecific hybridization is an important driver for genome evolution (Depotter *et*
59 *al.*, 2016b). Hybrid organisms experience a so-called "genomic shock" that incites major
60 genomic rearranges and changes in gene expression patterns (Doyle et al. 2008). Hybrids are
61 therefore especially adept in responding to environmental changes or invading novel niches.
62 Hybridization brings important additions to the toolset of pathogens; hence, host range and
63 pathogenicity are often altered in interspecific hybrids (Depotter et al. 2016b). Differences
64 between *V. longisporum* lineages may therefore originate from their separate hybrid origin.
65 Lineages A1/D1 and A1/D3 are found on various Brassicaceae species, whereas lineage
66 A1/D2 is only known from horseradish in the USA (Inderbitzin et al. 2011; Yu et al. 2016).
67 A1/D1 is the predominant lineage on oilseed rape and is also the most pathogenic *V.*
68 *longisporum* lineage of this crop (Novakazi et al. 2015).

69 In addition to its different genetic constitution, *V. longisporum* is unique amongst
70 Verticillium species for its disease symptom display on oilseed rape. Verticillium pathogens
71 are xylem colonizers inducing occlusions in the vessels, which hampers the water transport in
72 the xylem (Fradin and Thomma 2006). In response, Verticillium infections generally develop

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73 wilting symptoms. However, these symptoms are lacking from *V. longisporum* infections on
74 oilseed rape. Rather, black unilateral stripes appear on the plant stem at the end of the
75 growing season and, in a later stage, microsclerotia appear on the cortex under the stem
76 epidermis (Heale and Karapapa 1999; Depotter et al. 2016a). Hence, the new common name
77 "Verticillium stem striping" was coined to describe the *V. longisporum* disease on oilseed
78 rape (Depotter et al. 2016a). Intriguingly, Verticillium stem striping symptoms fail to appear
79 during pathogenicity tests when oilseed rape plants are grown under controlled conditions
80 and seedlings are inoculated by dipping the roots in a spore suspension (Eynck et al. 2007;
81 Eynck et al. 2009; Floerl et al. 2008; Zeise and Tiedemann 2002). Under those conditions,
82 plants exhibit chlorosis, vascular discoloration and stunting at an early stage. The reasons for
83 these differences in symptom development are currently unknown. Nevertheless, despite
84 differences in disease symptomatology, it has previously been determined that root-dip
85 pathogenicity tests in the glasshouse are a good proxy for oilseed rape cultivar resistance
86 under field conditions (Knüfer et al. 2016).

87 Emerging diseases pose threats to natural and cultivated ecosystems (Fisher et al.
88 2012). Pathogen emergence can be preceded by recent introductions if disease propagules
89 reach unaffected regions. For example, rusts are especially notorious for their natural ability
90 of long-distance dispersal as their wide-ranging airborne spread of spores makes that local
91 rust populations can change swiftly. Hence, dramatic shifts in the European wheat yellow rust
92 (*Puccinia striiformis* f. sp. *tritici*) populations have been observed in recent years as exotic
93 lineages have replaced former populations (Hubbard et al. 2015; Hovmøller et al. 2015).
94 Furthermore, disease dispersal is also facilitated through human activities. Globalization has
95 led to more inter-continental trade and transport of humans and commodities, which,
96 unquestionably, has contributed to an increasing frequency of pathogen introductions. For
97 example, the introduction of the North American forest pathogen *Heterobasidion irregulare*
98 into Italy coincided with an American military base in World War II (Garbelotto et al. 2013).
99 However, recent introductions are not prerequisites for new disease emergences (Anderson et
100 al. 2004). Environmental factors, such as weather and farming techniques, can give rise to
101 established, yet latent, pathogen populations. Verticillium stem striping occurred in the
102 United Kingdom (UK) in this fashion as a genetic diverse *V. longisporum* population
103 suddenly emerged for currently unknown reasons (Depotter et al. 2017).

104 Verticillium stem striping was reported in 2007 for the first time in the UK and is
105 currently widespread in England (Gladders et al. 2011, 2013). The recent and sudden rise of
106 this disease makes that hitherto little is known about the pathogenic traits of the British *V.*

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107 *longisporum* population. The aim of this study is to assess the threat of Verticillium stem
108 striping in the UK by comparing the pathogenicity British isolates with previously
109 characterized *V. longisporum* strains from different countries.
110
111 **Materials and methods**
112 *Pathogenicity tests*
113 Pathogenicity tests were conducted in order to compare the virulence of 9 *V. longisporum*
114 strains (Table 1). Three different *Brassica* crops were used: oilseed rape (*B. napus* var.
115 *oleifera*), cauliflower (*B. oleracea* var. *botrytis*) and Chinese cabbage (*B. rapa* subsp.
116 *Pekinensis*). Winter oilseed rape cultivars comprise open pollinating and hybrid types of
117 which one of each was tested: cv. Quartz and cv. Incentive, respectively. Furthermore, one
118 Chinese cabbage cultivar (cv. Hilton), and one cauliflower cultivar (cv. Clapton) were also
119 included. Thus, in total, four *Brassica* cultivars were tested. Plants were grown in climate-
120 controlled glasshouses under a 16 hrs light/ 8 hrs dark cycle with temperatures maintained
121 between 20 and 28°C during the day, and a minimum of 15°C overnight. Before sowing,
122 seeds were surface-sterilized during 1 min in 70% ethanol, followed by 15 min in 1%
123 commercial bleach and then rinsed four times with sterile water. These sterilized seeds were
124 then sown in trays with sterile compost and kept in the glasshouse for 14 days. These two-
125 week old seedlings were inoculated by dipping the roots for 30 min in a suspension of $1x10^6$
126 conidiospores ml^{-1}. Conidiospores were obtained from three-week old cultures grown on
127 potato dextrose agar plates. Fifteen plants were inoculated for every *V. longisporum* strain
128 and 15 control plants were dipped in sterile water instead of a conidiospore suspension.
129 Individual seedlings were planted in 9 cm square pots with 4:1:1 compost:sand:loam mixture
130 and pots were placed according to a random block design. The potting mixture had been
131 autoclaved twice for 60 min with 24h between each treatment. Plants were grown for 6 weeks
132 before harvesting. Plants were harvested by cutting the stem just above the hypocotyls and
133 pooled in 5 groups of three plants. Above ground dry weight was subsequently determined by
134 drying the samples at 100°C for a minimum of 12 h. Pathogenicity tests were executed twice
135 to confirm the virulence responses of the different strains. In addition, stem samples were
136 taken and pooled into 5 groups to determine the relative fungal colonization of the *V.*
137 *longisporum* strains. Stem pieces of 1 cm, just above the hypocotyls, were cut. During the
138 repeat experiment, Quartz plants were only grown until 25 days after inoculation to guarantee
139 sufficient plant biomass to extract DNA from as longer growth periods could lead to

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140 complete decay of the plants (Figure S1). Furthermore, in the second pathogenicity test,
141 Chinese cabbage plants were inoculated with a water suspension of $5x10^5$ conidiospores ml^{-1},
142 instead of $1x10^6$ conidiospores ml^{-1}.

143 *Arabidopsis thaliana* (Col-0) plants were grown in a different glasshouse where the
144 temperature was kept between 19 and 21°C and the same light/dark regime was used
145 (16h/8h). Four plants of every treatment were grown. Plant inoculation occurred three weeks
146 after sowing and roots were dipped for 10 min in a water suspension of 10^6 conidiospores ml^{-}
147 ^{1}. Above-ground plant material was harvested for real-time PCR analysis three weeks after
148 inoculation.

149

150 *Relative fungal quantification*

151 Samples were taken for fungal biomass quantification and ground in liquid nitrogen.
152 Approximately 200 mg ground plant material was dissolved in 500 µl of CTAB buffer (55
153 mM CTAB, 0.1 M Tris pH 8.0, 20 mM EDTA pH 8.0, 1.25 NaCl and 0.25 mM PVP 40). The
154 buffer/plant extract was incubated for 30 min at 65°C. Samples were centrifuged and
155 supernatant was subsequently transferred to a clean tube and 250 µl chloroform:isoamyl
156 alcohol solution (24:1) was added. Samples were mixed thoroughly by inversion and
157 subsequently centrifuged. Next, the supernatant was transferred into 50 µl ammonium acetate
158 (7.5M) and 500 µl ethanol. After mixing, DNA was precipitated and the supernatant was
159 removed. DNA pellets were washed twice with 70% ethanol and dissolved in DNase free
160 water.

161 The amount of *V. longisporum* DNA in stems was quantified relatively to the amount
162 of plant DNA by real-time PCR using QuantStudio™ Flex Real-Time PCR System (Applied
163 Biosystems, CA, USA). Fungal DNA was amplified with the *V. longisporum* specific primer
164 pair: VlTubF2/ VlTubR1 (GCAAAACCCTACCGGGTTATG /
165 AGATATCCATCGGACTGTTCGTA) (Debode et al. 2011) and the RuBisCO sequence
166 targeting primer pair RubF/RubR (TATGCCTGCTTTGACCGAGA /
167 AGCTACTCGGTTAGCTACGG) for plants. Real-time PCR was performed in reactions of
168 10 µl containing 500 nM of every primer and 5 µl of Power SYBR Green Master Mix
169 (Applied Biosystems, Foster City, CA, USA). The thermal programme of the real-time PCR
170 started with an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 15 s at
171 95°C, 1 min at 62°C and 30 s at 72°C. Specific amplification was verified running a melting

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172 curve: samples were heated to 95°C for 15 s, cooled down to 60°C for 1 min and heated
173 again to 95°C for 15 s. Signals above 36 cycles are considered below the detection limit.
174 DNA of the *Arabidopsis* plants was isolated according to Fulton et al. (1995).
175 Relative *V. longisporum* colonization was quantified according to Ellendorf et al. (2009)
176 using the qPCR core kit (Eurogentec, Liège, Belgium). Real-time PCR was executed on an
177 ABI7300 PCR System (Applied Biosystems, Foster City, CA, USA) with following thermal
178 conditions: an initial 95 °C denaturation step for 4 min, 30 cycles of denaturation for 15 s at
179 95 °C, annealing for 30 s at 60 °C, and extension for 30 s at 72 °C.
180
181 *Data analysis*
182 Significance levels for parametric data were calculated with the one-way analysis of variance
183 (ANOVA) or the t-test for unequal variances in R3.2.3 (R Core Team 2015). Non-parametric
184 data was analysed with the Mann-Whitney U-test. Correlations were calculated with the
185 Pearson correlation coefficient (r).
186
187 **Results**
188 The pathogenicity of four British *V. longisporum* isolates was compared with that of five
189 previously characterised isolates, including isolates of all three *V. longisporum* hybridization
190 lineages (Table 1). The isolates were tested on four different *Brassica* cultivars: two oilseed
191 rape (cv. Incentive and cv. Quartz), one cauliflower (cv. Clapton) and one Chinese cabbage
192 (cv. Hilton). Pathogenicity tests were repeated twice and similar pathogenicity outcomes for
193 the different *V. longisporum* strains were obtained on both occasions. Pathogenic *V.*
194 *longisporum* isolates stunted plant growth and leaves displayed chlorosis and necrosis (Figure
195 S1-4). Moreover, the more aggressive isolates caused complete decay of Quartz plants within
196 6 weeks of inoculation (Figure S1). Internally, vascular discoloration of diseased plant roots
197 was observed, ranging from light brown to black. Furthermore, the pathogenicity of the
198 British strains was also tested on the model plant *Arabidopsis thaliana* (Col-0): VLB1, VLB2
199 and VLB9 induced leaf curling and necrosis (Figure S5). The same symptoms were observed
200 for VLB3, but the plants were also heavily stunted indicating that VLB3 is more aggressive
201 than the other British strains on *Arabidopsis*.
202 The British *V. longisporum* isolates were pathogenic on all tested *Brassica* cultivars
203 (Figure 1). Disease symptoms such as stunted growth and necrosis led to significant
204 aboveground biomass reductions, which were amongst the highest for the *V. longisporum*

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205 strains tested. Similarly, severe biomass reductions were observed for the two A1/D1
206 representatives (PD639 and VLO1), illustrating the high virulence of this lineage on *Brassica*
207 hosts (Figure 1). In contrast, PD356 was the least virulent *V. longisporum* strain, especially
208 on oilseed rape, where no significant reductions in biomass accumulation were observed
209 upon inoculation (Figure 1A-B). PD356 inoculation significantly reduced the plant biomass
210 of Clapton and Hilton but nevertheless it was one of the least aggressive isolates (Figure 1C-
211 D). Disease responses of the two A1/D3 representatives (PD715 and PD589) were, in
212 contrast to the two A1/D1 representatives, dissimilar (Figure 1). The German isolate PD715
213 was a weak pathogen unable to cause significant biomass reduction on oilseed rape
214 (Figure1A-B) and was amongst the weakest strains tested on Clapton and Hilton (Figure 1C-
215 D). In contrast, the Japanese A1/D3 isolate, PD589, strongly affected *Brassica* crops as
216 PD589 was amongst the most severe isolates in Quartz, Clapton and Hilton (Figure 1A-C-D).
217 Intriguingly, although the devastating outcome on the oilseed cultivars Quartz, no significant
218 biomass reduction was observed when Incentive was infected with PD589 (Figure 1B).

219 To determine to what extent the symptomatology correlates with the amount of *V.*
220 *longisporum* biomass inside the plant, *V. longisporum* DNA was quantified inside the stems
221 relative to the amount of plant DNA. Verticillium spp. are xylem colonizers, hence stem
222 colonization is a good indication for strain aggressiveness. In correspondence with the
223 observed disease symptoms, PD356 and PD715 were weak *Brassica* colonizers, as both
224 strains were generally not detected in the stem, except for Hilton where PD715 was detected
225 in four of the five samples (Figure 2). In contrast, all other *V. longisporum* isolates could be
226 detected in most cases. In Quartz, PD589 was clearly the best colonizer, whereas the British
227 and the A1/D1 strains were approximately present in equal levels (Figure 2A). The
228 colonization of the detected isolates was negatively correlated with the aboveground biomass
229 of Quartz plants (r = -0.6526, p = 2.148x10^{-5}). In Incentive, significant differences in the
230 colonization of the pathogenic isolates were observed, although these were not significantly
231 correlated to plant biomass (r = -0.2745, p = 0.1105) (Figure 2B). Intriguingly, although no
232 significant biomass reduction was observed upon infection of PD589 on Incentive (Figure
233 1B), PD589 was able to colonize the stem to a similar extent as the A1/D1 strains. In Clapton,
234 high differences in colonization of the same isolates were observed between biological
235 replicates (Figure 2C). In correspondence with Quartz and the disease symptoms, PD589 had
236 the highest median colonization level, which was significantly higher than that of PD639 and
237 VLB3. The fungal colonization of the detected isolates was also negatively correlated to the
238 aboveground biomass of the cauliflower plants (r = -0.4793 p = 4,127 x10^{-3}). Hilton was the

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239 only *Brassica* cultivars with detection for PD715, however, no differences in colonization
240 between the isolates were observed (Figure 2D). Similarly, PD715 was also detected in
241 *Arabidopsis* for half of the inoculated plants and no significant differences in colonization
242 were found between the treatments (Figure 3).
243
244 **Discussion**
245 Emerging diseases are often received with concern as their rise is threatening and their final
246 impact uncertain. Hence, recent emergences of the humane virus diseases Ebola in 2014 and
247 Zika in 2015 led to global unsettlement (Attar 2016; Meyers et al. 2015). The majority of
248 emerging diseases originate from the introduction of pathogens in new geographic regions
249 (Anderson et al. 2004). Here, comparisons between emerging pathogens and their original
250 source can help to indicate to what extent the emerging disease may impact ecosystems.
251 However, environmental factors and ecosystem management can lead to sheer differences in
252 the disease outcome of the same pathogen. The recent outbreak of Verticillium stem striping
253 in the UK exemplifies the far-reaching consequences of environmental and anthropogenic
254 factors as the disease originates from a latent, established *V. longisporum* population
255 (Depotter et al. 2017). Changes in weather conditions and/or oilseed rape cultivation are
256 likely to have favoured conditions that supported the emergence of a British *V. longisporum*
257 population capable of initiating new disease epidemics. Similar to other newly emerging
258 diseases, Verticillium stem striping it threatening for British oilseed rape, as the impact on
259 yield is still relatively uncertain. In this study the pathogenicity of four British *V.*
260 *longisporum* isolates from different counties was compared with five previously
261 characterized strains including strains from all *V. longisporum* lineages (Table 1). The British
262 isolates caused significant reduction in the aerial biomass of all the cultivars tested and
263 colonized plants successfully (Figure 1-3). The disease level of British isolates resembled
264 those of the other two A1/D1 lineage isolates (PD639 and VLO1) as plant colonization
265 caused significant yield reductions on all *Brassica* crops (Figure 1). *V. longisporum* lineage
266 A1/D1 can therefore generally be considered pathogenic on *Brassica* crops. This corresponds
267 with a previous *V. longisporum* pathogenicity test that considered A1/D1 as the most virulent
268 *V. longisporum* lineage on oilseed rape and cauliflower (Novakazi et al. 2015).
269 Lineage A1/D2 has hitherto only been found on horseradish and causes the severest
270 disease on this crop of all *V. longisporum* strains (Novakazi et al. 2015). Isolate PD356 is a
271 relatively poor colonizer of *Brassica* crops and caused no or only a small reduction in plant
272 biomass (Figure 1-2). The strong disease symptoms on horseradish and the lack of disease in

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273 *Brassica* crops indicated that the host range of lineage A1/D2 is rather specialized. However,
274 A1/D2 came out as the most virulent *V. longisporum* lineage on cabbage (*B. oleracea* convar.
275 *capitata* var. *alba*, cv. Brunswijker) during pathogenicity tests of Novakazi *et al.* (2015)
276 indicating that pathogenicity may vary within different varieties or crop sub-species. Thus,
277 generalizations about the pathogenicity of the *V. longisporum* lineages should be treated with
278 caution. Lineage A1/D3 was considered non-pathogenic on oilseed rape (Zeise and
279 Tiedemann 2002). However, this pathogenicity study showed, along with a previous study,
280 that non-pathogenicity of lineage A1/D3 cannot be generalized on oilseed rape (Novakazi et
281 al. 2015). Lineage A1/D3 isolate PD715 was non-pathogenic on oilseed rape and was also not
282 detected in the stem after inoculation. In contrast, PD589 is a strong *Brassica* stem colonizer
283 (Figure 2) and caused severe disease symptoms in Quartz (Figure 1A). Intriguingly, this was
284 in contrast with the symptoms on Incentive that, despite extensive stem colonization,
285 tolerated infection by PD589.
286 Interspecific hybridization is an intrusive evolutionary mechanism that alters host
287 range and virulence of pathogens (Depotter et al. 2016b). Hybrid pathogens can therefore
288 have devastating outcomes on ecosystems. For example, the hybrid pathogen *Phytophthora*
289 x*alni*, lead to an epidemic of alder decline in riparian ecosystems of Europe since the early
290 1990s (Brasier et al. 1995). The hybrid fungus *V. longisporum* is currently gaining
291 momentum as it is causing Verticillium stem striping into previously unaffected regions
292 (Gladders et al. 2011; CFIA 2015). A decade since the first report of Verticillium stem
293 striping in the UK, the extent to which this disease may contribute to losses in British oilseed
294 rape is better understood; British *V. longisporum* strains are as pathogenic as other A1/D1
295 strains isolated from various geographical regions and given similar climatic constraints at
296 these locations, Verticillium stem striping is therefore expected to have similar outcomes as
297 in countries where the disease was previously established such as Germany and Sweden.
298 However, the British *V. longisporum* population is genotypically more diverse than the ones
299 in Germany and Swedish (Depotter et al. 2017). Conceivably, the heterogeneous character of
300 the British populations may therefore hamper disease management strategies more.
301 Nevertheless, these recent findings should be an incentive in oilseed rape breeding programs
302 to select for Verticillium stem striping resistance, especially as protective or curative control
303 by conventional fungicides is not possible for *Verticillium* diseases.
304
305 **Acknowledgments**

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311

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Table 1: *Verticillium longisporum* isolate information.

Isolate name	Region of origin	Country of origin	Original host	Collection year	Lineage	Reference*
PD639	Mecklenburg	Germany	Rape	1990	A1/D1	(Novakazi et al. 2015)
O1	Klein Brabant	Belgium	Cauliflower	/	A1/D1	(Tyvaert et al. 2014)
PD356	Illinois	USA	Horseradish	1997	A1/D2	(Novakazi et al. 2015)
PD715	Mecklenburg-Vorpommern	Germany	Rape	1988	A1/D3	(Novakazi et al. 2015)
PD589	Gunma	Japan	Cabbage	/	A1/D3	(Novakazi et al. 2015)
VLB1	Cambridgeshire	UK	Rape	2014	A1/D1	(Depotter et al. 2017)
VLB2	Leicestershire	UK	Rape	2014	A1/D1	(Depotter et al. 2017)
VLB3	Lincolnshire	UK	Rape	2014	A1/D1	(Depotter et al. 2017)
VLB9	Suffolk	UK	Rape	2014	A1/D1	(Depotter et al. 2017)

Figure 1: Aerial plant biomass accumulation of various Brassicaceae crops upon inoculation with *Verticillium longisporum*.

The bars indicate the average above ground dry weight at 6 weeks after inoculation for the cultivars Quartz, Clapton and Hilton (A, C and D), whereas the bars for Incentive give the median weight (B). Significant levels were calculated for Quartz, Clapton and Hilton with the t-test for unequal variances ($p<0.05$), whereas significance levels for Incentive were calculated Mann-Whitney U-test ($p<0.05$). Error bars represent the standard error. Different letter labels indicate significant differences.

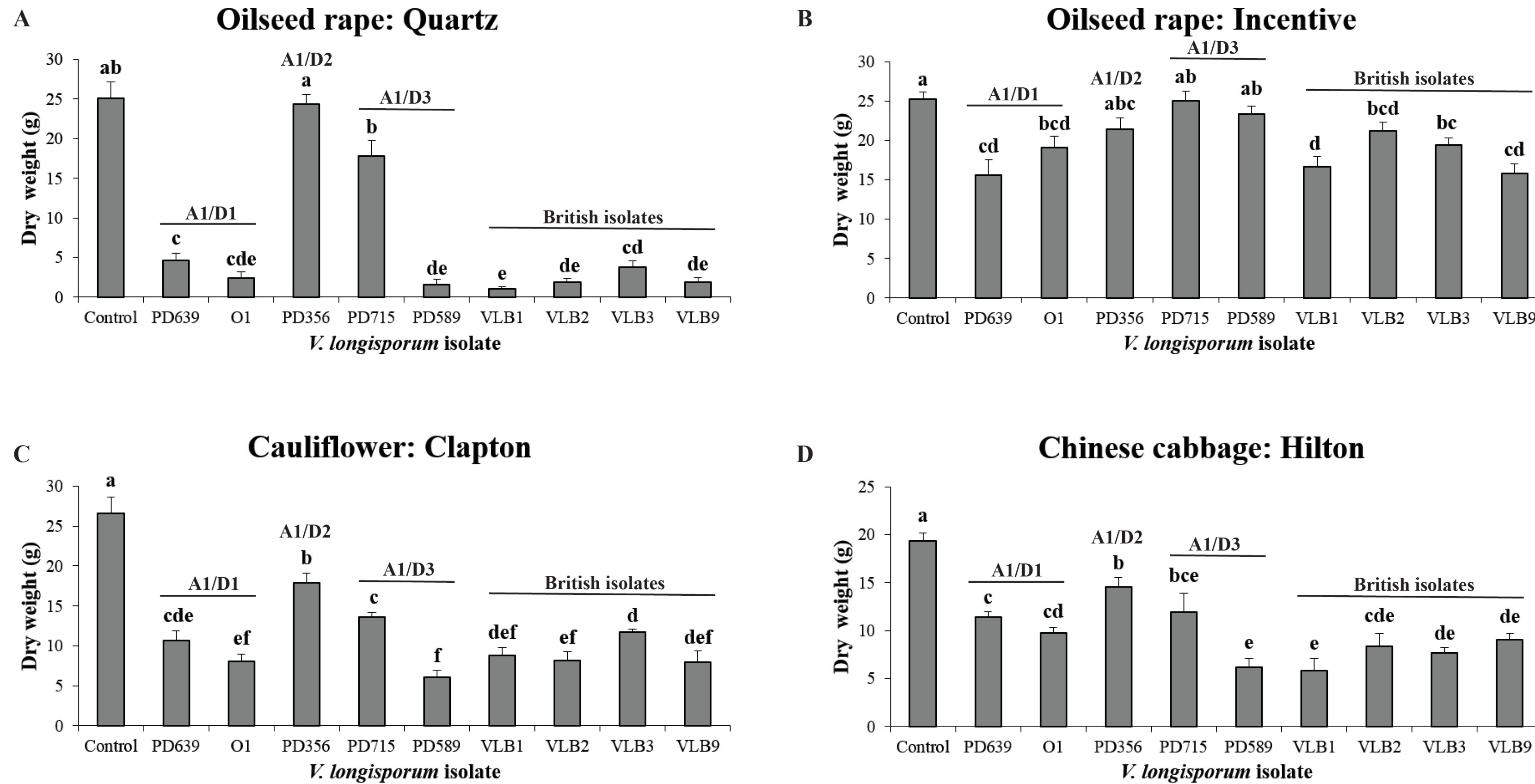

Figure 2: Fungal biomass accumulation of various *Verticillium longisporum* strains in plant stems.

The bars indicate the median *V. longisporum* biomass relatively to the stem biomass. Isolates with no bar and ND in the graph were not detected in all 5 biological replicates. Significant differences were calculated with the Mann-Whitney U-test ($p<0.05$) and depicted by different letter labels. No significant differences in colonization between isolates were found in Chinese cabbage cultivar Hilton. The number between brackets gives the amount of samples without detection. No number is given if the fungal colonization in all replicates was detected. Error flags represent the standard error.

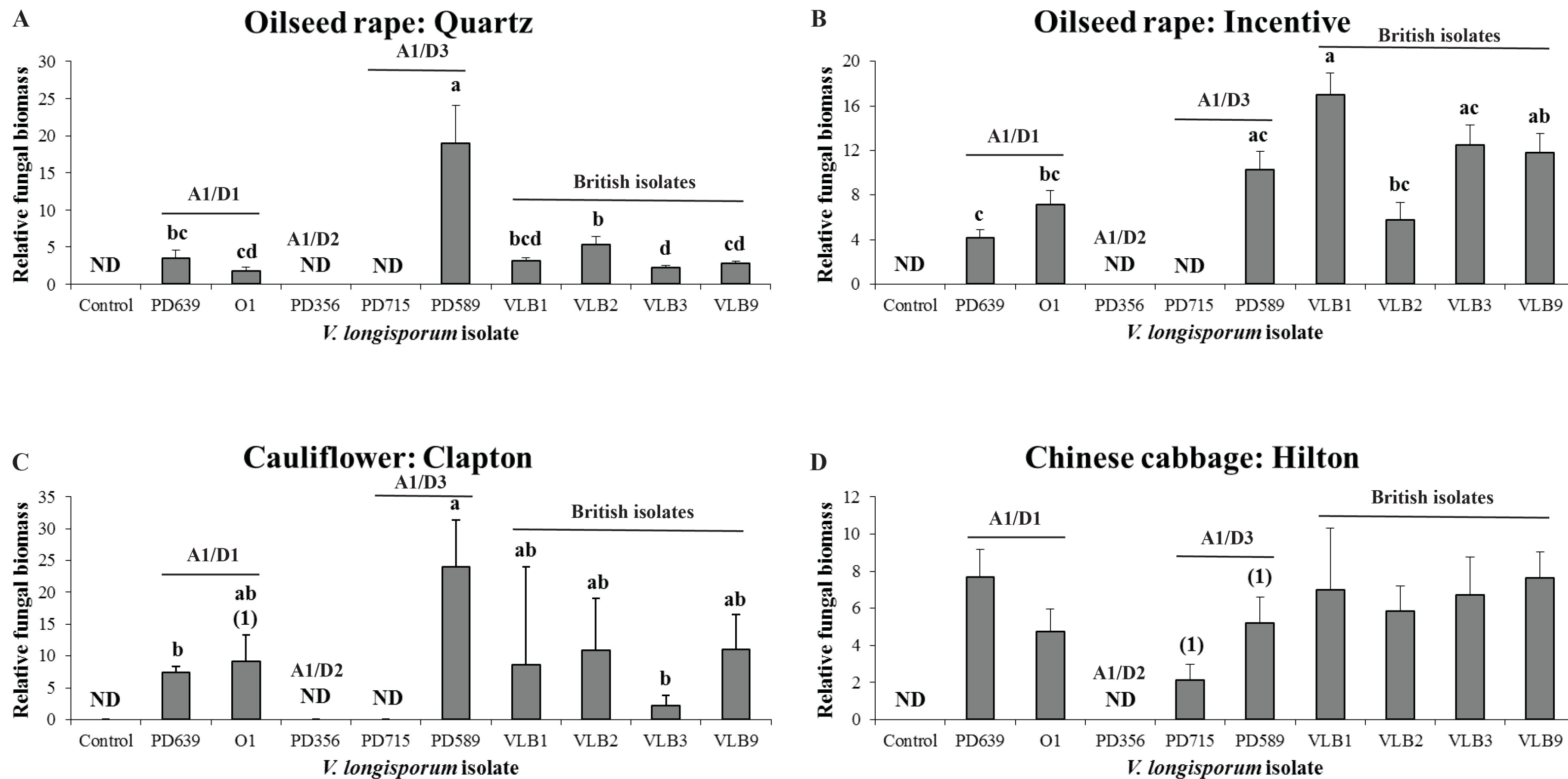

Figure 3: Fungal biomass accumulation of various *Verticillium longisporum* strains in *Arabidopsis* plants.

The bars indicate the median *V. longisporum* biomass relatively to plant biomass. Isolates with no bar and ND in the graph were not detected in all 5 biological replicates. No significant differences in colonization of the different isolates were found (Mann-Whitney U-test, $p<0.05$). The number between brackets gives the amount of samples without detection. No number is given if the fungal colonization in all replicates was detected. Error flags represent the standard error.