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1 **Besides stem canker severity, oilseed rape host genotype matters for the production of**
2 ***Leptosphaeria maculans* fruit bodies**

3

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19

20 **Abstract**

21

22 For fungal cyclic epidemics on annual crops, the pathogen carry-over is an important but poorly
23 documented step. Plant resistance affects the pathogen development within the epidemics but we lack
24 data on the inter-annual transmission of inoculum. For *Leptosphaeria maculans* on 15 oilseed rape
25 genotypes in field during 4 growing seasons, stem canker severity was visually scored at harvest. The
26 number of fruit bodies produced on incubated stubble was quantified using an automated image

27 analysis framework. Our results confirm that fruit body production increases with disease severity
28 and is significantly affected by host genotype and nitrogen supply. Tracking individual stems through
29 incubation, we confirm for the first time that the oilseed rape genotype has a direct effect on inoculum
30 production, not only disease severity. This major effect of genotype on inoculum carry-over should
31 be taken into account in models of varietal deployment strategies.

32
33 **Keywords:** blackleg, disease phenotyping, epidemiology, image analysis, inoculum potential,
34 transmission

36 **Introduction**

37 A key development in disease control has been the breeding and deployment of crop varieties
38 with genetically controlled resistance to pathogens, but most pathogens have repeatedly evolved and
39 overcome qualitative resistance genes (Burdon *et al.* 2016). Therefore, a critical challenge is to design
40 and implement durable crop protection strategies against rapidly evolving pathogens (Cowger &
41 Brown 2019). Any mitigation strategy that reduces the transfer of inoculum between seasons,
42 therefore the effective size of pathogen populations, should limit the evolutionary potential of
43 pathogens, and increase resistance durability (Bousset & Chèvre 2013; Zhan *et al.* 2015). In
44 experiments, combining quantitative with qualitative resistance (Brun *et al.* 2010; Delourme *et al.*
45 2014; Lasserre-Zuber *et al.* 2018), burying stubble to reduce inoculum transmission (Daverdin *et al.*
46 2012), or removing leaf litter in combination with reduced fungicide application (Didelot *et al.* 2016)
47 has delayed adaptation. In models and experiments, the spatio-temporal distribution of host resistance
48 in cultivated landscapes was optimised to mitigate disease transmission (Lô-Pelzer *et al.* 2010;
49 Bousset *et al.* 2018; Papaïx *et al.* 2018; Rimbaud *et al.* 2018; Watkinson-Powell *et al.* 2019).
50 However, model validation and accurate predictions rely on the availability of data that are difficult
51 and costly to collect at these large scales.

52 On annual crops, many plant diseases have cyclic epidemics (Zadoks & Schein 1979; Bousset
53 & Chèvre 2013; Fig. 1A). Their dynamics are highly influenced by both temporal and spatial
54 discontinuities, induced by the climate (e.g. seasonality) or by human actions (e.g. sowing and
55 harvesting). At the beginning of the cropping season, the primary inoculum level is a product of carry-
56 over from the prior crop(s) and/or dispersal. Mitigation strategies can thus target this carry-over of
57 the pathogen using resistant varieties (Marcroft *et al.* 2004a), biocontrol agents (Bailey *et al.* 2004),
58 or preventive management of crop residues (Wherrett *et al.* 2003). However, inoculum survival and
59 transmission are difficult to estimate in field and predict (Bailey *et al.* 2004; Bousset *et al.* 2015).

60 Host resistance impacts pathogen life history traits during the epidemic phase (e.g. infection
61 efficiency, latent period or sporulation) (Bruns *et al.* 2012; Delmas *et al.* 2016; Dumartinet *et al.* 2020;
62 Leclerc *et al.* 2019; Bove & Rossi 2020). Qualitative host resistance reduces the number of
63 compatible infections whereas quantitative host resistance reduces the amplification during the
64 epidemics (Fig. 1B). In addition, the selective deployment of resistant host genotypes can reduce
65 landscape connectivity following the intercrop (Lô-Pelzer *et al.* 2010; Bousset & Chèvre 2013;
66 Papaïx *et al.* 2018; Rimbaud *et al.* 2018; Watkinson-Powell *et al.* 2019). Little is known about the
67 direct effect of quantitative host resistance on initial amount of inoculum at the beginning of the next
68 cropping season (i.e. inter-annual transmission, Fig. 1B). Quantitative resistance affects transmission
69 in *Phytophthora infestans* through a trade-off between crop infection and survival on tubers (Pasco
70 *et al.* 2016). A trade off was detected in *Zymoseptoria tritici*, but varietal effects were not investigated
71 (Suffert *et al.* 2018a). In downy mildew, the effect of quantitative resistance was assessed on oospore
72 production (inoculum for inter-annual transmission; Delbac *et al.* 2019).

73 Inoculum transmission between seasons was described in *Leptosphaeria maculans* on winter
74 oilseed rape sown in autumn. This fungal pathogen initiates epidemics early in the cropping season
75 (in autumn) with stubble-borne ascospores that can spread between fields across the landscape (West
76 *et al.* 2001). It produces Phoma leaf spots on host leaves that are observed between autumn and early
77 spring. Then, stem cankers develop from spring to summer, up to the time of harvest, following

78 systemic growth of fungal hyphae from leaf spots to the leaf petiole through xylem vessels, and
79 subsequently to the stem base. The fungus can survive as hyphae in crop stubble, more specifically
80 in stems around crown level, forming two kinds of fruit bodies: pycnidia and pseudothecia.
81 Pseudothecia can only be formed following sexual reproduction if isolates of opposite mating types
82 co-occur in the same oilseed rape stem. Infected stubble ensures the carry-over of the fungus from
83 one cropping season to the next and serves as the main source of inoculum.

84 For *L. maculans*, several studies have shown that the number of produced pseudothecia, that
85 release initial inoculum ascospores, was correlated with Phoma stem canker severity (McGee &
86 Emmett 1977; Marcroft *et al.* 2004a; Lô-Pelzer *et al.* 2009) and that some quantitative host resistances
87 tends to decrease severity at harvest (Marcroft *et al.* 2004b; Lô-Pelzer *et al.* 2009). Host genotype
88 can significantly affect both the visual density of pseudothecia, and the number of released ascospores
89 (Marcroft *et al.* 2004b). However, properly disentangling the effects of host genotype on stem canker
90 severity and fruit body production remains to be achieved. Therefore, the question of a direct genetic
91 effect on the production of fruit bodies, and thus temporal transmission of inoculum between seasons,
92 is still unknown for this pathosystem. The development of high-precision and high-throughput
93 phenotyping methods could improve experimental quantification of plant pathogens, alleviate the
94 tedious manual quantification of fruit bodies on many genotypes, and improve knowledge and data
95 on pathogen carry-over. Imaging and processing algorithms to segment and count regions,
96 circumvented the long and tedious manual counting (Stewart *et al.* 2016; Karisto *et al.* 2018; Yang &
97 Hong 2018) and were applied to the automated quantification of fruit bodies of *L. maculans* (Bousset
98 *et al.* 2019). This offers a means to overcome the experimental bottleneck and assess the effect of
99 host quantitative resistance on pathogen carry-over.

100 In this study, we address the effect of host resistance on the carry-over of inoculum between
101 consecutive years, to understand how host genotype might affect fruit body production and the effects
102 of a common cropping practice, i.e. nitrogen fertilization, and disease severity at harvest in the
103 preceding growing season (year). We consider *L. maculans* on oilseed rape as an example

104 pathosystem and use an imaged-based phenotyping method for quantifying fruit bodies that appeared
105 on diseased stems collected in experimental field plots, after an incubation period. We analyse the
106 main drivers of this poorly known life-history trait of the pathogen.

107

108 **Materials and methods**

109 *Experimental fields and contamination of field plots*

110 This experiment was run on the INRAE UE La Motte experimental station located in Le Rheu
111 (48.1°N, 1.5°W), in Brittany, France. Winter *B. napus* genotypes were grown in 5-row plots (2.5 m²)
112 and assessed for stem canker (details are given in Table S1.1). The cropping season extends from
113 sowing in autumn to harvest the following summer and was repeated in the 4 years named 12-13, 13-
114 14, 14-15 and 15-16. We followed the same 15 genotypes in years 12-13, 13-14 and 14-15, (Table 1;
115 S1.2) and six genotypes were added in year 15-16 (Table 1). The varieties were chosen to represent a
116 wide range of winter oilseed rape diversity and derived from different breeding programs.

117 In the four years, nitrogen supply was normal (Nh) according to usual practices in
118 conventional cultivation to achieve yield potential of modern winter oilseed rape varieties (Bouchet
119 et al. 2014) (Table S1.3). In years 14-15 and 15-16, the panel was replicated with low (Nl) nitrogen
120 availability. On these Nl plots, no organic matter was spread on the fields for 3 years before the trials
121 and the previous crops were grown under a low input management system. This limited the amount
122 of mineral N in soil in the experimental plots, avoiding organic matter mineralisation into nitrogen
123 later available to the plants.

124 Homogeneous disease infection was achieved with contaminated stems collected from the
125 previous year on the susceptible Bristol and miscellaneous varieties. In the experimental area and
126 around Phoma stem cankered stubble, only *L. maculans* leaf spots were observed which supported
127 the hypothesis of the very small prevalence of the other causal agent *L. biglobosa* in this area (Bousset
128 et al. 2019). Stems were scattered throughout the plots in each cropping season at a density of two
129 stems per m² when the crop was at the two to three leaf growth stage. Each variety was grown on

130 three replicated plots, from which stems were pooled for the current analysis. Sprinklers were run
131 twice per day (4 mm per day) from plant emergence to the appearance of first leaf spots to promote
132 fruit body maturation and spore release. This kept the time from spreading the contaminated stems to
133 the appearance of leaf spots similar over years (Fig. S1E). No fungicides were applied throughout the
134 season. Phoma stem canker severity was assessed 2-3 weeks before crop maturity on a 1 to 6 scale as
135 follows: S1 = no disease, S2a = 1-5%, S2b = 6-25%, S3 = 26-50%, S4 = 51-75%, S5 = 76-99%, S6=
136 100% of crown cross section with Phoma stem canker symptoms. Severity classes 2a and 2b were
137 distinct for the analysis of fruit body formation on stems, but pooled before the calculation of plot
138 canker severity. The G2 aggregated disease index was calculated as follows (Aubertot et al. 2004):

$$139 \quad G2 = (0 \times n_1 + 1 \times n_2 + 3 \times n_3 + 5 \times n_4 + 7 \times n_5 + 9 \times n_6) / N$$

140 with n_i being the number of stems in severity class S_i , respectively and N the total number of stems.

141 The climate of the experimental area is oceanic. Meteorological data were obtained from the
142 INRAE CLIMATIK database, for Le Rheu weather station, on an hourly basis. Cumulative
143 temperature (Fig. S1ab), rainfall (Fig. S1cd), and number of days that are favourable for pseudothecia
144 maturation (Fig. S1e) were calculated from start (shortly after harvest in June; Table S1.1) to end of
145 incubation when stems were retrieved from the field (at the end of the following winter; Table S1.1).
146 A day was considered favourable if the mean temperature was between 2 and 20°C and if the
147 cumulative rainfall over the previous 11 days before (including the day in question) exceeded 4 mm
148 (Aubertot *et al.* 2006; Lô-Pelzer *et al.* 2009). Given these parameter values, 64 favourable days were
149 required for 50% of pseudothecia to reach maturation. We were able to compare the number of
150 calendar days needed to reach maturation in different years (Fig. S1E).

151

152 *Stubble incubation to promote fruit body formation*

153 Phoma stem canker severity was assessed at harvest, and then tracked for each individual stem
154 piece comprising the crown and upper 10 cm (Bousset *et al.* 2019). The sampling was constrained by
155 the availability of diseased stems across severity classes. After pooling the three replicated plots of

156 each variety, 30 stems in each of the 6 severity classes were randomly selected. If fewer than 30 stems
157 (per class) were available, then all were kept. Within each variety and severity class, groups of 5 stem
158 pieces attached together on BBQ sticks were labelled with a barcode (Bousset *et al.* 2019).

159 The selected stem pieces were incubated in field conditions at INRAE Le Rheu. Over summer,
160 they were placed on a 1:1:1 mix of sand, peat and compost spread on the ground to avoid weed
161 emergence, then transferred in autumn in experimental plots of winter oilseed rape to mimic
162 incubation under the following season's crop and to allow detection of released spores. Complete
163 maturation was indicated by the end of the appearance of new leaf spots on susceptible plants around
164 the incubating stem pieces. The stem pieces were recollected, washed, dried and stored (Table 1). All
165 stem pieces from a given year were incubated in the same time and location, starting in the summer
166 following harvest.

167 *Image acquisition, processing and post-processing*

168 The fruit bodies appeared on diseased stem pieces were quantified using image-based
169 phenotyping settings described in Bousset *et al.* (2019). In short, a picture of each group of 5 dry
170 stems was taken with the barcoded label, placed on a glass plate, 16 cm above a blue background
171 (PVC sheet Lastolite Colormatt electric blue). Two FotoQuantum LightPro 50 × 70cm softboxes were
172 placed on both sides of the stem pieces with 4 daylight bulbs each (5400K, 30W) within the lower
173 45° angle. Pictures were taken with a Nikon D5200 with an AF-S DX Micro Nikkor 40mm 1:2.8G
174 lens, on a Kaiser Repro stand, with a wired remote control. Aperture was set at F22 for maximal depth
175 of field, iso 125, daylight white balance. Pictures were saved as RGB images with a resolution of
176 6000 × 4000 pixels.

177 Pictures were pre-processed by reading the barcode to rename the files, splitting each digital
178 image in several new images, each containing only one stem segmented with an unsupervised method.
179 Finally, each new image was cropped to keep only the 5 cm portion on the crown end of the stem, i.e.
180 where pseudothecia are mainly located.

181 Then, stem pixels were classified either in state F (fruit bodies) or in state S (stem) using a
182 supervised machine learning algorithm. We did not re-train the algorithm for processing our images,
183 because learning and testing data used by Bousset et al. (2019) already included some images
184 collected for this study. Predicted images were post processed through a computer-assisted expert
185 curation using a graphical user interface. Each processed image was assigned a post-processed state,
186 i.e. "correct" or "incorrect", by visually evaluating the predicted image compared to the original one
187 (Bousset *et al.* 2019).

188 *Statistical analyses*

189 We analysed the effects of genotype, year and nitrogen fertilization on stem canker severity
190 using a proportional odds model. We used an ordered logit regression model that handles ordinal data.
191 The design of the experimental data only allowed us to estimate and test the first order effects and the
192 interaction between genotype and nitrogen fertilization.

193 The density of fruit bodies detected was analysed on the post-processed images validated by
194 the curator (state "correct"). For each image i we considered the number of pixels in states F (fruit
195 bodies) and S (stem), i.e. $n_{F,i}$ and $n_{S,i}$, and used a likelihood function based on $n_{F,i} \sim B[(n_{F,i} + n_{S,i}), p]$
196 with a logit link function to build Generalised Linear Models and analyse the effects of selected
197 factors with Wald tests. We first considered the complete unbalanced dataset that only enables the
198 identification and test of first order effects (i.e. year, severity prior incubation, plant genotype and
199 nitrogen fertilization) and the interactions between genotype and nitrogen fertilization, and stem
200 severity with nitrogen fertilization (Table 1). Second, in order to go further and investigate second
201 and third order effects (i.e. interactions) we split the 4 years data into 3 datasets: Data14-16 (2 years
202 14-15 and 15-16, 2 nitrogen fertilization levels Nh and Nl, 15 genotypes), Data12-16 (4 years 12-13,
203 13-14, 14-15 and 15-16, 15 genotypes, all on high level of fertilization Nh), and Data15-16 (year 15-
204 16, 21 genotypes and 2 nitrogen fertilization levels Nh and Nl). Note that for all these subdatasets the
205 severity prior incubation (7 levels) was also included as a categorical variable in the regression
206 models.

207 The whole image processing was developed in Python (Van Rossum & Drake 1995) whereas
208 statistical analyses were performed using R (R Core Team 2019).

209

210 *Prediction of inoculum potential*

211 We simulated the production of inoculum in a population of infected host plants by coupling
212 two stochastic processes. We first considered 10 experimental plots of 100 plants, whose severity
213 drawn from a Multinomial distribution with event probabilities corresponding to those estimated with
214 the ordered logistic regression model (Tables S5.1 and S5.2). Second, we assumed that the size of
215 each individual stem is 700 000 pixels, i.e. twice the average observed number of pixels per processed
216 image, and for each stem we drew the number of fruit body pixels using a Binomial distribution, with
217 probability parameter previously estimated in the GLM model (Tables S5.3 and S5.3). To illustrate
218 the importance of host genotype on fruit body production, we compared the output of the simulation
219 with versus without host genotype.

220

221 **Results**

222 *Severity data at harvest*

223 The observed distribution of canker severities was assessed at harvest, before the sampling of
224 stems selected for fruit body formation assays (See Materials and Methods). Model of the complete
225 ordinal dataset indicated effects (p-values <0.05) of the genotype, the year, the fertilisation and an
226 interaction between genotype and fertilisation on canker severity at harvest (Aggregated G2 index,
227 Table 2; Fig. 2; Fig. S2.1; Fig. S2.2).

228 *Fruit body formation was strongly influenced by year, genotype and disease severity*

229 Across datasets, fruit body formation was affected by host genotype, year, and disease severity
230 at harvest. The year was the most influential parameter, with about 20% of the explained deviance for
231 all analyses (Table 3). Host genotype explained between 6 and 17% of the explained deviance of fruit
232 body formation. Finally, severity of infected stems before incubation explained between 5 and 10 %

233 of the total deviance. Raw data as well as pairwise comparisons of modalities with least-squares
234 means indicated differences between genotypes and years when stems were collected. The production
235 of fruit bodies was lowest in year 15-16 and highest in year 13-14 (Fig. 3; Supplementary Information
236 S3).

237 The amount of fruit bodies produced increased with stem canker severity as already shown by
238 Bousset et al. (2019). Stem canker severity classes were ranked from the lowest to highest, except for
239 classes S6 and S5 that were in reversed order (Fig. 4a; Supplementary Information Fig. S3.1; 3.4;
240 3.7). However, for each severity class, the fruit bodies produced depended on the genotype (Fig. 4;
241 5; Supplementary Information Fig. S4.1; S4.2). Some genotypes like Al and Fa produced greater
242 numbers of fruit bodies at each severity class than genotypes like As and Av. Noteworthy, here the
243 stem pieces have been individually tracked, so the production of a genotype can be compared at each
244 severity class. One can also note that a severely infected genotype like Yu (having no stem in classes
245 S1 and S2a) is not the one producing the greatest amount of fruit bodies at a given severity class. In
246 contrast, a mildly infected genotype like Da (having no stem in classes S5 and S6) produced more
247 fruit bodies at a given severity class than e.g. genotypes As or Av.

248 Because several genotypes did not occur either in the first two (S1-S2) or the last two (S5-S6)
249 severity classes, it was not possible to estimate all the coefficients of the genotype \times severity
250 interaction on the full dataset. However, when considering the genotypes for which this interaction
251 was identifiable, we found a significant genotype \times severity interaction, suggesting that the amount
252 of produced fruit bodies by each severity class may change with host genotypes.

253

254 *Nitrogen fertilization of the crop has a significant but minor effect on fruit body formation*

255 We detected a significant fertilization \times host genotype interaction, indicating that fertilization
256 has varying effects on disease symptoms of different host genotypes. (Fig. 2). The fertilization \times
257 disease severity and fertilization \times year interactions were also significant. However, as shown by the
258 little deviance explained by these first, second and third order factors, nitrogen fertilization had a

259 minor influence on fruit body production (Table 3; Fig. 6). In fact, nitrogen explained below 1% of
260 the deviance in the Data15-16 dataset, and only the nitrogen × genotype interaction explained 5% of
261 the deviance (Table 3).

262

263 *Simulated fruit body formation*

264 The stochastic simulation of fruit body production based on fitted event probabilities
265 (proportional odds and generalized linear models) allowed us to predict the potential level of
266 inoculum produced by field plots for each genotype x year x nitrogen fertilization case (Table S5.1;
267 S5.2; S5.3; S5.4). Simulations outputs illustrate the variability of fruit body formation and confirm
268 the importance of host genotype on this life-history trait of the fungal pathogen (Fig. 7; Fig. S5.1).

269 When the simulated fruit body production was plotted over canker severity at harvest using
270 the aggregated G2 index, among the genotypes, either susceptible (high canker severity) or with
271 higher levels of quantitative resistance (low canker severity), the pixels of fruit bodies are variable
272 (Fig. 8). A very susceptible genotype is not always the one producing the greatest amount of fruit
273 bodies for the following year. Moreover, genotypes with higher levels of quantitative resistance have
274 low canker severity at harvest but are still diverse with regard to fruit body production (Fig. 8).

275

276 **Discussion**

277

278 With field experiments and image-based automated quantification of fruit bodies on individual
279 stems, we disentangled the role of the year, the genotype, the disease severity and the nitrogen
280 fertilization. We confirmed for the first time that the oilseed rape genotype has a direct effect, not
281 only through disease severity, on a seldom-informed trait of the fungal pathogen life cycle. The effect
282 of host genotype on both the visual density of pseudothecia and the numbers of released ascospores
283 had already been observed by Marcroft *et al.* (2004b). However, given the experimental design used,
284 the authors could not clearly distinguish the effect of stem canker severity from the effect of host

285 genotype. Lô-Pelzer *et al.* (2009) followed by Bousset *et al.* (2019) detected the effect of host
286 quantitative resistance and field on disease severity and on fruit body production. Yet, those studies
287 were unable to disentangle the role of host genotype, cropping practices as well as the between-season
288 variability. In our study, because all genotypes were planted in the same field and later incubated at
289 the same place, we could exclude confusion with an effect of the environment. Furthermore, as stems
290 were tracked individually, we were able to separate the effect of disease severity from the effect of
291 genotype. One way forward would be assessing canker severity as a quantitative measure for each
292 stem, without losing part of the information into severity classes, for instance by using image-based
293 phenotyping. Then, it would be possible to assess the quantitative relationship between severity and
294 fruit body production among genotypes.

295 The number of ascospore per pseudothecium ranged from 1000 to 10 000 (Lô-Pelzer *et al.*
296 2009) and 1000 to 14 000 (Schneider 2005). Estimates were based either on calculating the minimal
297 and maximal volumes of pseudothecia considered as a sphere and asci considered as a spheroid, or
298 on crushing 50 pseudothecia in water with bleach and counting the ascospores with a haemocytometer
299 (Lô-Pelzer *et al.* 2009). Therefore, the numbers of ascospores available for release in cropping areas
300 are huge. Nevertheless, clear gradients of decrease in leaf spot numbers on farmer's oilseed rape fields
301 were observed with increasing distance from emitting spore sources (previous years' oilseed rape
302 fields) (Bousset *et al.* 2015). Further, how far a source would spill ascospores depends on the number
303 of emitted ascospores. This indicates that however large the number of ascospores is, decreasing their
304 number by the means of genotype will contribute to decreasing disease transmission between
305 successive years. In the panel of genotypes considered in the study, the host modulates the production
306 of fruit bodies rather than suppressing it. Therefore, plant genotypes cannot prevent epidemics but
307 may help to mitigate them. As stated later in the discussion, our findings can help identify conditions
308 (i.e. arrangements of cultivars and fields) within a landscape allowing a durable control of the
309 pathogen, which is a current challenge.

310 Differences in host phenology may contribute to the observed variability among host
311 genotypes. From the biotrophic and asymptomatic presence of the fungus in the stem, visible cankers
312 appear progressively when the crop matures. Moreover, the delay between infection and symptom
313 appearance (i.e. asymptomatic phase) is known to vary between host-plants in a population, and the
314 distribution of the incubation period could also change with some variables like the host-age when
315 the infection occurs (Leclerc *et al.* 2014). When collecting disease data from genotypes differing in
316 phenology, the sampling can occur at different stages in these processes, and thus introduce more
317 variability in the relationship between the visual Phoma stem canker severity and the resulting fruit
318 bodies produced. In oilseed rape stems, pathogen load, i.e. the amount of fungal mycelium, remains
319 low throughout winter and starts to increase as *L. maculans* changes from biotrophic to necrotrophic
320 after host flowering (Gervais *et al.* 2017). Nevertheless, in our study neither the time of flowering nor
321 the time between flowering and harvest appeared as a major determinant of the genotype effect (Table
322 S1.2). A more precise quantification of mycelium, by qPCR or by the visualisation of cankers through
323 non-destructive imaging like MRI, could be helpful to better understand the mechanisms involved in
324 the genotype effect.

325 Genotype effect could be due to a difference in the amount of nutrients available for the
326 fungus. Indeed, in *P. infestans*, the most severely affected plants during the cropping season were the
327 least prone to the intercrop survival of the pathogen on tubers. As quantitative host resistance reduces
328 disease severity during the cropping season, it thus seems to promote intercrop survival (Pasco *et al.*
329 2016). However, in our study some genotypes may be severely infected but produce a moderate
330 quantity of fruit bodies (Yu) while others may produce more fruit bodies (Da) than expected based
331 on their Phoma stem canker severity class. Quantitative host resistance does affect inoculum
332 production by decreasing disease severity, as number of pseudothecia on stems increase with Phoma
333 stem canker severity (McGee & Emmett 1977; Marcroft *et al.* 2004a; Lô-Pelzer *et al.* 2009; Bousset
334 *et al.* 2019). In our study, the genotypes Av, Cb, Da, Gr, Jn, Ko had higher than average levels of
335 quantitative resistance on stem canker severity (Table S1.2; Fopa-Fomeju *et al.* 2015; Kumar *et al.*

336 2018), but they did not show any specific trend regarding fruit body production at a given severity
337 class (Fig. 4; 5). Further, the nitrogen supply affected both disease severity at harvest and fruit body
338 production, though the magnitude of the effect on fruit bodies was relatively small. It could be worth
339 considering this for breeding cultivars for low-input systems. Genetic analyses are needed to identify
340 the determinants underlying fruit body production in the genotypes. So far, plant resistance is still
341 only characterised during the epidemics, e.g. in grapevine (Bove & Rossi 2020). However, pathogen
342 life-cycle stages related to intercrop transmission, like the production of oospores in *Plasmopara*
343 *viticola*, are starting to be evaluated (Delbac *et al.* 2019). In pathosystems such as downy mildew on
344 grapevine or stem canker on oilseed rape, as host resistance affects pathogen transmission and
345 survival, a low production of fruit bodies could be worth selecting in breeding schemes. At least,
346 discarding the most fruit body-prone genetic backgrounds would be a step forward.

347 The highlighted effect of host genotype on inoculum carry-over may also be explained by a
348 bias in mating of the fungal pathogen. Because the fungus is heterothallic, mating only occurs when
349 the two mating types are present. A small canker with mycelia of both mating types is thus suitable
350 for mating. In contrast, if a large canker is caused by only one individual, then no pseudothecia could
351 be produced. The occurrence of Allee effects in fungi, i.e. reduced success of mating at low population
352 density, has started to be investigated under controlled conditions for *Zymoseptoria tritici* (Suffert *et*
353 *al.* 2018b) but should deserve further investigations. In the particular case of *L. maculans*, mycelial
354 growth in the petiole has started to be investigated (Huang *et al.* 2019), but the precise location of the
355 fungus in stem and its consequences on mating remains unknown. Further studies would be
356 interesting and could rely on the use of GFP-transformed strains in controlled conditions or
357 sequencing methods combined with population genetics analyses in natural monitored epidemics.

358

359 In agreement with previous findings (Lô-Pelzer *et al.* 2009; Bousset *et al.* 2019), our study
360 confirmed the importance of between-year variability that may be related to variable environmental
361 conditions. The highest fruit bodies numbers (Fig. 2) and rainfall during incubation (Fig. S1d) were

362 observed in year 13-14. In contrast, the lowest fruit bodies numbers and rainfall during incubation
363 were observed in year 15-16. While the effects of climatic variables on maturation of the fruit bodies
364 have been modelled (Aubertot *et al.* 2006), their influences on the pathogen during the cropping
365 season and during the intercrop still deserve further investigations. In particular, besides influencing
366 the development of the host plant, some main climatic variables such as temperature may drive
367 within-host pathogen growth, influence pathogen load at stem base, thus influence mating of the
368 fungus and therefore fruit body production. Linking environmental conditions with host and pathogen
369 development is a current challenge for most pathosystems to improve predictions of epidemics and
370 yields. In the particular case of oilseed rape stem canker, further studies are needed to address the
371 influence of crop growth in real situations as well as intercrop practices of the production of fruit
372 bodies (McCredden *et al.* 2017).

373 As plant genotype appeared to be an important driver of the production of inoculum, this
374 should be taken into account in models used to compare strategies for the deployment of varieties in
375 the landscape (Lô-Pelzer *et al.* 2010; Papaïx *et al.* 2018; Rimbaud *et al.* 2018; Watkinson-Powell *et*
376 *al.* 2019). So far, the effect of qualitative host resistance on infection and some effects of quantitative
377 host resistance on pathogen development are considered. Our results suggest that it would be worth
378 considering the effect of host quantitative resistance on the pathogen during the intercrop. One
379 striking result of our stochastic simulations of fruit body production is that the ranking of the
380 genotypes (Fig. 7) appeared to be very different from the ranking obtained when considering the
381 disease severity at harvest (Fig. 2). For example, the genotypes Ja, Pr and Yu with a high disease
382 severity at harvest (Fig.2) produce fewer fruit bodies than others (Fig.7). In contrast, the genotypes
383 Fa, Fr and Po have a moderate disease severity at harvest (Fig.2) but later produce higher amounts of
384 fruit bodies than others (Fig.7). Recognition of this trade-off in pathogen resistance properties may
385 help breeding varieties less prone to inoculum production and guide further breeding schemes.

386

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388

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397 phoma) en situation de contrainte azotée).

398

399 **Authors' contributions**

400

401 LB, PV and RD carried out experiments, ML carried out statistical analyses, LB, ML, NP and MP
402 analysed pictures. LB conceived and designed the study and prepared the manuscript, read and
403 approved by all authors. The authors declare the absence of conflict of interest.

404

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406

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547

548

549 **Figure Legends**

550 **Fig. 1.** Schematic representation of cyclic epidemics and their control by plant resistance. **(A)** Cyclic
551 epidemics are characterized over years by the disease being present in the landscape with a stable
552 long term dynamics (black dashed line), but short term dynamics alternating between epidemic phase
553 (red lines) when the crop is present and decrease (blue lines) during the intercrop (Ic.). **(B)** Plant
554 genetic resistance can contribute to the control of cyclic epidemics during the crop with qualitative
555 resistance reducing infection and quantitative resistance reducing the increase. During the intercrop,
556 the selective deployment of genotypes reduces landscape connectivity, and the plant genotype
557 influence the amount of inoculum produced (this study).

558

559 **Fig. 2.** Canker severity (G2 index) on field plots depending on the nitrogen level and the genotype.
560 The model was adjusted to the data of 12-13 to 15-16 years. Full names of genotypes and flowering
561 dates are available (Supplementary Information Table S1.2).

562

563 **Fig. 3.** Fraction of fruit body pixels depending on year of sampling (12-13, 13-14, 14-15 and 15-16)
564 for the fifteen genotypes in the Data12-16 dataset. Full names of genotypes are available
565 (Supplementary Information Table S1.2).

566

567 **Fig. 4.** Fraction of fruit body pixels depending for the S1 to S6 severity classes in the Data14-16
568 dataset. (A) For all genotypes; (B) to (H) per genotype for each of the six severity classes. Full
569 names of genotypes are available (Supplementary Information Table S1.2).

570

571 **Fig. 5.** Fraction of fruit body pixels depending on the severity before incubation exemplified for the
572 fifteen genotypes in the Data14-16 dataset. This result was observed for all datasets (Supplementary
573 Information Fig. S4.1; S4.2). Full names of genotypes are available (Supplementary Information
574 Table S1.2).

575

576 **Fig. 6.** Predicted fraction of fruit body pixels depending on the twenty one genotypes in the Data15-
577 16 dataset for (A) normal (Nh) or (B) reduced (Nl) nitrogen fertilization of the crop. Full names of
578 genotypes are available (Supplementary Information Table S1.2).

579

580 **Fig. 7** Boxplot of simulated numbers of fruit body pixels depending on the normal (Nh) or reduced
581 (Nl) nitrogen level and on the genotype (21 genotypes). The effect of genotype was taken into account
582 both for the distribution of stems in canker severity classes and on the prediction of fruit body pixels,
583 adjusting the model on observed data. Overall are values without the genotype effect. Data are means
584 of 10 simulated field plots with 100 plants of 700 000 pixels. Full names of genotypes are available
585 (Supplementary Information Table S1.2).

586

587 **Fig. 8** Average simulated numbers of fruit body pixels depending on the canker severity at harvest
588 (G2 index calculated for the ensemble of stems) for the fifteen genotypes over all years. In
589 simulations, the effect of genotype was taken into account both for the distribution of stems in canker
590 severity classes and on the prediction of fruit body pixels, adjusting the model on observed data. Data
591 are means of 10 simulated field plots with 100 plants of 700 000 pixels. Among the genotypes, either
592 susceptible (high canker severity) or with higher levels of quantitative resistance (low canker

593 severity), the pixels of fruit bodies are variable. A very susceptible genotype (Yu) is not the one
594 leaving the greatest amount of fruit bodies.
595
596

Fig 1

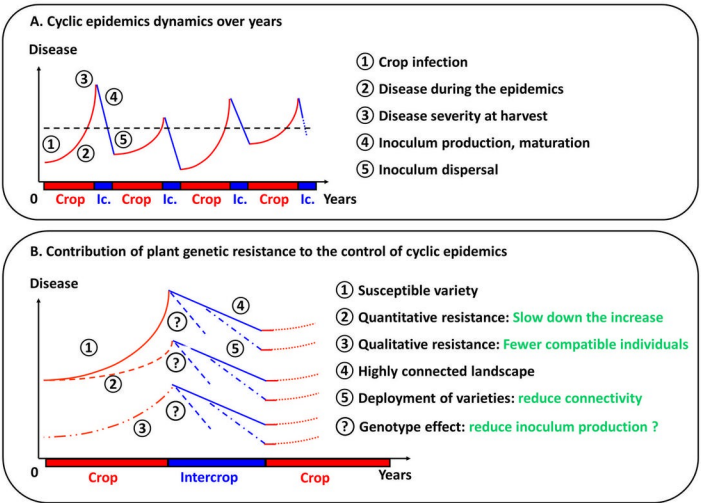
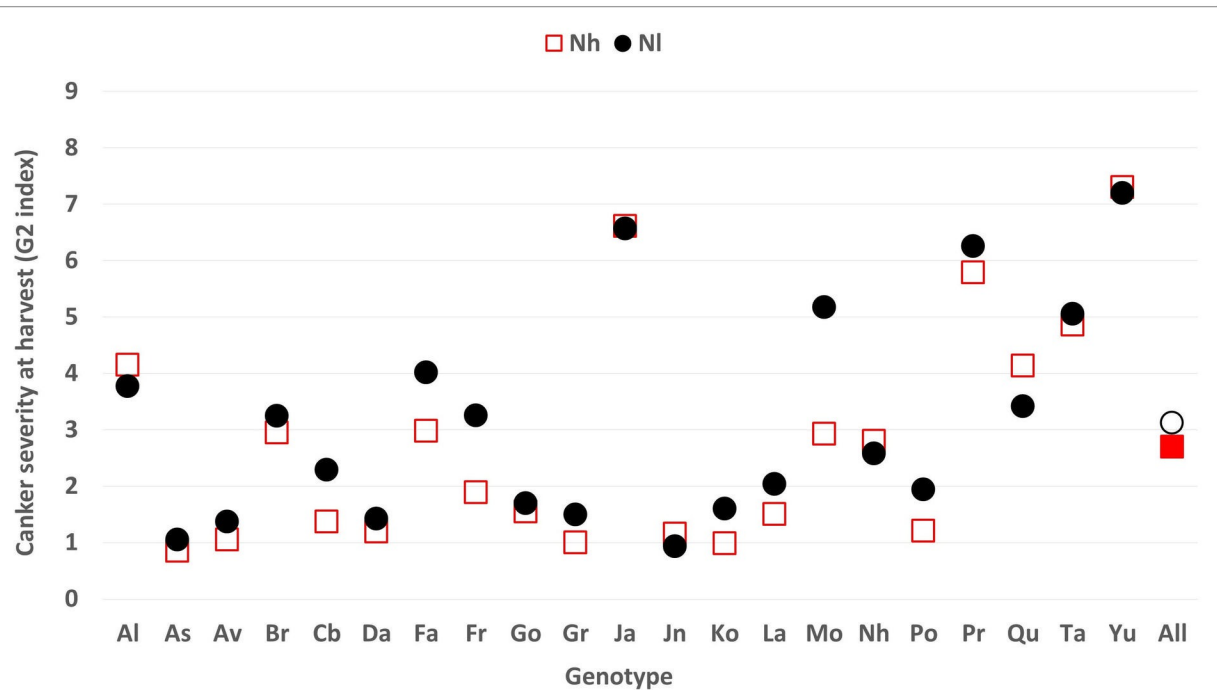
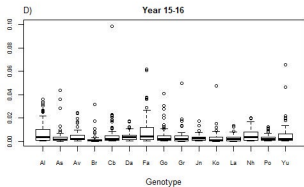
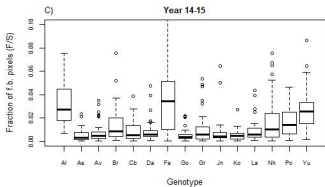
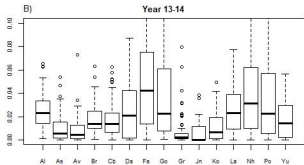
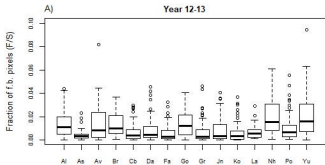
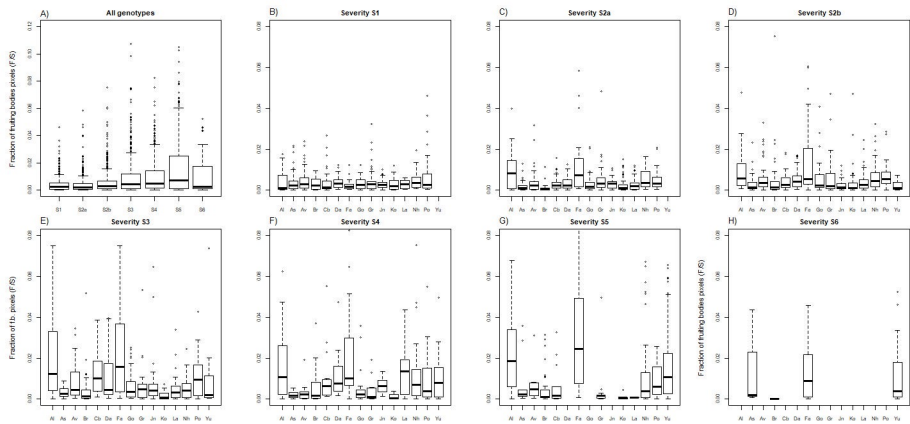
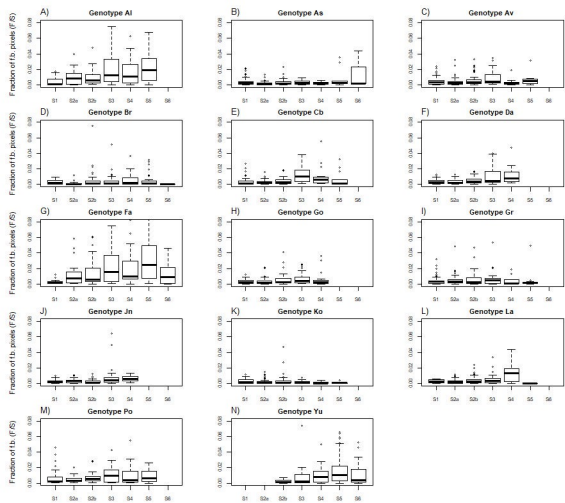


Fig 2









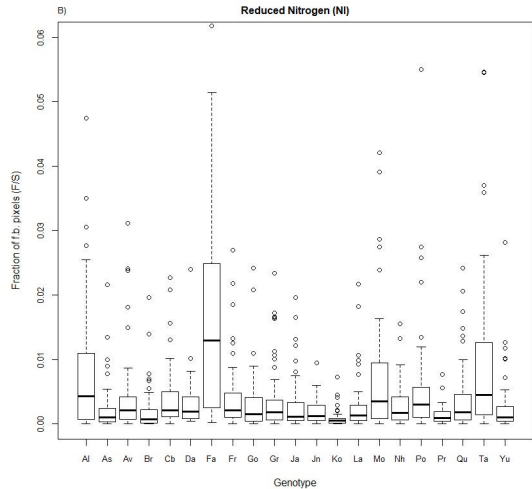
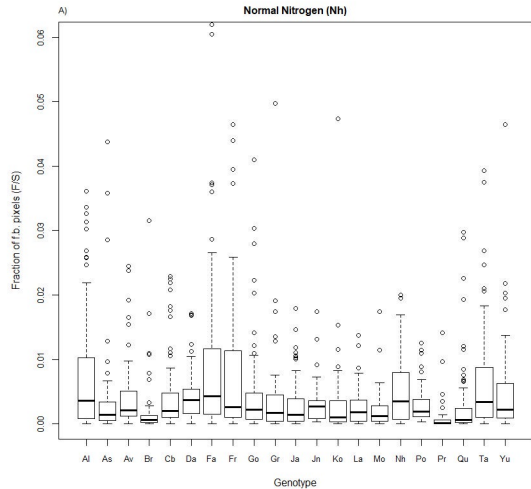


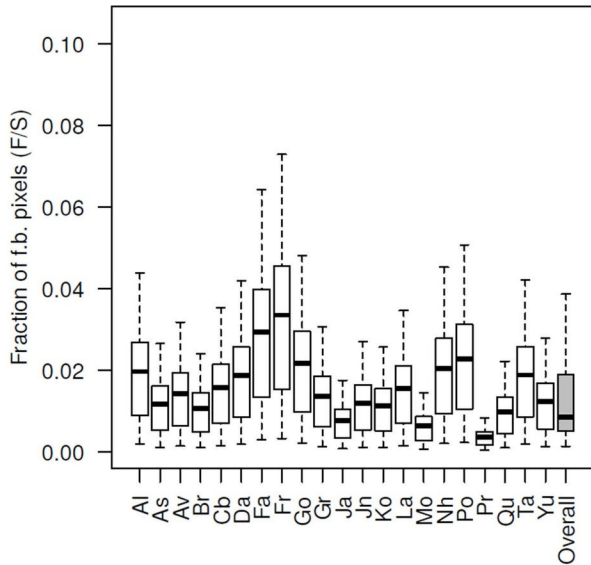
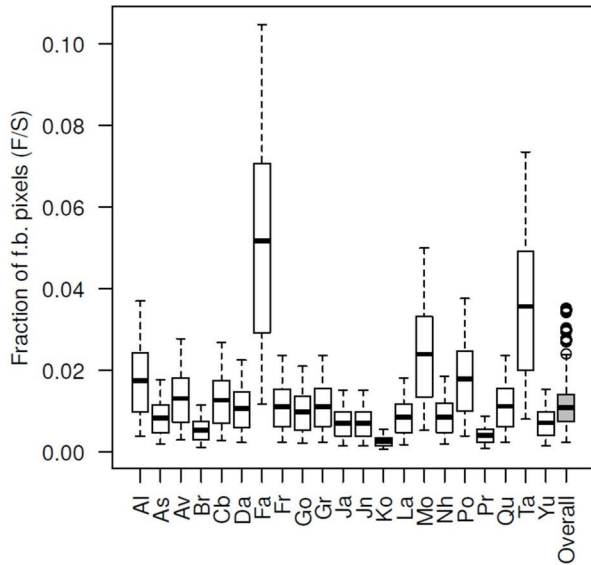
Fig 7**A) Normal Nitrogen (Nh)****B) Reduced Nitrogen (NI)**

Fig 8

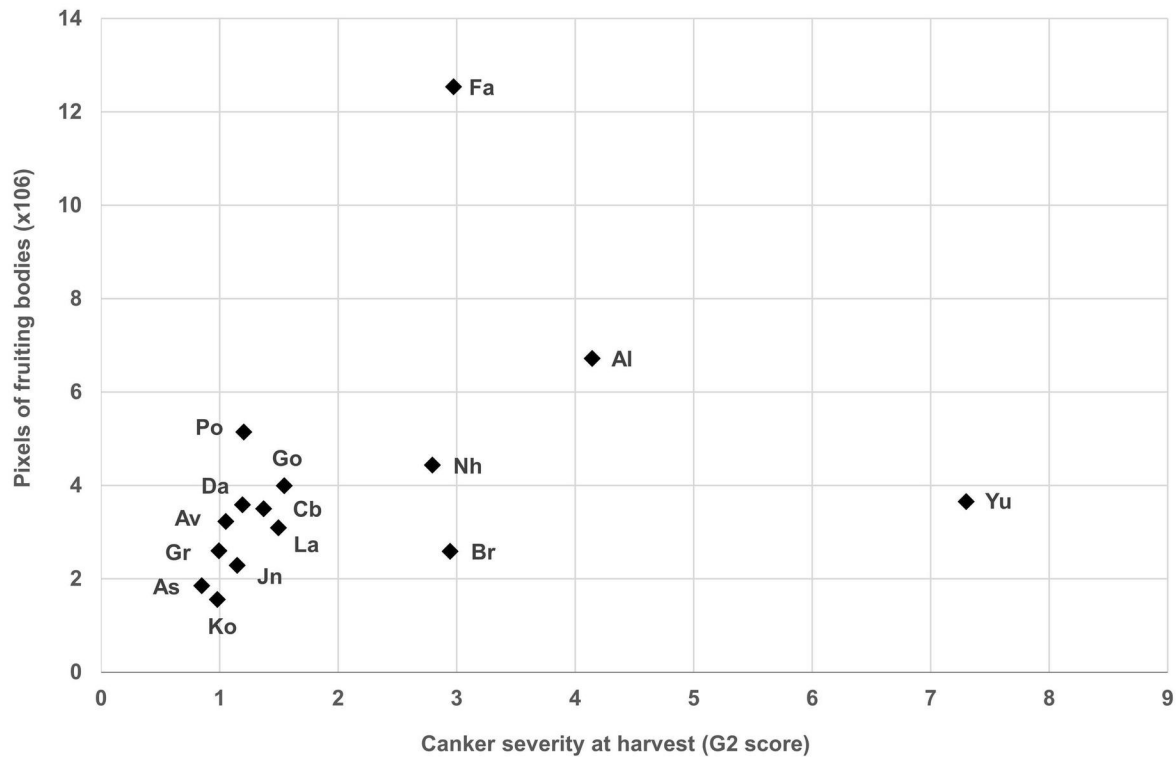


Table 1. Numbers of stems for the complete and the three sub datasets (Data14-16, Data12-16, Data15-16) given overall (italicised), but also by year and nitrogen fertilization treatment (Nl and Nh) and by genotype.

Dataset	Year	Nitrogen	Post process			Genotype																				
			Before	Discard	After	Al	As	Av	Br	Cb	Da	Fa	Go	Gr	Jn	Ko	La	Nh	Po	Yu	Fr	Ja	Mo	Pr	Qu	Ta
Complete	All		6758	18.3%	5525	390	318	366	325	356	286	338	290	348	247	291	320	363	291	273	114	133	137	76	181	82
	12-13	Nh	938	36	902	96	50	70	58	67	64	56	49	54	60	58	44	77	60	39	-	-	-	-	-	-
	13-14	Nh	919	48	871	49	59	75	52	88	48	73	42	65	12	31	80	86	70	41	-	-	-	-	-	-
	14-15	Nh	826	222	604	72	41	37	15	37	40	57	40	40	43	27	29	57	35	34	-	-	-	-	-	-
	14-15	Nl	789	318	471	38	31	30	47	23	25	39	23	32	24	27	36	24	23	49	-	-	-	-	-	-
	15-16	Nh	1626	240	1386	67	78	77	77	73	57	61	66	71	44	78	71	76	47	59	48	68	91	35	96	46
15-16	Nl	1660	369	1291	68	59	77	76	68	52	52	70	86	64	70	60	43	56	51	66	65	46	41	85	36	
Data1416	All		4045	10.1%	3029	245	209	221	215	201	174	209	199	229	175	202	196	200	161	193	-	-	-	-	-	-
	14-15	Nh	826	222	604	72	41	37	15	37	40	57	40	40	43	27	29	57	35	34	-	-	-	-	-	-
	14-15	Nl	789	318	471	38	31	30	47	23	25	39	23	32	24	27	36	24	23	49	-	-	-	-	-	-
	15-16	Nh	1208	206	1002	67	78	77	77	73	57	61	66	71	44	78	71	76	47	59	-	-	-	-	-	-
	15-16	Nl	1222	270	952	68	59	77	76	68	52	52	70	86	64	70	60	43	56	51	-	-	-	-	-	-
Data1216	All		3685	3.1%	3379	284	228	259	202	265	209	247	197	230	159	194	224	296	212	173	-	-	-	-	-	-
	12-13	Nh	938	36	902	96	50	70	58	67	64	56	49	54	60	58	44	77	60	39	-	-	-	-	-	-
	13-14	Nh	919	48	871	49	59	75	52	88	48	73	42	65	12	31	80	86	70	41	-	-	-	-	-	-
	14-15	Nh	826	222	604	72	41	37	15	37	40	57	40	40	43	27	29	57	35	34	-	-	-	-	-	-
	15-16	Nh	1002	206	1002	67	78	77	77	73	57	61	66	71	44	78	71	76	47	59	-	-	-	-	-	-
Data1516	All		3286	6.1%	2677	135	137	154	153	141	109	113	136	157	108	148	131	119	103	110	114	133	137	76	181	82
	15-16	Nh	1626	240	1386	67	78	77	77	73	57	61	66	71	44	78	71	76	47	59	48	68	91	35	96	46
	15-16	Nl	1660	369	1291	68	59	77	76	68	52	52	70	86	64	70	60	43	56	51	66	65	46	41	85	36

Table 2. Analysis of deviance for the observed distribution of canker severities at harvest

Factor	Df	χ^2	P-value
Year	3	7742	< 2.2e-16
Nitrogen	1	211	< 2.2e-16
Genotype	20	8828	< 2.2e-16
Nitrogen \times Genotype	20	1971	< 2.2e-16

Table 3: Analysis of deviance for the three datasets, with percentage of deviance explained by each factor and by the model

Dataset	Analysis of deviance				Model
	Factor	Df	P-value	Explained deviance	R ²
All	Year	3	<0.05	0.20	0.34
	Genotype	20	<0.05	0.061	
	Severity	6	<0.05	0.069	
	Nitrogen	1	<0.05	<0.001	
	Nitrogen × Genotype	20	<0.05	0.014	
	Nitrogen × Severity	6	<0.05	<0.001	
Data14-16	Nitrogen	1	<0.05	0.01	0.51
	Year	1	<0.05	0.20	
	Genotype	14	<0.05	0.17	
	Severity	6	<0.05	0.08	
	Nitrogen × Year	1	<0.05	0.0002	
	Nitrogen × Genotype	14	<0.05	0.003	
	Year × Genotype	14	<0.05	0.01	
	Year × Severity	6	<0.05	0.01	
	Nitrogen × Severity	6	<0.05	0.01	
	Nitrogen × Year × Genotype	14	<0.05	0.02	
	Nitrogen × Year × Severity	6	<0.05	0.002	
Data12-16	Year	3	<0.05	0.235	0.52
	Genotype	14	<0.05	0.115	
	Severity	6	<0.05	0.096	
	Year × Genotype	42	<0.05	0.062	
	Year × Severity	18	<0.05	0.011	
Data15-16	Nitrogen	1	<0.05	0.0003	0.28
	Genotype	20	<0.05	0.17	
	Severity	6	<0.05	0.05	
	Nitrogen × Genotype	20	<0.05	0.05	
	Nitrogen × Severity	6	<0.05	0.009	