

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

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- 2 Leptosphaeria maculans fruit bodies
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- 21

For fungal cyclic epidemics on annual crops, the pathogen carry-over is an important but poorly documented step. Plant resistance affects the pathogen development within the epidemics but we lack data on the inter-annual transmission of inoculum. For *Leptosphaeria maculans* on 15 oilseed rape genotypes in field during 4 growing seasons, stem canker severity was visually scored at harvest. The number of fruit bodies produced on incubated stubble was quantified using an automated image analysis framework. Our results confirm that fruit body production increases with disease severity and is significantly affected by host genotype and nitrogen supply. Tracking individual stems through incubation, we confirm for the first time that the oilseed rape genotype has a direct effect on inoculum production, not only disease severity. This major effect of genotype on inoculum carry-over should be taken into account in models of varietal deployment strategies.

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

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36 Introduction

A key development in disease control has been the breeding and deployment of crop varieties 37 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 and implement durable crop protection strategies against rapidly evolving pathogens (Cowger & 40 41 Brown 2019). Any mitigation strategy that reduces the transfer of inoculum between seasons, therefore the effective size of pathogen populations, should limit the evolutionary potential of 42 pathogens, and increase resistance durability (Bousset & Chèvre 2013; Zhan et al. 2015). In 43 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. 2012), or removing leaf litter in combination with reduced fungicide application (Didelot et al. 2016) 46 47 has delayed adaptation. In models and experiments, the spatio-temporal distribution of host resistance in cultivated landscapes was optimised to mitigate disease transmission (Lô-Pelzer et al. 2010; 48 Bousset et al. 2018; Papaïx et al. 2018; Rimbaud et al. 2018; Watkinson-Powell et al. 2019). 49 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 & Chèvre 2013; Fig. 1A). Their dynamics are highly influenced by both temporal and spatial 53 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 carryover from the prior crop(s) and/or dispersal. Mitigation strategies can thus target this carry-over of 56 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 landscape connectivity following the intercrop (Lô-Pelzer et al. 2010; Bousset & Chèvre 2013; 65 66 Papaïx et al. 2018; Rimbaud et al. 2018; Watkinson-Powell et al. 2019). Little is known about the direct effect of quantitative host resistance on initial amount of inoculum at the beginning of the next 67 cropping season (i.e. inter-annual transmission, Fig. 1B). Quantitative resistance affects transmission 68 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).

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

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

In this study, we address the effect of host resistance on the carry-over of inoculum between consecutive years, to understand how host genotype might affect fruit body production and the effects of a common cropping practice, i.e. nitrogen fertilization, and disease severity at harvest in the preceding growing season (year). We consider *L. maculans* on oilseed rape as an example pathosystem and use an imaged-based phenotyping method for quantifying fruit bodies that appeared
 on diseased stems collected in experimental field plots, after an incubation period. We analyse the
 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

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

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

Homogeneous disease infection was achieved with contaminated stems collected from the previous year on the susceptible Bristol and miscellaneous varieties. In the experimental area and around Phoma stem cankered stubble, only *L. maculans* leaf spots were observed which supported the hypothesis of the very small prevalence of the other causal agent *L. biglobosa* in this area (Bousset et al. 2019). Stems were scattered throughout the plots in each cropping season at a density of two stems per m^2 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 season. Phoma stem canker severity was assessed 2-3 weeks before crop maturity on a 1 to 6 scale as 134 135 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

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

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 temperature (Fig. S1ab), rainfall (Fig. S1cd), and number of days that are favourable for pseudothecia 143 144 maturation (Fig. S1e) were calculated from start (shortly after harvest in June; Table S1.1) to end of incubation when stems were retrieved from the field (at the end of the following winter; Table S1.1). 145 146 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 required for 50% of pseudothecia to reach maturation. We were able to compare the number of 149 150 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 piece comprising the crown and upper 10 cm (Bousset et al. 2019). The sampling was constrained by 154 155 the availability of diseased stems across severity classes. After pooling the three replicated plots of each variety, 30 stems in each of the 6 severity classes were randomly selected. If fewer than 30 stems
(per class) were available, then all were kept. Within each variety and severity class, groups of 5 stem
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, they were placed on a 1:1:1 mix of sand, peat and compost spread on the ground to avoid weed 160 161 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

The fruit bodies appeared on diseased stem pieces were quantified using image-based 168 phenotyping settings described in Bousset et al. (2019). In short, a picture of each group of 5 dry 169 170 stems was taken with the barcoded label, placed on a glass plate, 16 cm above a blue background (PVC sheet Lastolite Colormatt electric blue). Two FotoQuantum LightPro 50 × 70cm softboxes were 171 placed on both sides of the stem pieces with 4 daylight bulbs each (5400K, 30W) within the lower 172 45° angle. Pictures were taken with a Nikon D5200 with an AF-S DX Micro Nikkor 40mm 1:2.8G 173 174 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 6000×4000 pixels. 176

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

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

188 Statistical analyses

We analysed the effects of genotype, year and nitrogen fertilization on stem canker severity using a proportional odds model. We used an ordered logit regression model that handles ordinal data. The design of the experimental data only allowed us to estimate and test the first order effects and the 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_{Ei} and $n_{S,i}$, and used a likelihood function based on $n_{Ei} \sim B[(n_{Ei} + n_{S,i}), p]$ 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 15-16, 21 genotypes and 2 nitrogen fertilization levels Nh and Nl). Note that for all these subdatasets the 204 205 severity prior incubation (7 levels) was also included as a categorical variable in the regression 206 models.

The whole image processing was developed in Python (Van Rossum & Drake 1995) whereas
 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 the ordered logistic regression model (Tables S5.1 and S5.2). Second, we assumed that the size of 214 215 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

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

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

Across datasets, fruit body formation was affected by host genotype, year, and disease severity at harvest. The year was the most influential parameter, with about 20% of the explained deviance for all analyses (Table 3). Host genotype explained between 6 and 17% of the explained deviance of fruit body formation. Finally, severity of infected stems before incubation explained between 5 and 10 % of the total deviance. Raw data as well as pairwise comparisons of modalities with least-squares
means indicated differences between genotypes and years when stems were collected. The production
of fruit bodies was lowest in year 15-16 and highest in year 13-14 (Fig. 3; Supplementary Information
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 contrast, a mildly infected genotype like Da (having no stem in classes S5 and S6) produced more 246 247 fruit bodies at a given severity class than e.g. genotypes As or Av.

Because several genotypes did not occur either in the first two (S1-S2) or the last two (S5-S6) severity classes, it was not possible to estimate all the coefficients of the genotype × severity interaction on the full dataset. However, when considering the genotypes for which this interaction was identifiable, we found a significant genotype × severity interaction, suggesting that the amount 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

We detected a significant fertilization × host genotype interaction, indicating that fertilization has varying effects on disease symptoms of different host genotypes. (Fig. 2). The fertilization × disease severity and fertilization × year interactions were also significant. However, as shown by the little deviance explained by these first, second and third order factors, nitrogen fertilization had a minor influence on fruit body production (Table 3; Fig. 6). In fact, nitrogen explained below 1% of
the deviance in the Data15-16 dataset, and only the nitrogen × genotype interaction explained 5% of
the deviance (Table 3).

262

263 Simulated fruit body formation

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

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

275

276 **Discussion**

277

With field experiments and image-based automated quantification of fruit bodies on individual stems, we disentangled the role of the year, the genotype, the disease severity and the nitrogen fertilization. We confirmed for the first time that the oilseed rape genotype has a direct effect, not only through disease severity, on a seldom-informed trait of the fungal pathogen life cycle. The effect of host genotype on both the visual density of pseudothecia and the numbers of released ascospores had already been observed by Marcroft *et al.* (2004b). However, given the experimental design used, 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 quantitative resistance and field on disease severity and on fruit body production. Yet, those studies 286 287 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 on crushing 50 pseurothecia in water with bleach and counting the ascospores with a haemocytometer 298 299 (Lô-Pelzer et al. 2009). Therefore, the numbers of ascospores available for release in cropping areas are huge. Nevertheless, clear gradients of decrease in leaf spot numbers on farmer's oilseed rape fields 300 301 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 of emitted ascospores. This indicates that however large the number of ascospores is, decreasing their 303 number by the means of genotype will contribute to decreasing disease transmission between 304 305 successive years. In the panel of genotypes considered in the study, the host modulates the production of fruit bodies rather than suppressing it. Therefore, plant genotypes cannot prevent epidemics but 306 307 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.

Genotype effect could be due to a difference in the amount of nutrients available for the 325 326 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. 2016). However, in our study some genotypes may be severely infected but produce a moderate 329 330 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 et al. 2019). In our study, the genotypes Av, Cb, Da, Gr, Jn, Ko had higher than average levels of 334 quantitative resistance on stem canker severity (Table S1.2; Fopa-Fomeju et al. 2015; Kumar et al. 335

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 fungus in stem and its consequences on mating remains unknown. Further studies would be 355 356 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

In agreement with previous findings (Lô-Pelzer *et al.* 2009; Bousset *et al.* 2019), our study confirmed the importance of between-year variability that may be related to variable environmental 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 the development of the host plant, some main climatic variables such as temperature may drive 366 367 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 the landscape (Lô-Pelzer et al. 2010; Papaïx et al. 2018; Rimbaud et al. 2018; Watkinson-Powell et 375 376 al. 2019). So far, the effect of qualitative host resistance on infection and some effects of quantitative host resistance on pathogen development are considered. Our results suggest that it would be worth 377 378 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 disease severity at harvest (Fig. 2). For example, the genotypes Ja, Pr and Yu with a high disease 381 382 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 that 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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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.

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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

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

562

Fig. 3. Fraction of fruit body pixels depending on year of sampling (12-13, 13-14, 14-15 and 15-16)
for the fifteen genotypes in the Data12-16 dataset. Full names of genotypes are available
(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

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

575

Fig. 6. Predicted fraction of fruit body pixels depending on the twenty one genotypes in the Data1516 dataset for (A) normal (Nh) or (B) reduced (Nl) nitrogen fertilization of the crop. Full names of
genotypes are available (Supplementary Information Table S1.2).

579

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

586

Fig. 8 Average simulated numbers of fruit body pixels depending on the canker severity at harvest (G2 index calculated for the ensemble of stems) for the fifteen genotypes over all years. In simulations, the effect of genotype was taken into account both for the distribution of stems in canker severity classes and on the prediction of fruit body pixels, adjusting the model on observed data. Data are means of 10 simulated field plots with 100 plants of 700 000 pixels. Among the genotypes, either 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 2





Genotype

Genotype











Genotype

Genotype

Fig 7



Fig 8



Dataset	Year	Nitrogen	Post process			Genoty	pe																			
			Before	Discard	After	Al	As	Av	Br	Cb	Da	Fa	Go	Gr	Jn	Ко	La	Nh	Ро	Yu	Fr	Ja	Мо	Pr	Qu	Та
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	NI	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	NI	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	NI	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	Nİ	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	NI	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 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 (NI and Nh) and by genotype.

Factor	Df	χ2	P-value
Year	3	7742	< 2.2e-16
Nitrogen	1	211	< 2.2e-16
Genotype	20	8828	< 2.2e-16
Nitrogen × Genotype	20	1971	< 2.2e-16

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

Table 3: Analysis of deviance for the three datasets, with percentage of deviance explained by

 each factor and by the model

	Analysis of deviance				Model
Dataset	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 X Genotype	20	<0.05	0.014	
	Nitrogen X 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 X Genotype	14	<0.05	0.01	
	Year $ imes$ Severity	6	<0.05	0.01	
	Nitrogen X Severity	6	<0.05	0.01	
	Nitrogen X Year X Genotype	14	<0.05	0.02	
	Nitrogen X Year X 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 X Genotype	42	<0.05	0.062	
	Year X 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 X Genotype	20	<0.05	0.05	
	Nitrogen X Severity	6	<0.05	0.009	