Title: Sexual reproduction in the fungal foliar pathogen *Zymoseptoria tritici* is driven by antagonistic density-dependence mechanisms

Running head: Density dependence in *Zymoseptoria tritici* sexuality

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Abstract

This study provides empirical evidence for antagonistic density-dependence mechanisms driving sexual reproduction in the wheat fungal pathogen *Zymoseptoria tritici*. Biparental crosses with 12 increasing inoculum concentrations, in controlled conditions, showed that sexual reproduction in *Z. tritici* was impacted by an Allee effect due to mate limitation and a competition with asexual multiplication for resource allocation. We found that asexual multiplication was itself affected by competition for host resources between the two parental isolates, as illustrated by the decrease in asexual fruiting body density and sporulation intensity observed with increasing lesion density. Consistent with these results for controlled co-inoculation, we found that the intensity of sexual reproduction varied with both cropping period and the vertical position of the host tissues in the field. An optimal lesion density maximizing offspring (ascospores) number was established, and its eco-evolutionary consequences are considered here. Two ecological mechanisms may be involved: competition for resources between the two modes of reproduction (decrease in the host resources available for sexual reproduction due to their prior use in asexual multiplication), and competitive disequilibrium between the two parental isolates, due to differential interaction dynamics with the host, for example, leading to an imbalance between mating types. Conceptual models based on these results suggest that sexual reproduction plays a key role in the evolution of pathogenicity traits, including virulence and aggressiveness. Ecological knowledge about the determinants of sexual reproduction in *Z. tritici* may, therefore, open up new perspectives for the management of other fungal foliar pathogens with dual modes of reproduction.

Keywords

Allocation resource, asexual multiplication, competition, fungal pathogen, plant disease epidemiology, sexual reproduction

Introduction

An ability to reproduce is one of the most fundamental features of life. It has been suggested that the benefits of sexual reproduction include the purging of deleterious mutations from the genome and the production of a recombinant progeny well-adapted to a changing environment. Density-dependent mechanisms are common in microbial ecology and play a crucial role in sexual reproduction. The modeling of density-dependent selection would shed light on the ability of populations to persist despite "sex"-related conflicts, but simple models seem unable to predict the diversity of the responses observed in nature [1]. A few empirical studies have focused on the role of sex in influencing fungal population and community structures [2, 3].

Several density-dependent mechanisms may operate simultaneously in a population, and it is important to understand how they act, both independently and together. Positive (facilitation) density-dependent mechanisms, such as demographic Allee effects (increasing relationship between overall individual fitness and population density), linked to mate-finding, for example [4], are frequently taken into account in population dynamics studies. Negative (restriction) density-dependent mechanisms, such as competition for resources in cases of high population density, have also been widely described. The effects of competition for resources on individual fitness at low and high densities are a key, complex question in population and evolutionary ecology. For instance, Neiman et al. [5] detected a strong positive effect of high population density on reproductive output in the presence of adequate food supplies in *Potamopyrgus antipodarum* (freshwater snail), but they also identified food

limitation as the primary source of negative density dependence. Generally, in situations of high population density, negative density dependence often erases the demographic Allee effect through resource competition.

The impact of density-dependent mechanisms on sexual reproduction has mostly been studied in animal ecology, where encounters between male and female animals result from active behavior influenced by sexual dimorphism or mating advantage [1]. For instance, Stelzer [6] focused on two different mechanisms of density-dependent population regulation — resource exploitation and sexual reproduction — in populations of the rotifer *Brachionus calyciflorus* displaying different investments in sex: populations reproduce clonally at low densities, with the proportion of sexual individuals increasing at higher densities. These two mechanisms have been shown to apply to several facultatively sexual animals, but similar antagonistic density-dependent mechanisms may also operate in fungal microorganisms with dual modes of reproduction.

* * *

Competitive interactions and their effect on fitness during reproduction, particularly for asexual reproduction, have been investigated in the emblematic fungal plant pathogens *Puccinia graminis* f. sp. *tritici*, the causal agent of wheat stem rust [7], and *Phytophthora infestans*, the causal agent of potato late blight [8]. The findings obtained were consistent with the ecological principle that resource availability decreases over time, with increasing host tissue mortality, but also during multiple infections, in which different parasite strains share the host resources. Clément et al. [8] focused on the consequences of multiple infections on asexual reproduction, and dissociated the effects of single-site and double-site infections and those of competition between identical and different isolates during double-site infections.

Allee effects due to mate limitation have been reported to affect the dynamics of some fungal pathogens at large spatial scales, such as *Hymenoscyphus fraxineus*, the causal agent of ash dieback [9], and *Mycosphaerella fijiensis*, the causal agent of black Sigatoka disease in banana plants [10]. Sexual spores for dispersal are produced only when compatible mating types come into direct contact. However, mate limitation at the plant scale was not confirmed by experimental studies, probably because there are still many gaps in our knowledge concerning the ways in which sexual reproduction occurs in host tissues and its determinants. However, this effect forms the theoretical basis of demogenetic models combining sexual and asexual reproduction, in which the impact of mate-finding limits the speed of pathogen spread [11, 12].

Thus, on the one hand, pathogen population density can affect competition between individuals for limited host resources at a given reproductive stage (e.g. asexual reproduction) or the competition between asexual and sexual stages. On the other hand, it may also affect the probability of finding a mate and, thus, the intensity of sexual reproduction. Antagonistic interactions between negative and positive density-dependent mechanisms have never been studied experimentally in the reproduction of fungal plant pathogens. They could have been investigated by Clément et al. [8] for *P. infestans* co-infections with isolates of mating types A1 and A2 [13], but sexual reproduction was deliberately prevented by inoculation with incompatible isolates only.

* * *

Sexual reproduction in *Zymoseptoria tritici*, the causal agent of Septoria leaf blotch of wheat, requires a physical encounter between two compatible strains (Mat1-1 and Mat1-2; [14, 15]). It generally occurs on senescent tissues (leaf and stem debris), with pseudothecia (sexual fruiting bodies producing ascospores) appearing 46 to 76 days after the initial infection in

114 field conditions [16]. The very active sexual cycle of *Z. tritici* has crucial consequences for
115 the epidemiology of Septoria leaf blotch, because it determines the amount of primary
116 inoculum (wind-dispersed ascospores produced on wheat debris) available for the initiation of
117 subsequent epidemics [17]. Sexual reproduction also plays an essential role in maintaining the
118 diversity of local pathogen populations and determining their rate of adaptation to selection
119 pressures, such as those exerted by resistant hosts or fungicide treatments. Given the high
120 population diversity, whatever the spatial scale considered [18], a wheat plant should be
121 considered to be subject to "multiple infections". The sex ratio is consequently close to 1 at
122 the local scale (Morais et al., unpubl.) and the imbalance effects sometimes reported for other
123 organisms are negligible.

124 Avirulent *Z. tritici* strains were recently shown to cross with virulent strains on wheat
125 cultivars carrying the corresponding resistance gene [19]. This key finding calls into question
126 current views about the determinants of sexual reproduction, including, in particular, the
127 common belief that individuals unable to infect wheat tissues cannot reproduce sexually.
128 However, the proportion of crossing events resulting from the "exclusive paternal
129 parenthood" model was not quantified at the leaf scale in the natural context of highly diverse
130 populations. The vast majority of crossing events probably occur when leaf lesions caused by
131 two compatible strains either coalesce or are located very close together. In practice, it
132 remains unclear how close together infections must be for effective mating to occur. Metcalfe
133 [20] found internal hyphae spanning mean distances of no more than 11 mm in a susceptible
134 cultivar just before the production of pycnidia, suggesting that infection foci located more
135 than 20 mm apart may be isolated. The number of offspring (i.e. the number of ascospores)
136 should be proportional to the number of crossing events, and correlated with disease severity,
137 which is a good proxy for lesion density. This has been shown at field scale: more severe
138 epidemics are associated with higher levels of ascospore production during epidemics in the

following year [21, 22]. Moreover, the fitness (aggressiveness) of the *Z. tritici* parental isolates and a time lag between the inoculations with the two parental strains can affect sexual reproduction intensity [23].

The density-dependent mechanisms driving the interaction between *Z. tritici* and wheat during the asexual stage remain unclear. In particular, it remains unknown how the fungus exploits the resources of it host to obtain nutrients for growth, how it invades the apoplast during the long asymptomatic infection phase, and how the necrotrophic phase triggered [24].

Objective and strategy

We investigated the density dependence of both asexual and sexual reproductive mechanisms in *Z. tritici*, a relevant fungal model organism for such eco-epidemiological investigations. What impact does lesion density in host tissues have on sexual reproduction at the leaf scale? Could we generate empirical data for the establishment of a density-dependence relationship? Would this relationship be monotonic or more complex, due to competition for resources between the two modes of reproduction? This study addressed these questions. We first analyzed epidemiological datasets collected in field conditions, to confirm our initial assumptions. We then assessed the intensities of asexual and sexual reproduction after five *Z. tritici* biparental crosses on adult wheat plants over a period of three years.

Materials & methods

Preliminary analysis of field epidemiological data

Field epidemiological data were analyzed to assess the impact of disease severity (proxy of asexual multiplication intensity) and the vertical position of the host tissues on the intensity of

162 subsequent sexual reproduction, during the epidemic period and then during the interepidemic
163 period. We reanalyzed a dataset obtained in Danish field conditions by Eriksen & Munk [16],
164 together with the data obtained in our three-year experiment in France.

165 *Epidemic period* – The relationship between the proportion of pseudothecia among the total
166 fruiting bodies (pycnidia and pseudothecia) on wheat leaves during a growing season and
167 mean disease severity (expressed as the percentage of the leaf area covered by pycnidia and
168 pseudothecia) were determined with the experimental data for the field experiment performed
169 by Eriksen & Munk [16] (Table 1).

170 *Interepidemic period* – In a field plot sown with a monoculture of wheat cv. Soissons from
171 2007 to 2016 at the Grignon experimental station [22], standing stubble was left over an area
172 of 20 m^2 during the fall of 2015-2016, 2016-2017 and 2017-2018. Stubble samples were
173 collected three or four times each year, from September to January. Stems and leaves were
174 partitioned from lower to upper layers, according to their vertical position (0-5 cm, 5-10 cm,
175 10-15 cm, 15-25 cm, 25-35 cm, 35-50 cm, 50-65 cm). The dry tissues of each layer of wheat
176 stubble were weighed, and ascospore production, as a proxy of sexual reproduction intensity,
177 was quantified as described by Suffert et al. [17].

178

179 **Fungal material**

180 Five crosses were performed, with eight *Z. tritici* isolates (FS0802 × FS1006, FS0813 ×
181 FS0732, FS0808 × FS0806, FS1022 × FS1008 [25]) collected in 2010 from wheat cv.
182 Soissons in France, and two isolates (3D7 × 1A5 [26]) collected in 1999 from wheat cv.
183 Galaxie and Lono (Table 1). The compatibility of the five pairs of isolates was determined by
184 PCR amplification of the two mating-type idiomorphs [15]. The effective ability of the eight
185 French isolates to reproduce sexually on adult plants was first checked in semicontrolled

186 conditions [23]. Subcultures of each isolate stored at -80°C were grown for five days in Petri
187 dishes containing PDA (potato dextrose agar, 39 g L^{-1}) at 18°C in the dark. For each isolate,
188 an initial conidial suspension was prepared with a Malassez counting chamber. Serial
189 dilutions (1:5) of each initial monoparental suspension were prepared, from 10^7 conidia mL^{-1}
190 to 50 conidia mL^{-1}. Twelve biparental suspensions were then prepared by mixing 25 mL each
191 of the appropriate monoparental suspensions and adding two drops of surfactant (Tween 20;
192 Sigma, France).

193

194 **Plant material**

195 Seeds of the wheat cv. Soissons or Runal (moderately susceptible to Septoria tritici blotch)
196 were sown on December 18 2014, December 14 2015, and December 15 2016 (Table 1) in
197 Jiffy peat pots, which were then kept in greenhouse conditions for two weeks. Seedlings were
198 vernalized in a growth chamber for 8 weeks at 8°C, with a 10-h light/14-h dark photoperiod.
199 They were then returned to the greenhouse and left to acclimate for one week before
200 transplantation into individual pots filled with 1 liter of commercial compost (Klasmann Peat
201 Substrat 4; Klasmann France SARL, France). We added 4 g of slow-release fertilizer
202 (Osmocote Exact 16-11-11N-P-K 3MgO Te) to each pot. The plants were also watered with
203 Hydrokani C2 fertilizer (Hydro Agri Spécialités, France), diluted 1:100 and poured into the
204 saucers under the pots. Plants were sprayed once with spiroxamine (Flexity SC at 300 g L^{-1};
205 BASF, France) for the specific prevention of powdery mildew (*Blumeria graminis* f.sp.
206 *tritici*), no later than six weeks before inoculation. During the growth period, the plants were
207 illuminated with natural daylight supplemented with 400 W sodium vapor lamps between
208 6.00 a.m. and 9.00 p.m. The air temperature was kept below 20°C during the 15-h light period
209 and above 12°C during the 9-h dark period. Plants were thinned to three stems per pot during
210 the growth period.

211

212 **Inoculation procedure**

213 Co-inoculations with the five biparental suspensions were carried out after the wheat heads
214 had fully emerged (one in 2015, on April 30; two in 2016, on May 3; two in 2017, on April
215 25; Table 1). In 2017, two complete sets of wheat plants cv. Soissons were also inoculated
216 with each monoparental suspension (FS1022 and FS1008). The suspensions were applied
217 with an atomizer (Ecospray, VWR, France), on three adult plants (nine stems), as described
218 by Suffert et al. [23]. Plants were turned during the 10-second spraying event, to ensure even
219 coverage with inoculum. Infection was promoted and cross-contamination prevented by
220 enclosing each trio of plants inoculated with the same suspension in a sealed transparent
221 polyethylene bag humidified with distilled water for 72 h. Wheat plants were then kept in a
222 greenhouse for about 13 weeks (from April 30 to July 24 2015, from 3 May to July 28 2016,
223 from 25 April to August 2 2017; Figure 1a). Air temperature was kept above 18°C during the
224 spring, and reached 30°C several times during summer.

225

226 **Assessment of fitness traits during the asexual stage**

227 Disease severity on leaves was assessed by eye five weeks after inoculation (on June 2 2015,
228 June 6 2016 and May 27 2017), as the percentage of the leaf area displaying necrosis (mean
229 for the F1, F2, and F3 leaves for each stem; 1, 2, 3 and 5%, thereafter by increments of 5% up
230 to 100%; Figure 2). In 2016, the density of pycnidia (number of pycnidia divided by the
231 necrotic area) and the proportion of pycnidia exuding a cirrhus (Figure 2b-c) were also
232 estimated for plants inoculated with each of the two biparental suspensions, FS0813 × FS0732
233 and FS0808 × FS0806 (Table 1), from the necrotic area in a 6-cm leaf section from four
234 randomly selected F1 leaves per inoculum concentration.

235

236 **Promotion of sexual reproduction and ascosporogenesis**

237 Co-inoculated plants were placed outdoors during the summer and fall (from July 24 2015 to
238 October 9 2015, from July 28 2016 to November 14 2016, and from July 28 2017 to
239 December 4 2017) to induce the maturation of pseudothecia (Figure 1d). Each trio of co-
240 inoculated plants was tied up with stiff wire, with the stems and leaves clamped so as to
241 prevent contact with other trios of plants. On October 14 2015, November 16 2016, and
242 December 6 2017, dry leaves and stems from each trio of plants were cut into 2-cm fragments
243 and allowed to dry, in separate open boxes, in a laboratory atmosphere, at 18°C for one week.
244 Ascospore release was assessed immediately after drying, to check the maturity of the
245 pseudothecia. The pseudothecia were considered mature in 2015 and 2017. By contrast, in
246 2016, the plant debris was placed outdoors again until January 16 2017, to promote
247 pseudothecium maturation.

248

249 **Assessment of the intensity of sexual reproduction**

250 For each inoculum concentration, the debris was weighed, soaked in water for 20 min and
251 spread on dry filter paper in a box (24 × 36 cm), the lid of which was left half open to
252 decrease the relative humidity of the air around the debris. Eight Petri dishes (90 mm in
253 diameter) containing PDA medium were then placed upside down 1 cm above the debris. The
254 boxes were placed in the dark, at 18°C for 19 h. The Petri dishes were then closed and
255 incubated in the same conditions. Each discharge event was replicated four times (from
256 November 2015 to January 2016, from January 2016 to February 2017, and from December
257 2017 to January 2018).

The ascospores released onto the Petri dishes germinated after 24 h. Six days later, yeast-like colonies resembling cream-colored convoluted heaps were observed. The colonies were counted under a microscope four and seven days after ascospore discharge. It was assumed that each colony resulted from the germination of a single ascospore, and that clusters of colonies appeared just above mature pseudothecia from which several ascospores had been discharged. A cumulative ascospore discharge index (ΣADI), defined as the total number of ascospores discharged per gram of wheat debris [17, 23], was calculated for each discharge event, as follows:

$$\Sigma\mathrm{ADI} = \sum_{e=1}^{n} \frac{1}{8} \cdot \frac{x.y}{\pi.r^2} \cdot \frac{\mathrm{NBcol}}{\mathrm{DW}} \qquad \text{eq. 1}$$

where NBcol is the total number of colonies in the eight Petri dishes, DW is the total dry weight of debris spread out in the box, *r* is the radius of a Petri dish (4.5 cm), *x* and *y* are the width and length of the box (24 cm and 36 cm, respectively), and *n* is the number of discharge events ($n = 4$).

Assessment of yield components

Yield components were assessed in 2016 and 2017 (Table 1). At maturity, the spikes were cut and hand-threshed to separate the kernels from the chaff. Kernels were then counted and weighed to estimate the grain weight per spike (GWS) and thousand-kernel weight (TKW) for each cross and inoculum concentration.

Data analysis

A quadratic model (eq. 2) was used to describe empirically the relationship between sexual reproduction intensity (SRI) and asexual multiplication intensity (AMI).

$$\mathrm{SRI} = \mathrm{a.AMI}^2 + \mathrm{b.AMI} + \mathrm{c} \qquad \text{eq. 2}$$

where *a, b* and *c* are parameters.

A second model integrating two types of density-dependent constraints (eq. 3) was used. It was based on a logistic function f(α,β,γ,AMI) (eq. 4) describing a positive density-dependent mechanism and coupled with a function g(μ,σ,ρ,AMI) (eq. 5) describing a negative density-dependent mechanism.

$$\mathrm{SRI} = \mathrm{f}(\alpha, \beta, \gamma, \mathrm{AMI}) \times \mathrm{g}(\mu, \sigma, \rho, \mathrm{AMI}) \qquad \text{eq. 3}$$

$$\mathrm{f}(\alpha, \beta, \gamma, \mathrm{AMI}) = \alpha.\, \mathrm{e}^{\left(-\beta.\mathrm{e}^{-\gamma.\mathrm{IAM}}\right)} \qquad \text{eq. 4}$$

$$\mathrm{g}(\mu, \sigma, \rho, \mathrm{AMI}) = \mu - (\mu - \sigma)\left(\mathrm{e}^{\left(-\ln \mu.\mathrm{e}^{(-\rho.\mathrm{IAM})}\right)} - 0.01\right) \qquad \text{eq. 5}$$

where α, β, γ, μ, σ, and ρ are parameters.

Both models were adjusted with the data generated by the five crosses, with the estimation of SRI by determination of the cumulative ascospore discharge index (ΣADI) and of AMI with the percentage of the total area displaying necrosis.

Results

Sexual reproduction in field conditions varies according to the cropping period and the vertical position of the infected host tissues in the canopy

298 The epidemiological data obtained by Eriksen & Munk [16] in field conditions showed that
299 the proportion of pseudothecia (proxy for the intensity of sexual reproduction) present on
300 wheat leaves varies with disease severity (proxy for asexual multiplication intensity; Figure 3)
301 over the cropping season. Our reanalysis of these data made it possible to limit bias due to
302 fruiting dynamics (the latent period is much longer for pseudothecia than for pycnidia). The
303 proportion of pseudothecia was maximal for severities of 50 to 80%. This proportion
304 remained stable or even decreased at severities above 80%.

305 Monitoring of the dynamics of sexual reproduction on standing stubble over three
306 interepidemic periods revealed a vertical structuring of mean ascospore production on
307 senescent wheat tissues (Online Resource 1). This vertical structuration changed over time.
308 Ascospore numbers peaked in November (2015, 2016) or December (2017). Ascospore
309 production generally increased with distance from the ground, up to a height of 25 cm (Figure
310 4). This layer corresponds to the part of the stubble not usually exported at harvest. The 25-35
311 cm layer made the largest contribution to sexual reproduction. Above this height, ascospore
312 production follows the opposite trend, gradually decreasing between 35 and 65 cm. The
313 lowest layer of standing stubble, which includes the crown and what remains of the first
314 leaves to emerge before tillering, generated less than 5% of the total number of ascospores.

315

316 **The general interaction between *Z. tritici* and wheat is density-dependent**

317 The interaction between the pathogen and the host plant is clearly density-dependent.
318 Regardless of the year in which the cross was performed (2015, 2016 or 2017), the biparental
319 suspension used and the host cultivar (Soissons or Runal), inoculum concentration (total
320 number of *Z. tritici* conidia in the biparental suspension per mL) was positively related to the
321 estimated mean disease severity (percentage of necrotic area) for the three uppermost leaves

322 of the adult wheat plants (Figure 5). Soissons was particularly susceptible to the isolates with
323 which it was co-inoculated (mean sporulation area of 60% for an inoculum concentration of 5
324 × 10^5 conidia.mL^{-1}), whereas Runal was more resistant (35%). For both varieties, disease
325 severity (80%) was maximal at an inoculum concentration of 10^7 conidia.mL^{-1}.

326 Mean severity on the three uppermost leaves of adult wheat plants cv. Soissons and Runal
327 was inversely correlated with total grain weight per spike (GWS), and thousand kernel weight
328 (TKW): late (inoculation after flowering) but intense attacks (sporulation over 80% of the
329 area) decreased grain yield by up to 20-25% (Figure 6). *Z. tritici* attacks had a strong impact
330 on wheat yield, demonstrating that the interaction between the pathogen and the host plant
331 was density-dependent, i.e. a function of pathogen inoculum pressure.

332

333 **Competition for resources affects asexual multiplication in *Z. tritici***

334 We observed robust, negative relationships between mean disease severity and the density of
335 pycnidia and between mean disease severity and the percentage of exuding pycnidia in 2016
336 (Figure 7): more intense *Z. tritici* attacks were associated with a lower density of pycnidia,
337 and a lower percentage of exuding pycnidia. For both FS813 × FS732 and FS808 × FS806
338 crosses, severe attacks (mean sporulation area of 80%) halved the density of pycnidia (150 vs.
339 300 pycnidia.cm^{-2}) and the percentage of exuding pycnidia (45 vs. 90%). Thus, the asexual
340 development of *Z. tritici* is affected by competition for resource allocation.

341 A comparison of the disease severity reached after inoculation with a biparental suspension
342 and after inoculation with a suspension containing only one isolate (with an equivalent
343 number of conidia to the mixture) highlighted competitive disequilibrium between the two
344 parental isolates. This finding provides a second line of evidence that the asexual
345 development of *Z. tritici* is driven by density-dependent processes. Soissons appeared to be

346 more susceptible to FS1008 than to FS1022, regardless of the concentration of the inoculum:
347 in practice, FS1008 was more aggressive than FS1022 (e.g. disease severity with FS1008 was
348 twice that with FS1022 for inoculum concentrations of 10^4 to 10^6 conidia.mL^{-1}; Figure 8). At
349 inoculum concentrations of 50 to 10^5 conidia.mL^{-1}, FS1008 induced a less severe attack (<
350 20% on average) if the other, less aggressive isolate (FS1022) was present at the same
351 concentration. This difference was significant provided that the number of FS1022 conidia
352 was the same. It disappeared at the highest inoculum concentrations (> 10^5 conidia.mL^{-1},
353 corresponding to a disease severity of 35-40%).

354

355 **Competition with asexual multiplication for resource allocation affects sexual**
356 **reproduction in *Z. tritici***

357 Ascospores were collected after each of the five crosses in 2015, 2016 and 2017. The number
358 of ascospores was largest for the FS802 × FS1006 cross in 2015 and lowest for the FS808 ×
359 FS806 cross in 2016 (Online Resource 2; Figure 9). Moreover, in 2016, the first ascospores
360 were collected later in the season (January), due to relatively the dry weather from August to
361 September 2016 (65 mm of rainfall, versus the mean of 170 mm generally observed this
362 period). A similar effect of year was also observed with the ascomycetes *Leptosphaeria*
363 *maculans* (Marie-Hélène Balesdent, INRA BIOGER, pers. comm.) and *Septoria linicola*
364 (Annette Penaud, Terres Inovia, pers. comm.) in the same experimental area.

365 For all crosses, the number of ascospores discharged increased with inoculum concentration.
366 The smallest numbers of ascospores were collected for the lowest inoculum concentrations
367 (50 or 10^2 conidia.mL^{-1}). The number of ascospores discharged at the highest inoculum level
368 (10^7 conidia.mL^{-1}) was however systematically below the maximum number. For crosses on
369 Soissons, this maximum was reached for inoculum concentrations of 5×10^4 conidia.mL^{-1}

370 (FS802 × FS1006 and FS813 × FS732) to 10^6 conidia.mL^{-1} (FS813 × FS732 and FS1022 ×
371 FS1008). It was reached for an inoculum concentration of 5 × 10^6 conidia.mL^{-1} for the cross
372 performed on Runal (3D7 × 1A5), on which disease severity was significantly lower than on
373 Soissons for inoculum levels of 5 × 10^2 to 5 × 10^6 conidia.mL^{-1} (Figure 5). This pattern was
374 even more marked for the relationship between effective disease severity (proxy for asexual
375 multiplication intensity) and the normalized number of ascospores (proxy for sexual
376 reproduction intensity; Figure 10). The quadratic model and a model integrating two density-
377 dependent constraints converged for three crosses (FS802 × FS1006, FS808 × FS806, FS1022
378 × FS1008), with the intensity of sexual reproduction increasing to a peak for disease severities
379 of 35 to 50% and then decreasing thereafter. This pattern was particularly marked for the
380 FS802 × FS1006 and FS808 × FS806 crosses. The initial ascending part of the curves
381 illustrates an Allee effect (difficulty finding mates at low pathogen densities), whereas the
382 second, descending part of the curve highlights competition for resource allocation between
383 the two modes of reproduction (fewer host resources available for sexual reproduction due
384 their previous mobilization for asexual multiplication).

385

386 # Discussion

387 In field conditions, the different layers of wheat plants, from the soil to the head, contribute
388 differently to *Z. tritici* reproduction. Mean *Z. tritici* ascospore production was maximal
389 between 25 and 35 cm above the ground. This significant impact of vertical positioning is
390 complementary to the findings of Pfender & Wootke [27], who established that the
391 pseudothecia of *Pyrenophora tritici-repentis* survived well in standing stubble and upper
392 mulch layers, but not in straw or mulch located in the soil or directly on its surface. At least
393 two antagonistic mechanisms are likely to account for the increase and subsequent decrease in

394 sexual reproduction intensity with increasing height of the canopy layer above the ground.
395 Higher positions in the canopy are associated with a lower density of lesions. The probability
396 of crosses occurring, therefore, also decreases with height. Conversely, higher positions in the
397 canopy are occupied by younger wheat tissues, on which infections are likely to be more
398 recent, with a lower probability of ascosporogenesis having had time to occur. Lower down in
399 the canopy, the wheat tissues are older and the pseudothecia are already empty or degraded.
400 These empirical observations confirmed our initial assumption of antagonistic density-
401 dependent mechanisms controlling *Z. tritici* sexual reproduction in natural conditions.

402 * * *

403 Traits relating to asexual multiplication *in planta,* such as the density and exudation capacity
404 of pycnidia, are affected by competition between the two parental isolates for resources. This
405 result is consistent with the lower level of pycnidial coverage observed after inoculation with
406 mixtures of isolates than after inoculation with single isolates [28, 29]. The competitive
407 disequilibrium between the two parental isolates may also account for the density dependence
408 of asexual development in *Z. tritici*. This finding is consistent with previous results obtained
409 in the same experimental conditions in a previous study [23], in which an interval of two to
410 three weeks between inoculation with the first parental isolate and inoculation with the second
411 was found to be detrimental to ascosporogenesis. In this case, the host tissues were likely to
412 be colonized by the first isolate, leaving fewer host resources available for the second isolate,
413 consistent with the establishment of competition during the asexual stage of the disease cycle.
414 Interactions of this type were studied by Clement et al. [8], who identified two basic response
415 patterns, consisting of an increase or decrease in reproductive fitness in multiple infections of
416 potato leaves with *P. infestans,* depending on pathogen genotype. In the case of *Z. tritici,* the
417 activation of host defenses by the less virulent or less aggressive isolate may limit the
418 subsequent development of a second more aggressive isolate at low inoculum pressure.

419 Conversely, at high inoculum pressure, the host sites for infection are likely to be saturated,
420 regardless of the aggressiveness of the isolates causing the infection. The less aggressive
421 isolate (i.e. to which the host is less susceptible) may trigger defense responses that ultimately
422 prevent the more aggressive isolate from developing to the extent that it would have if used
423 alone for inoculation. The highest inoculum pressure was so high that this mechanism was not
424 expressed.

425 * * *

426 Sexual reproduction is driven by at least two density-dependent mechanisms with opposite
427 impacts: a positive Allee effect due to mate limitation, and with a negative effect due to
428 competition with asexual multiplication for resources. We provide here the first experimental
429 evidence that sexual reproduction in a fungal foliar pathogen is driven by antagonistic
430 density-dependent mechanisms.

431 *Positive density dependence of sexual reproduction* - Higher densities of *Z. tritici* lesions on
432 leaves and stems are associated with a higher probability of encounter and a higher intensity
433 of sexual reproduction. Mate limitation has been identified as the most common mechanism
434 underlying Allee effects [4]. This experimental result, obtained at the plant scale in semi-
435 controlled conditions, is supported by the positive correlations previously established in
436 natural conditions at both the field and landscape scales: ascospore production is generally
437 greatest after the most severe epidemics [21, 22].

438 *Negative density dependence of sexual reproduction* - Strong Septoria tritici blotch attacks (i.e
439 intense asexual multiplication) limit the resources available for sexual reproduction, resulting
440 in a detrimental effect. We showed that competition between the two parental isolates during
441 asexual multiplication (accentuated, for example, by differences in aggressiveness or a time
442 interval between the two inoculations) is plausible and may lead to an imbalance between

443 mating types. However, this explanation appears to be less relevant in natural conditions,
444 given the high diversity of the pathogen population even at the leaf scale (sex ratio close to 1).
445 As illustrated by the negative impact on fruiting and sporulating capacity, competition also
446 limits the potential development of parental isolates in a similar way: host resources are
447 overexploited during the asexual phase, to the detriment of sexual reproduction.

448 The optimal equilibrium between asexual and sexual reproduction in semi-controlled
449 conditions corresponds to a disease severity of 30 to 45%. This optimum severity is lower
450 than that observed in the field on the leaves of the lower plant layers, on which severity may
451 reach very high levels (80-100%) and which senesce faster than the leaves of the upper layers.
452 Eriksen & Munk [16] reported that pseudothecia form only at disease severities exceeding
453 38%, following the coalescence of lesions, and that the highest proportion of pseudothecia
454 occurs on leaves with a disease severity of 50 to 80%. Furthermore, the model we fitted to
455 these experimental data revealed no negative density-dependent effect, perhaps because the
456 epidemiological survey stopped earlier (August) than in our own experiment conditions,
457 suggesting that the pseudothecia may not have been mature in the upper plant layers.

458 * * *

459 As a final step in this study, we developed an overall synthetic representation of the
460 relationship between the investments of *Z. tritici* in the two modes of reproduction at the host-
461 tissue scale, taking into account the ecological processes discussed above. In Figure 11, we
462 propose a theoretical relationship between asexual multiplication intensity (disease severity)
463 and sexual reproduction intensity (ability to produce offspring). The number of fruitful
464 encounters between parental isolates initially increases with increasing disease severity (1).
465 During this first stage, competition between isolates may lead to mating-type disequilibrium,
466 with an impact on sexual reproduction (2). At disease severities of more than 30-45%, a high-

467 density pathogen population becomes disadvantageous, due to competition for host resources
468 between the asexual and sexual modes of reproduction (3). This model is complementary to
469 the findings of Suffert et al. [23], formalized by the title "Fashionably late partners have more
470 fruitful encounters". We can now extend the metaphor by providing *Z. tritici* with additional
471 advice for even more fruitful encounters: "Do not live as a hermit but avoid crowded places!"
472 This view was already put forward by Kokko & Rankin [1], in their study entitled "Lonely
473 hearts or sex in the city?"
474 Is an optimal disease severity of 30-45% an appropriate ecological equilibrium determining
475 the evolutionary endpoints of selection in *Z. tritici*? This theoretical question needs to be
476 addressed in an evolutionary ecology perspective, and we can now use empirical data to try to
477 answer it. Indeed, a successful parasitic life depends on optimal exploitation of the host to
478 satisfy key functions directly involved in reproductive fitness. Theoretically, both weakly
479 aggressive isolates causing only a few small lesions, and very aggressive isolates causing
480 large numbers of large lesions, have a lower probability of transmitting a part of their genetic
481 background by sexual reproduction than isolates with intermediate levels of aggressiveness.
482 This view is complementary to the findings of Kema et al. [19], which explained the
483 maintenance of avirulent individuals in the pathogen population despite the widespread
484 deployment of resistance genes (e.g. AvrStb6 despite the extensive presence of Stb6 in the
485 wheat lines used in breeding programs worldwide [30]; Thierry Marcel, INRA BIOGER,
486 com. pers.). Sexual reproduction therefore plays a key role in both the evolution of virulence
487 and aggressiveness. We can hypothesize that sexual reproduction is less intense on very
488 susceptible wheat varieties than on less susceptible varieties, but this counter-intuitive
489 hypothesis requires testing in future experiments.
490 A previous study revealed an absence of functional trade-offs between the two modes of
491 reproduction in *Z. tritici*: no adaptive compromise was established between pathogenicity

492 (asexual multiplication on adult wheat plants) and transmission (intensity of sexual
493 reproduction on wheat debris [23]), in analyses of pathogen life traits at the individual level.
494 An overall epidemiological trade-off was, however, established between intra- and
495 interannual scales over a larger spatiotemporal scale (field and surrounding area), probably
496 driven by the consequences of sexual reproduction for local population dynamics relating to
497 selection and counter-selection [22]. According to the findings reported here, the potential
498 antagonism between the asexual and sexual modes of reproduction and its epidemiological
499 consequences appear to be more complex, due to a dependence on scale, from host tissues up
500 to agricultural landscape scale. Sexual reproduction is clearly a key process in the eco-
501 epidemiology of Septoria tritici blotch. An understanding of its determinants may open up
502 new perspectives for the management of other foliar fungal pathogens with dual modes of
503 reproduction.
504

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588 **Table 1**. List of fitness traits and yield components assessed during the asexual and sexual stages for the five *Zymoseptoria tritici* crosses on
589 adult wheat plants.

590

Year	Parental isolate	Mating type	Host cv.	Fitness traits			Yield components	
				Asexual stage		Sexual stage		
				Necrotic area	Density and exudation capacity of pycnidia	Ascospore production	GWS [1]	TKW [2]
2015	INRA09-FS0802 INRA09-FS1006	Mat1-1 Mat1-2	Soissons	×		×		
2016	INRA09-FS0813 INRA09-FS0732	Mat1-1 Mat1-2	Soissons	×	×	×	×	×
2016	INRA09-FS0808 INRA09-FS0806	Mat1-1 Mat1-2	Soissons	×	×	×	×	×
2017	INRA09-FS1022 INRA09-FS1008	Mat1-1 Mat1-2	Soissons	× [3]		×	× [3]	× [3]
2017	ST99CH-3D7 ST99CH-1A5	Mat1-1 Mat1-2	Runal	×		×	×	×

591 [1] grain weight per spike

592 [2] thousand-kernel weight

593 [3] also assessed on two sets of plants inoculated separately with each monoparental suspension

Fig.1 (a) Adult wheat plants cv. Soissons (three replicates per inoculum concentration) five weeks after their inoculation in the greenhouse with 12 biparental suspensions of *Zymoseptoria tritici* isolates (FS808 and FS806), at concentrations of 50 to 10^7 conidia.mL^{-1}. Plants are ranked from the lowest (left) to the highest concentration (right). (b-c) Sporulating area on the flag leaf, characterized by a high density of pycnidia, with high (b) and low (c) exudation capacities. (d) Trios of plants stored outdoors to induce sexual reproduction and ascosporogenesis.

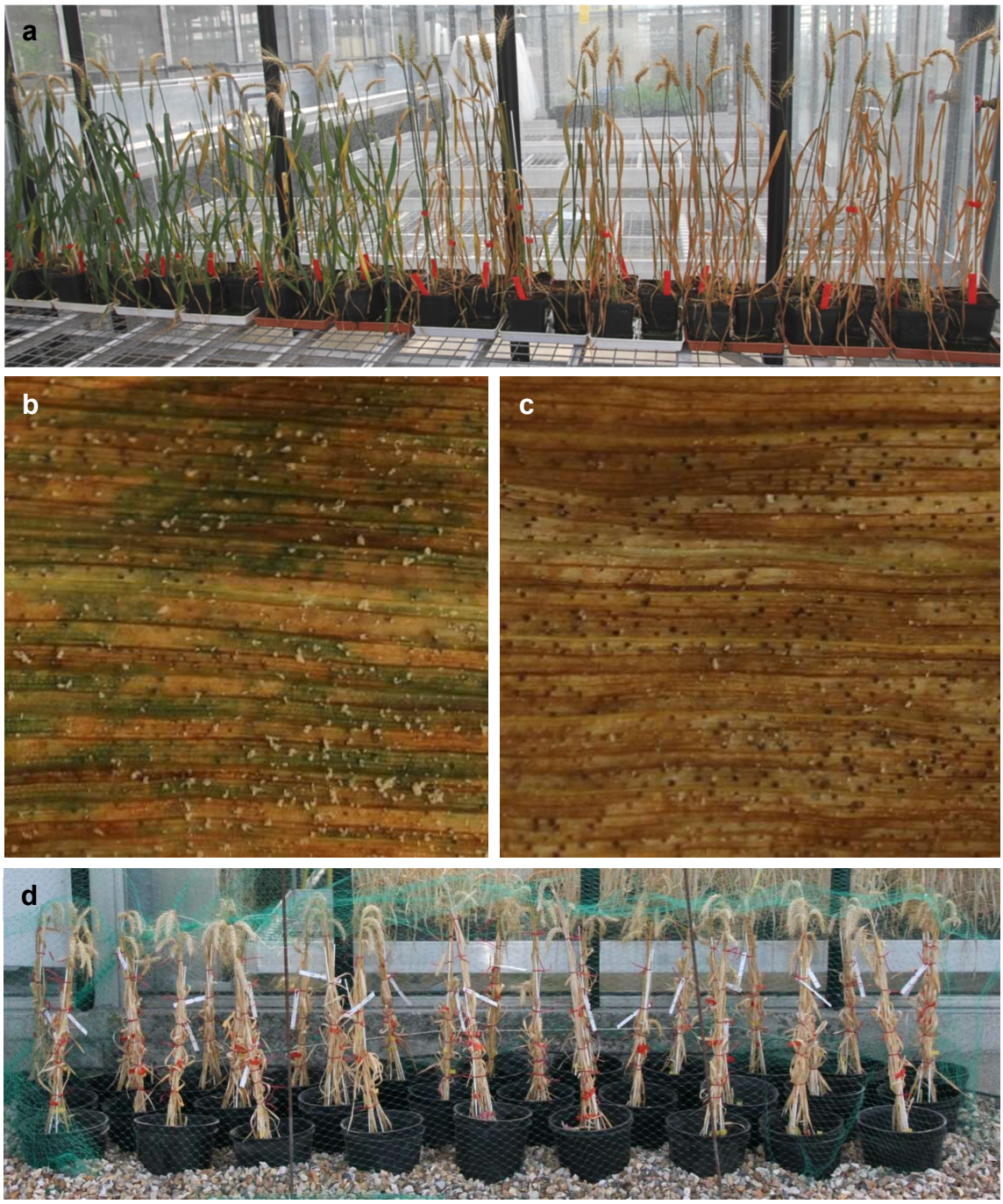
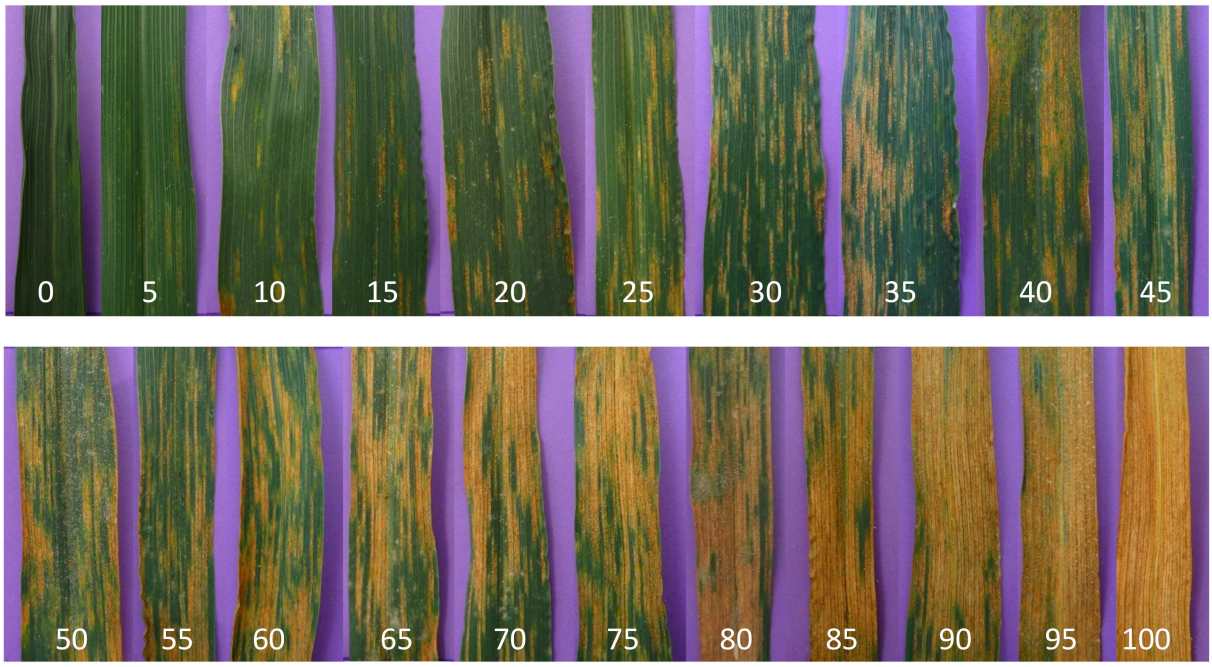


Fig.2 Visual rating scale used to quantify the intensity of *Zymoseptoria tritici* asexual multiplication (disease severity assessed as a percentage of necrotic area) on the three uppermost leaves (F1, F2 and F3) of adult wheat plants. The images show the 2-7 cm section of the flag leaves over their point of insertion on the stem.



611 **Fig.3** Relationship between the proportion of pseudothecia among the total fruiting bodies on
612 wheat leaves in field conditions and the mean severity of Septoria tritici blotch expressed as a
613 percentage of the leaf area covered by pycnidia or pseudothecia. A model (bold line)
614 integrating different density-dependent constraints (see eq. 3 in Material & Methods) was
615 fitted to some of the experimental data (◆) obtained by Eriksen et al. [16] (Table 1) in a two-
616 year field survey in Denmark. Data for leaves L8 to L11 (basal leaf layers) were excluded (×)
617 because no pseudothecia were found whatever the level of disease severity, probably due to
618 their advanced state of decomposition; data for leaves L5 to L7 (collected before mid-June),
619 L1 (flag leaf), and L2 (collected before August), were also excluded (+), because sexual
620 reproduction was probably incomplete (29-53 days between the appearance of the first
621 pycnidia and the appearance of the first pseudothecia [16]).

622

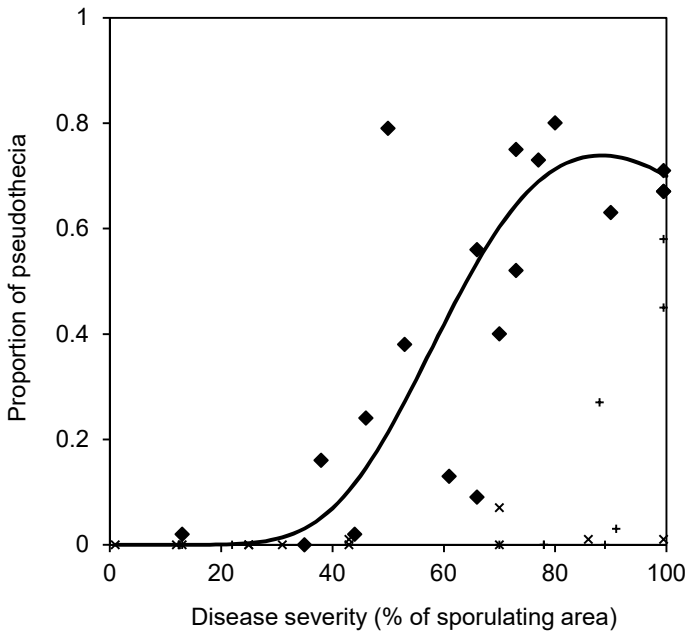


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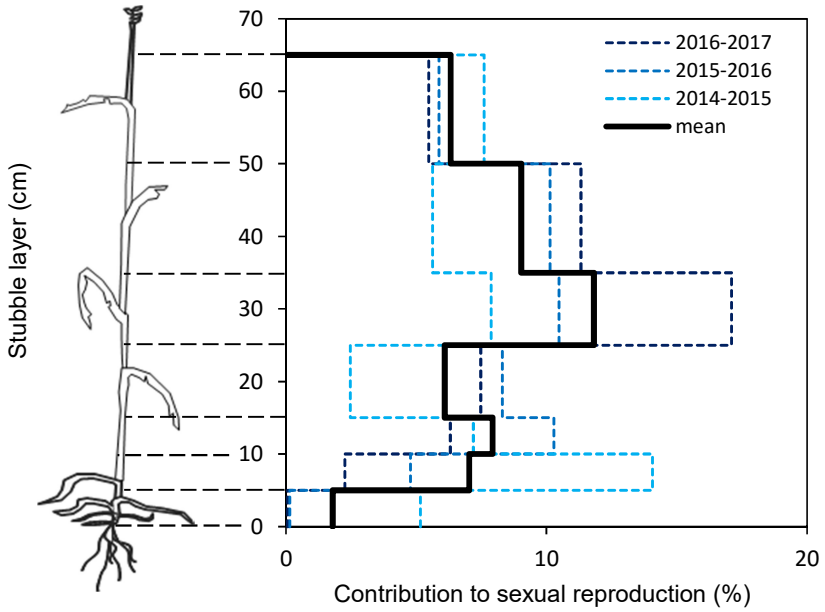
Fig.4 Impact of the vertical position of wheat tissues in standing stubble left in the field in the fall (stems and leaves, from lower to upper layers: 0-5 cm, 5-10 cm, 10-15 cm, 15-25 cm, 25-35 cm, 35-50 cm, 50-65 cm) on the intensity of *Zymoseptoria tritici* sexual reproduction expressed as the number of ascospores collected per gram of debris (logarithmic scale) on three or four dates (from September to January) over three interepidemic periods (2015-2016, 2016-2017, 2017-2018). The mean contribution of each stubble layer to sexual reproduction is expressed as a percentage of the total number of ascospores collected during each interepidemic period.



634 **Fig.5** Relationship between *Zymoseptoria tritici* inoculum concentration (total number of
635 conidia per mL) and mean intensity of asexual multiplication (disease severity assessed as a
636 % of necrotic area) estimated on the three uppermost leaves of adult wheat plants. Inoculation
637 with biparental suspensions of isolates FS802 × FS1006 (2015), FS813 × FS732 (2016),
638 FS808 × FS806 (2016), and FS1022 × FS1008 (2017) were performed on cv. Soissons;
639 Inoculations with biparental suspensions of isolates 3D7 × 1A5 (2017) were performed on cv.
640 Runal.

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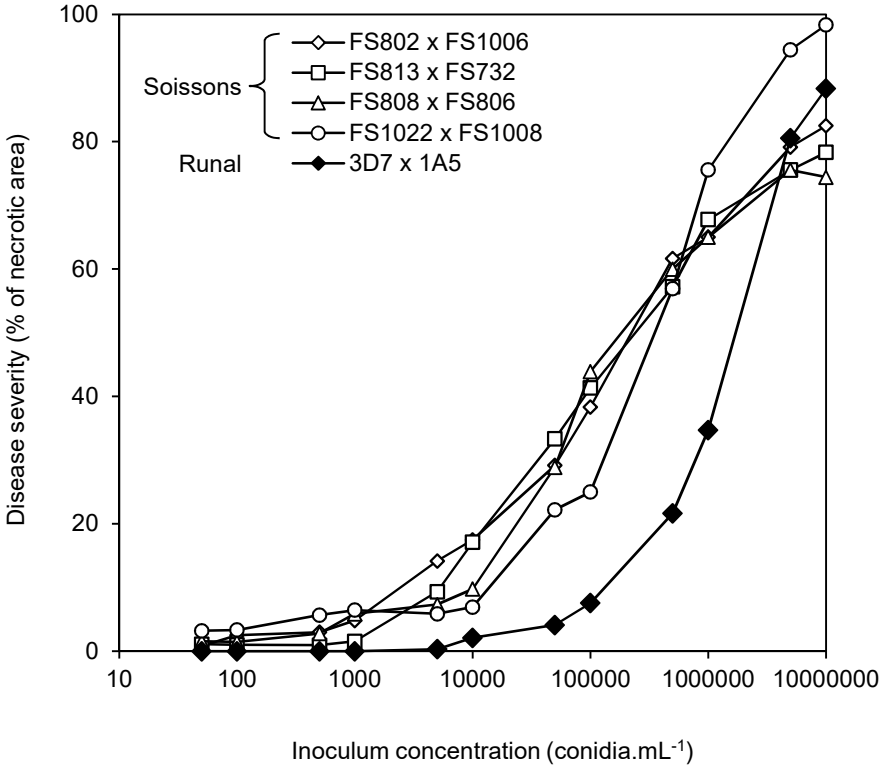

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Fig.6 Relationship between the mean intensity of asexual multiplication in *Zymoseptoria tritici* (disease severity assessed as a % of necrotic area) on the three uppermost leaves of adult wheat plants cv. Soissons (black symbols) or Runal (white symbol), and (a, c, e) total grain weight per spike (GWS) or (b, d, f) thousand-kernel weight (TKW). Disease severity was assessed five weeks after inoculation with monoparental or biparental suspensions of isolates FS806, FS808, FS732, and FS813 in 2016, and FS1022, FS1008, 1A5, and 3D7 in 2017. Vertical bars represent the standard deviation.

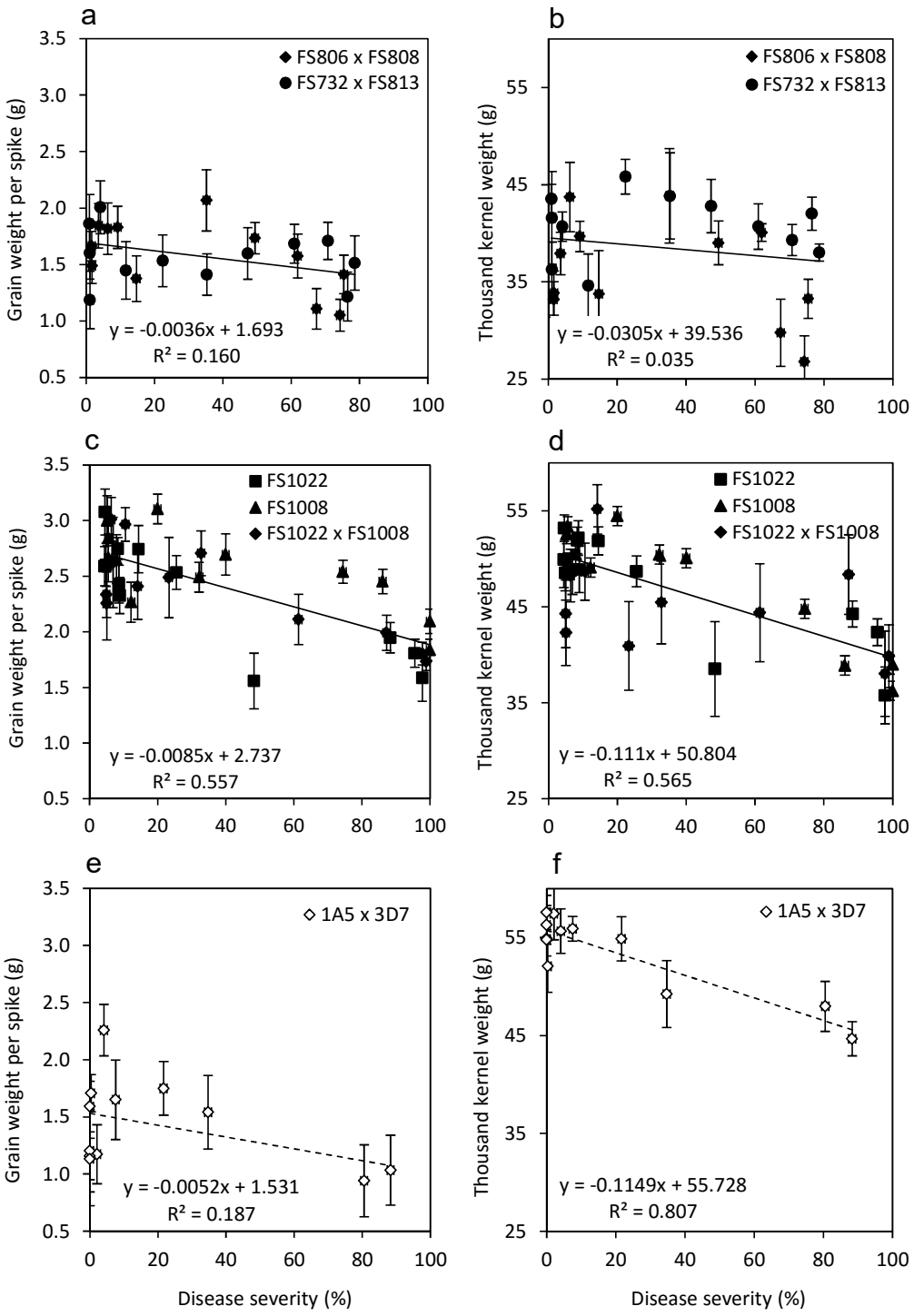


Fig.7 Relationship between the mean severity of Septoria tritici blotch (percentage of necrotic area) and (a) density of pycnidia (number of pycnidia per cm^2) or (b) percentage of pycnidia displaying exudation. Assessments were performed five weeks after the inoculation of adult wheat plants cv. Soissons with biparental suspensions of *Zymoseptoria tritici* isolates FS813 × FS732 (2016) and FS808 × FS806 (2016). Vertical bars represent the standard deviation.

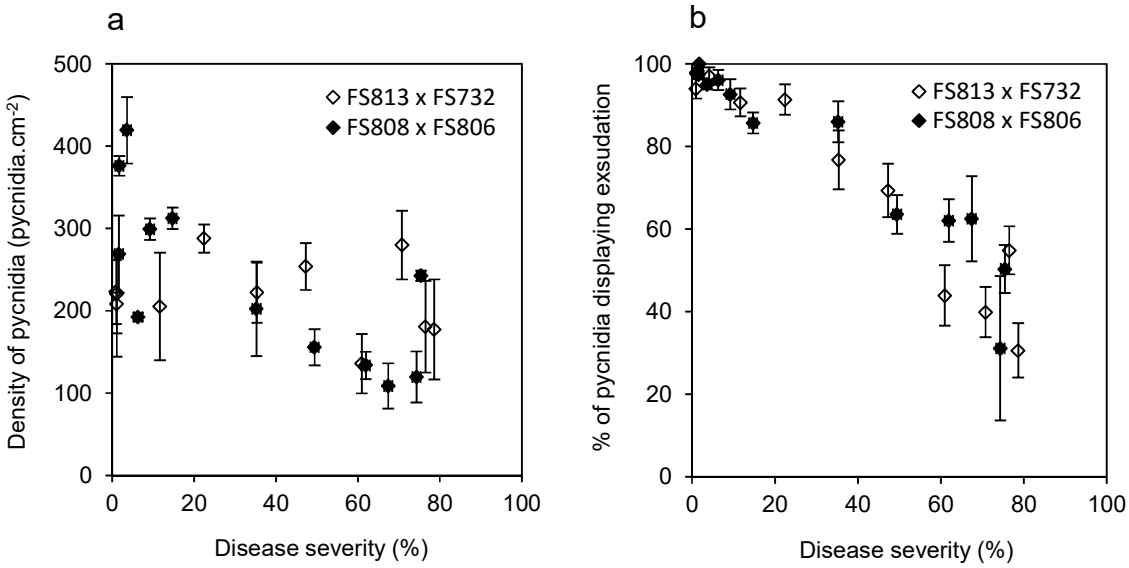


Fig.8 Relationship between inoculum concentration of *Zymoseptoria tritici* FS1008 or FS1022 isolate (total number of conidia per mL) in suspension alone or mixed with a second isolate present in the same amount and the mean severity of Septoria tritici blotch (percentage of necrotic area) estimated on the three uppermost leaves of adult wheat plants cv. Soissons. The number indicates the difference (in %) between the severity of the disease induced by FS1008 (the most aggressive isolate) inoculated alone and the severity of the disease induced by the FS1008 × FS1022 mixture (for the same number of FS1008 conidia); a negative value indicates that disease severity was lower after inoculation with a mixture.

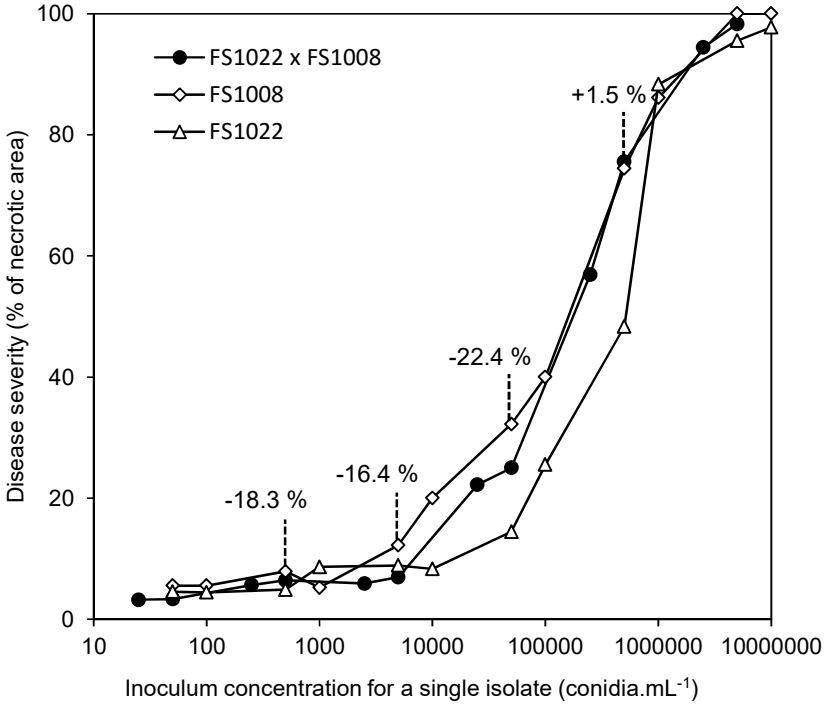


Fig.9 Impact of inoculum concentration (total number of conidia per mL) on the intensity of sexual reproduction in *Zymoseptoria tritici* (number of ascospores per g of wheat debris). Inoculation with biparental suspensions of isolates (a) FS802 × FS1006 (2015), (b) FS813 × FS732 (2016), (c) FS808 × FS806 (2016), and (d) FS1022 × FS1008 (2017) were performed on cv. Soissons, and with (e) 3D7 × 1A5 (2017) on cv. Runal. Each bar corresponds to the cumulative number of ascospores collected per gram of wheat debris over four independent discharge events (rep1 to rep4). The complete dataset is given in Table S1.

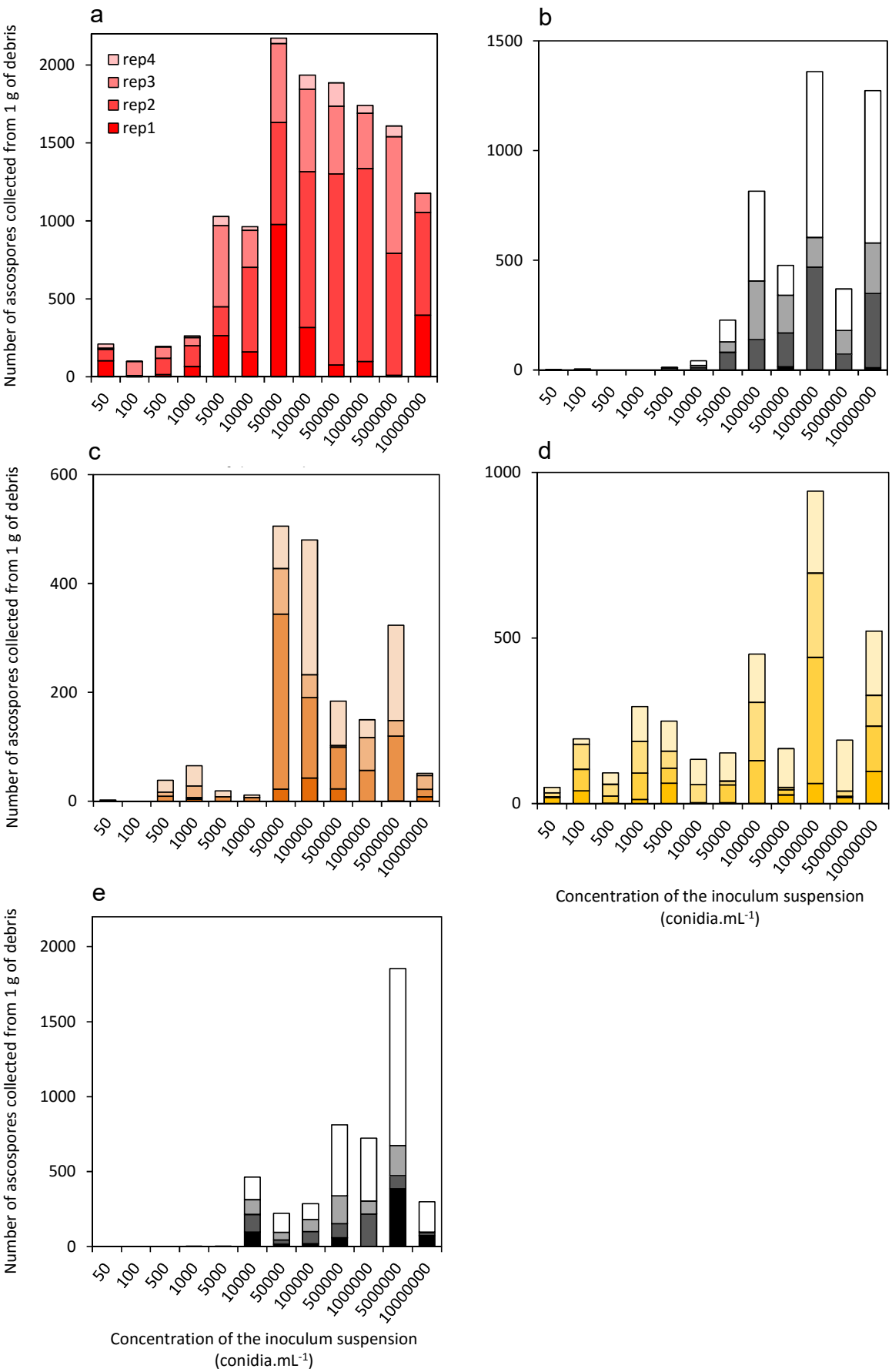
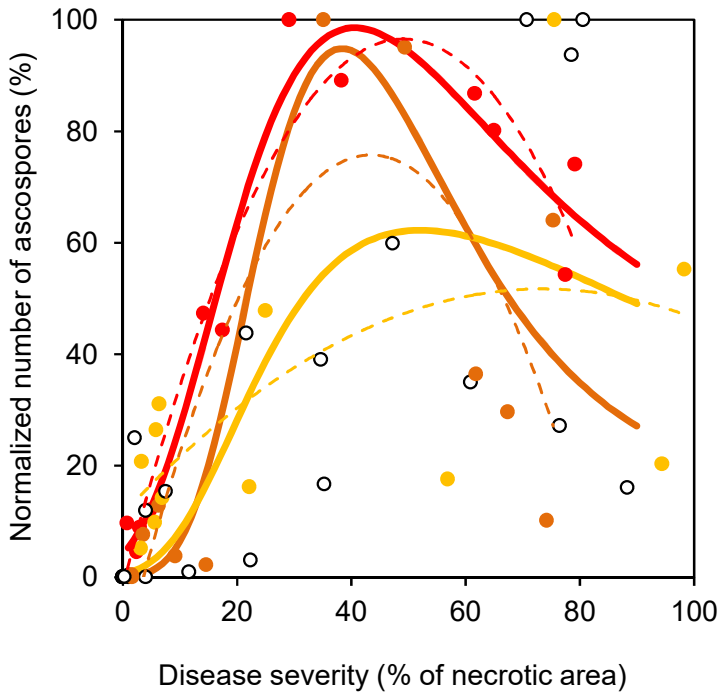
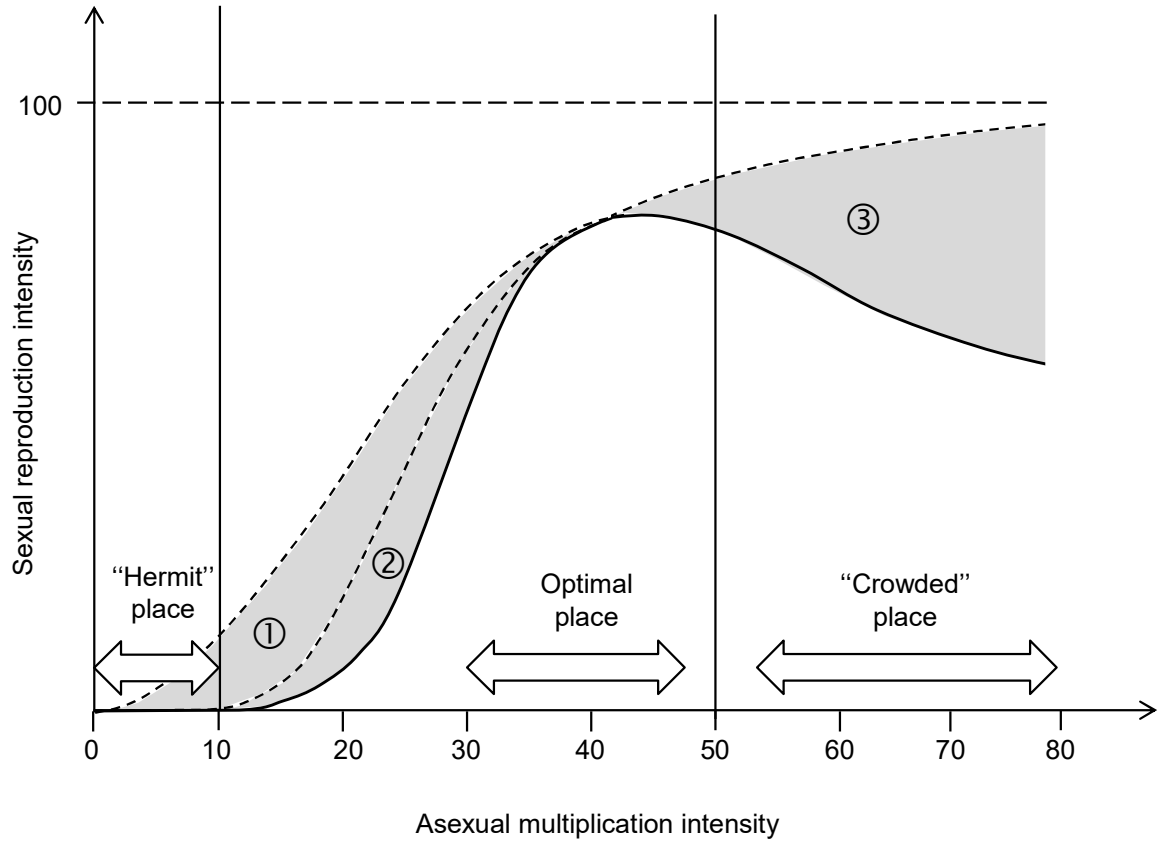

a
b
c
d
e
rep4
rep3
rep2
rep1
Number of ascospores collected from 1 g of debris
Concentration of the inoculum suspension
(conidia.mL-1)
0
500
1000
1500
2000
200
400
600
50
100
500
1000
5000
10000
50000
100000
500000
1000000
5000000
10000000

Fig.10 Empirical relationship between asexual multiplication intensity in *Zymoseptoria tritici* (disease severity assessed as a percentage of necrotic area) and sexual reproduction intensity (normalized number of ascospores for each cross expressed in %). A quadratic model (dotted lines; see eq. 2 in Material & Methods) and a model integrating two density-dependent constraints (bold lines; see eq. 3) were adjusted on the data for three crosses (FS802 × FS1006 in red, FS808 × FS806 in orange, FS1022 × FS1008 in yellow). Convergence was not obtained for the other two crosses (white points).



688 **Fig.11** Theoretical relationship between asexual multiplication intensity in *Zymoseptoria*
689 *tritici* (disease severity assessed as a percentage of necrotic area) and sexual reproduction
690 intensity (normalized number of ascospores produced per g of wheat tissue), assuming that
691 the conidia of two compatible parental isolates were deposited in equal proportions on leaves.
692 Grey areas represent the decrease in sexual reproduction intensity due to: (1) an Allee effect
693 (difficulty finding the opposite mating type at low population densities); (2) induced
694 resistance (activation of host defense mechanisms by one of the parental isolates, leading to a
695 mating-type disequilibrium); (3) competition for host resource allocation between asexual
696 multiplication and sexual reproduction. Sexual reproduction intensity is expected to be
697 optimal for disease severities of 30 to 45%.

698



699

700 **Online Resource 1**. Number of *Zymoseptoria tritici* ascospores collected per gram of wheat debris during four independent discharge events for
701 the five crosses (FS802 × FS1006, FS813 × FS732, FS808 × FS806, FS1022 × FS1008, 3D7 × 1A5) performed with 12 inoculum concentrations.

702

FS802 × FS1006 on wheat cv. Soissons (2015)							
Inoculum concentration [1]	Disease severity [2]	Mean number of ascospores collected per gram of debris					
Date	05-02-2015	11-12-2015	11-19-2015	12-03-2015	01-21-2016	-	Total
50	0.8 ± 0.3	103.2	72.5	7.9	26.6		210.2
10^2	2.5 ± 0.6	0.3	7.5	91.2	0.3		99.2
5×10^2	3.0 ± 0.5	13.5	105.6	71.5	3.7		194.3
10^3	4.8 ± 1.3	66.4	132.9	51.2	11.4		261.9
5×10^3	14.2 ± 2.2	265.0	184.6	518.7	60.9		1029.1
10^4	17.5 ± 3.5	159.2	543.2	236.3	24.6		963.3
5×10^4	29.2 ± 3.4	976.8	656.1	504.4	35.5		2172.7
10^5	38.3 ± 4.7	316.2	999.9	529.3	90.8		1936.1
5×10^5	61.7 ± 3.1	76.3	1225.2	433.8	150.0		1885.2
10^6	65.0 ± 4.6	98.7	1238.5	353.6	49.9		1740.7
5×10^6	79.2 ± 4.2	10.1	781.8	747.6	69.3		1608.8
10^7	77.5 ± 4.9	395.0	660.1	120.6	3.1		1178.7

703 [1] total number of conidia per mL
704 [2] percentage of necrotic area ± standard deviation

FS813 × FS732 on wheat cv. Soissons (2016)							
Inoculum concentration [1]	Disease severity [2]	Mean number of ascospores collected per gram of debris					
Date	05-06-2016	11-17-2016	01-19-2017	02-15-2017	03-01-2017	03-15-2017	Total
50	1.1 ± 0.4	0.0	0.0	2.0	0.0	0.0	2.0
10^2	1.0 ± 0.5	0.0	0.0	0.0	4.5	0.0	4.5
5×10^2	0.9 ± 0.3	0.0	0.0	0.0	0.0	0.0	0.0
10^3	1.6 ± 0.4	0.0	0.0	0.0	0.0	0.0	0.0
5×10^3	9.3 ± 2.0	0.0	0.0	9.6	0.2	2.9	12.7
10^4	17.1 ± 2.6	0.0	0.0	10.6	10.0	21.2	41.8
5×10^4	33.3 ± 2.1	0.0	0.0	80.6	47.8	98.5	227.0
10^5	41.3 ± 3.8	0.0	0.0	138.8	266.9	409.0	814.7
5×10^5	57.2 ± 3.7	0.0	14.4	154.7	172.4	134.8	476.3
10^6	67.8 ± 1.7	0.0	0.7	467.9	135.4	755.9	1360.0
5×10^6	75.6 ± 1.5	0.0	0.0	72.2	108.1	189.6	369.9
10^7	78.3 ± 2.4	0.0	10.6	338.2	230.3	694.5	1273.7

705 [1] total number of conidia per mL

706 [2] percentage of necrotic area ± standard deviation

FS808 × FS806 on wheat cv. Soissons (2016)							
Inoculum concentration [1]	Disease severity [2]	Mean number of ascospores collected per gram of debris					
Date	05-06-2016	11-17-2016	01-19-2017	02-15-2017	03-01-2017	03-15-2017	Total
50	1.6 ± 0.3	0.0	0.0	0.2	2.1	0.0	2.3
10^2	1.4 ± 0.3	0.0	0.0	0.0	0.0	0.0	0.0
5×10^2	2.8 ± 0.7	0.0	0.0	9.0	7.6	21.9	38.5
10^3	5.9 ± 1.5	0.0	4.1	2.6	21.5	36.9	65.0
5×10^3	7.3 ± 1.5	0.0	0.0	8.1	0.0	11.1	19.2
10^4	9.8 ± 2.6	0.0	0.0	6.9	0.0	4.2	11.2
5×10^4	28.9 ± 2.6	0.0	22.2	321.2	83.9	78.0	505.3
10^5	43.9 ± 3.1	0.0	42.6	147.7	42.3	247.6	480.2
5×10^5	60.0 ± 2.9	0.0	22.7	76.5	3.1	81.8	184.0
10^6	65.0 ± 3.3	0.0	0.0	56.7	60.2	32.8	149.7
5×10^6	75.6 ± 1.6	0.0	0.6	119.2	28.3	175.4	323.5
10^7	74.4 ± 2.2	0.0	8.2	13.6	25.5	4.0	51.2

707 [1] total number of conidia per mL

708 [2] percentage of necrotic area ± standard deviation

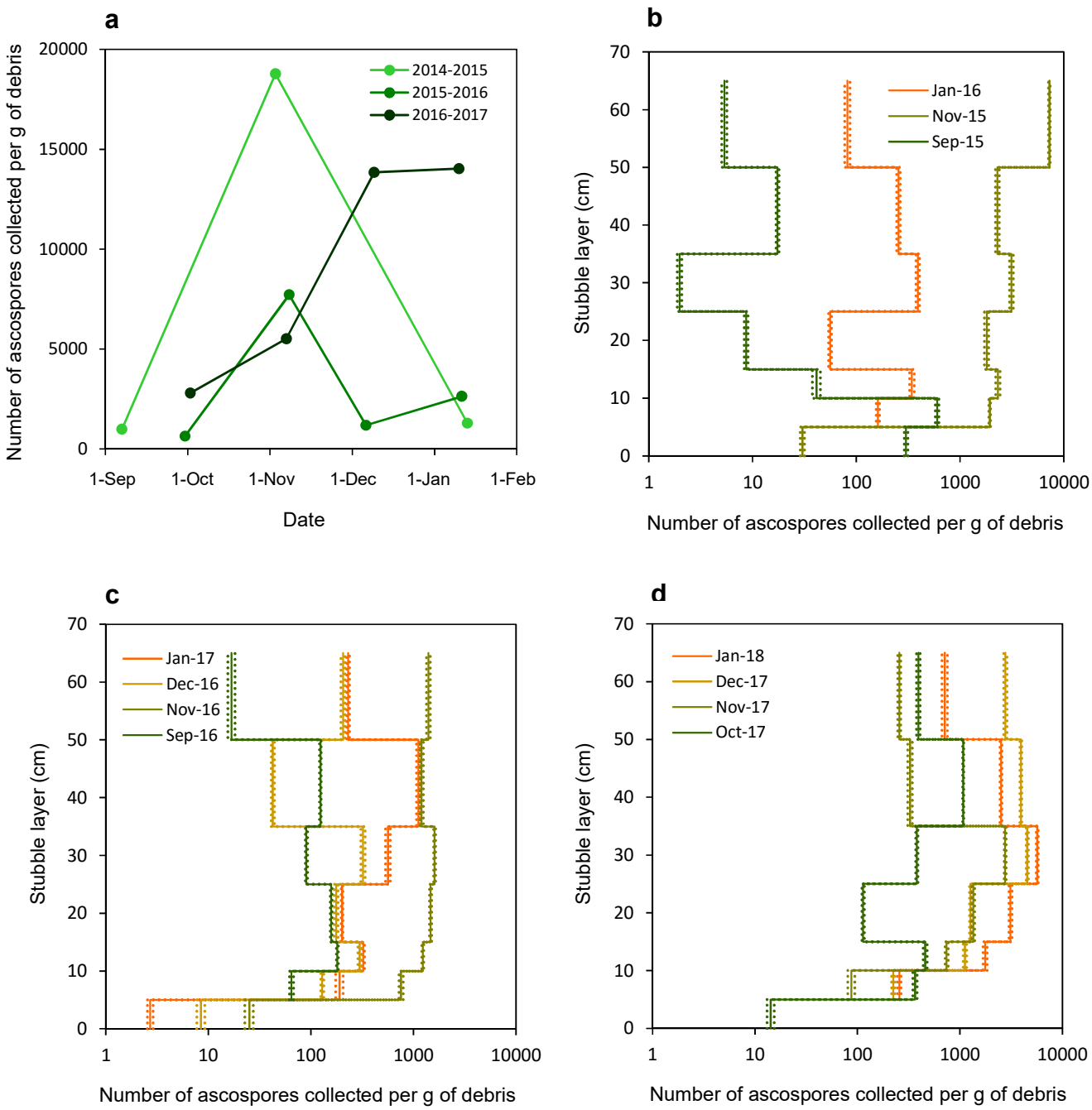
FS1022 × FS1008 on wheat cv. Soissons (2017)							
Inoculum concentration [1]	Disease severity [2]	Mean number of ascospores collected per gram of debris					
Date	05-27-2017	11-09-2017	12-07-2017	01-04-2017	01-01-2018	01-24-2018	Total
50	3.2 ± 0.2	0.0	18.3	1.7	12.7	16.1	48.8
10^2	3.3 ± 0.2	0.0	38.8	64.6	75.6	16.8	195.7
5×10^2	5.7 ± 0.5	0.0	0.9	22.0	35.0	34.8	92.8
10^3	6.4 ± 0.7	0.0	12.5	79.2	96.6	104.8	293.1
5×10^3	5.9 ± 0.5	0.0	61.2	45.5	51.4	90.9	249.0
10^4	6.9 ± 1.2	0.0	2.2	0.4	54.2	77.0	133.8
5×10^4	22.2 ± 2.2	0.0	2.8	53.6	11.6	84.9	152.8
10^5	25.0 ± 2.4	0.0	0.1	129.5	176.1	145.6	451.3
5×10^5	56.9 ± 3.6	0.0	26.2	15.5	7.3	117.0	166.0
10^6	75.6 ± 5.0	0.0	60.6	381.1	254.4	247.5	943.6
5×10^6	94.4 ± 0.4	0.0	18.4	3.2	16.3	153.5	191.4
10^7	98.3	0.0	97.3	136.4	92.9	194.3	521.0

709 [1] total number of conidia per mL

710 [2] percentage of necrotic area ± standard deviation

3D7 × 1A5 on wheat cv. Runal (2017)							
Inoculum concentration [1]	Disease severity [2]	Number of ascospores collected per gram of debris					
Date	05-27-2017	11-09-2017	12-07-2017	01-04-2017	01-01-2018	01-24-2018	Total
50	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10^2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5×10^2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10^3	0.0	0.0	0.0	0.0	0.0	2.2	2.2
5×10^3	0.3 ± 0.1	0.0	0.0	1.4	0.0	0.2	1.6
10^4	2.1 ± 0.3	0.0	97.6	117.2	98.0	150.2	463.0
5×10^4	4.1 ± 0.2	0.0	15.5	29.5	51.4	124.8	221.2
10^5	7.6 ± 0.3	0.0	19.2	81.0	79.5	106.2	286.0
5×10^5	21.7 ± 0.7	0.0	58.4	95.0	185.0	473.8	812.1
10^6	34.7 ± 2.8	0.0	0.0	217.4	85.8	420.5	723.7
5×10^6	80.6 ± 0.4	0.0	386.2	87.5	200.2	1181.4	1855.3
10^7	88.3 ± 1.8	0.0	73.2	19.6	4.2	201.5	298.5

[1] total number of conidia per mL

[2] percentage of necrotic area ± standard deviation

Online Resource 2. (a) Spatiotemporal dynamics of *Zymoseptoria tritici* sexual reproduction in standing stubble left in the field during the fall, over three interepidemic periods (2015-2016, 2016-2017, 2017-2018). (b, c, d) Impact of the vertical position of wheat tissues (stems and leaves, from lower to upper layers: 0-5 cm, 5-10 cm, 10-15 cm, 15-25 cm, 25-35 cm, 35-50 cm, 50-65 cm) on the number of ascospores collected per gram of debris (logarithmic scale) on three or four sampling dates (from September to January) over the three interepidemic periods. The dotted lines indicate the standard error.