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. The highest number of ascospores discharged was reached at intermediate inoculum concentrations (from 5.10^4 conidia.mL^{-1} to 10^6 conidia.mL^{-1}). 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, with a maximum between 25 and 35 cm above the ground. An optimal lesion density (disease severity of 30 to 45%) 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. A conceptual model 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

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

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

82 * * *

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

93 effects due to mate limitation have been reported to affect the dynamics of some fungal
94 pathogens at large spatial scales, such as *Hymenoscyphus fraxineus*, the causal agent of ash
95 dieback [9], and *Mycosphaerella fijiensis*, the causal agent of black Sigatoka disease in
96 banana plants [10]. Sexual spores for dispersal are produced only when compatible mating
97 types come into direct contact. However, mate limitation at the plant scale was not confirmed
98 by experimental studies, probably because there are still many gaps in our knowledge
99 concerning the ways in which sexual reproduction occurs in host tissues and its determinants.
100 However, this effect forms the theoretical basis of demogenetic models combining sexual and
101 asexual reproduction, in which the impact of mate-finding limits the speed of pathogen spread
102 [11, 12].

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

112 * * *

113 Sexual reproduction in *Zymoseptoria tritici*, the causal agent of Septoria leaf blotch of wheat,
114 requires a physical encounter between two compatible strains (Mat1-1 and Mat1-2; [14, 15])
115 and generally occurs on senescent tissues, with pseudothecia appearing 46 to 76 days after
116 the initial infection in field conditions [16, 17]. It determines the amount of primary inoculum

(wind-dispersed ascospores) available for the initiation of subsequent epidemics and plays an essential role in maintaining the diversity of local pathogen populations and determining their rate of adaptation to selection pressures, such as those exerted by resistant hosts or fungicide treatments.

Avirulent *Z. tritici* strains were recently shown to cross with virulent strains on wheat cultivars carrying the corresponding resistance gene [18]. This key finding calls into question current views about the determinants of sexual reproduction, including, in particular, the common belief that individuals unable to infect wheat tissues cannot reproduce sexually. Another recent study has cast doubt on this point given the extent of epiphytic growth seen in some strains, suggesting finally that meeting would be possible without penetration [19]. The proportion of crossing events resulting from the "exclusive paternal parenthood" model [18] was however not quantified at the leaf scale in the natural context of highly diverse populations. In the absence of other factual elements it should be considered that the vast majority of crossing events occur when leaf lesions caused by two compatible strains either coalesce or are located very close together. In practice, it remains unclear how close together infections must be for effective mating to occur. Metcalfe [20] found internal hyphae spanning mean distances of no more than 11 mm in a susceptible cultivar just before the production of pycnidia, suggesting that infection foci located more than 20 mm apart may be isolated. The number of offspring (i.e. the number of ascospores) should be proportional to the number of crossing events, and correlated with disease severity, which is a good proxy for lesion density. This has been shown at field scale: more severe 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 its 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 subsequent sexual reproduction, during the epidemic period and then during the interepidemic period. We reanalyzed a dataset obtained in Danish field conditions by Eriksen & Munk [16], together with the data obtained in our three-year experiment in France.

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

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

Fungal material

Five crosses were performed, with eight *Z. tritici* isolates (FS0802 × FS1006, FS0813 × FS0732, FS0808 × FS0806, FS1022 × FS1008 [25]) collected in 2010 from wheat cv. Soissons in France, and two isolates (3D7 × 1A5 [26]) collected in 1999 from wheat cv. Galaxie and Lono (Table 1). The compatibility of the five pairs of isolates was determined by PCR amplification of the two mating-type idiomorphs [15]. The effective ability of the eight French isolates to reproduce sexually on adult plants was first checked in semicontrolled conditions [23]. Subcultures of each isolate stored at -80°C were grown for five days in Petri dishes containing PDA (potato dextrose agar, 39 g L^{-1}) at 18°C in the dark. For each isolate, an initial conidial suspension was prepared with a Malassez counting chamber. Serial

188 dilutions (1:5) of each initial monoparental suspension were prepared, from 10^7 conidia mL^{-1}
189 to 50 conidia mL^{-1}. Twelve biparental suspensions were then prepared by mixing 25 mL each
190 of the appropriate monoparental suspensions and adding two drops of surfactant (Tween 20;
191 Sigma, France).

192

193 **Plant material**

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

210

211 **Inoculation procedure**

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

224

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

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

234

235 **Promotion of sexual reproduction and ascosporogenesis**

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

Assessment of the intensity of sexual reproduction

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

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

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 and visualization

Statistical analysis - ANOVA or Kruskal-Wallis test when relevant and post hoc multiple comparisons - were performed using the R software.

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

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

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

An alternative equation integrating two types of density-dependent constraints (eq. 3) was also 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.

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

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

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

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

Both equations 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

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

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

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

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

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

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

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

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

Disease severity reached after inoculation with an isolate inoculated alone (FS1008) was compared to the disease severity reached after inoculation with a biparental suspension (FS1008 × FS1022) containing the same number conidia of the previous isolate (FS1008). Each of the five groups of plants that were compared was thus exposed to a single or double

348 *Z. tritici* inoculum pressure, but to the same amount of FS1008 conidia. The comparison
349 highlighted competitive disequilibrium between the two parental isolates (Figure 8). This
350 finding provides a second line of evidence that the asexual development of *Z. tritici* is driven
351 by density-dependent processes. Soissons appeared to be more susceptible to FS1008 than to
352 FS1022, regardless of the concentration of the inoculum: in practice, FS1008 was more
353 aggressive than FS1022 (e.g. disease severity with FS1008 was twice that with FS1022 for
354 inoculum concentrations of 5.10^3 to 5.10^5 conidia.mL^{-1}). From inoculum concentrations of
355 5.10^3 to 5.10^4 conidia.mL^{-1}, FS1008 induced a less severe attack if the other, less aggressive
356 isolate (FS1022) was present at the same concentration. This difference was significant
357 provided that the number of FS1022 conidia was the same. It disappeared at the highest
358 inoculum concentrations (> 10^5 conidia.mL^{-1}, corresponding to a disease severity of 35-40%).
359 Furthermore, the results for the mixture are actually very close to what would be seen with the
360 same inoculum pressure (10^4 and 10^5 conidia.mL^{-1}) with only the less aggressive isolate
361 (FS1022).

362

363 **Competition with asexual multiplication for resource allocation affects sexual**
364 **reproduction in *Z. tritici***

365 Ascospores were collected after each of the five crosses in 2015, 2016 and 2017. The number
366 of ascospores was largest for the FS802 × FS1006 cross in 2015 and lowest for the FS808 ×
367 FS806 cross in 2016 (Online Resource 2; Figure 9). Moreover, in 2016, the first ascospores
368 were collected later in the season (January), due to relatively the dry weather from August to
369 September 2016 (65 mm of rainfall, versus the mean of 170 mm generally observed this
370 period). A similar effect of year was also observed with the ascomycetes *Leptosphaeria*

371 *maculans* (Marie-Hélène Balesdent, INRA BIOGER, pers. comm.) and *Septoria linicola*
372 (Annette Penaud, Terres Inovia, pers. comm.) in the same experimental area.

373 For all crosses, the number of ascospores discharged increased with inoculum concentration.
374 The smallest numbers of ascospores were collected for the lowest inoculum concentrations
375 (50 or 10^2 conidia.mL^{-1}). The number of ascospores discharged at the highest inoculum level
376 (10^7 conidia.mL^{-1}) was however systematically below the maximum number. For crosses on
377 Soissons, this maximum was reached for inoculum concentrations of 5×10^4 conidia.mL^{-1}
378 (FS802 × FS1006 and FS813 × FS732) to 10^6 conidia.mL^{-1} (FS813 × FS732 and FS1022 ×
379 FS1008). It was reached for an inoculum concentration of 5×10^6 conidia.mL^{-1} for the cross
380 performed on Runal (3D7 × 1A5), on which disease severity was significantly lower than on
381 Soissons for inoculum levels of 5×10^2 to 5×10^6 conidia.mL^{-1} (Figure 5). This pattern was
382 even more marked for the relationship between disease severity (proxy for asexual
383 multiplication intensity) and the normalized number of ascospores (proxy for sexual
384 reproduction intensity; Figure 10). The quadratic model and a model integrating two density-
385 dependent constraints converged for three crosses (FS802 × FS1006, FS808 × FS806, FS1022
386 × FS1008), with the intensity of sexual reproduction increasing to a peak for disease severities
387 of 35 to 50% and then decreasing thereafter. This pattern was particularly marked for the
388 FS802 × FS1006 and FS808 × FS806 crosses. The initial ascending part of the curves
389 illustrates an Allee effect (difficulty finding mates at low pathogen densities), whereas the
390 second, descending part of the curve highlights competition for resource allocation between
391 the two modes of reproduction (fewer host resources available for sexual reproduction due
392 their previous mobilization for asexual multiplication).

393

394 # Discussion

In field conditions, the different layers of wheat plants, from the soil to the head, contribute differently to *Z. tritici* reproduction. We showed that mean *Z. tritici* ascospore production was maximal between 25 and 35 cm above the ground. This significant impact of vertical positioning is complementary to the findings of Pfender & Wootke [27], who established that the pseudothecia of *Pyrenophora tritici-repentis* survived well in standing stubble and upper mulch layers, but not in straw or mulch located in the soil or directly on its surface. At least two antagonistic mechanisms are likely to account for the increase and subsequent decrease in sexual reproduction intensity with increasing height of the canopy layer above the ground. Higher positions in the canopy are associated with a lower density of lesions. The probability of crosses occurring, therefore, also decreases with height. Furthermore, higher positions in the canopy are occupied by younger wheat tissues, on which infections are likely to be more recent, with a lower probability of ascosporogenesis having had time to occur. Lower down in the canopy, the wheat tissues are older and the pseudothecia are already empty or degraded. These empirical observations thus complete the results obtained by reanalyzing the dataset of Eriksen & Munk [16].

* * *

Traits relating to asexual multiplication *in planta*, such as the density and exudation capacity of pycnidia, are affected by competition between the two parental isolates for resources. This result is consistent with the lower level of pycnidial coverage observed after inoculation with mixtures of isolates than after inoculation with single isolates [28, 29]. The competitive disequilibrium between the two parental isolates may also account for the density dependence of asexual development in *Z. tritici*. This finding is consistent with previous results obtained in the same experimental conditions in a previous study [23], in which an interval of two to three weeks between inoculation with the first parental isolate and inoculation with the second was found to be detrimental to ascosporogenesis. In this case, the host tissues were likely to

420 be colonized by the first isolate, leaving fewer host resources available for the second isolate,
421 consistent with the establishment of competition during the asexual stage of the disease cycle.
422 Interactions of this type were studied by Clement et al. [8], who identified two basic response
423 patterns, consisting of an increase or decrease in reproductive fitness in multiple infections of
424 potato leaves with *P. infestans*, depending on pathogen genotype. In the case of *Z. tritici*, the
425 activation of host defenses by the less virulent or less aggressive isolate may limit the
426 subsequent development of a second more aggressive isolate at low inoculum pressure.
427 Conversely, at high inoculum pressure, the host sites for infection are likely to be saturated,
428 regardless of the aggressiveness of the isolates causing the infection. The less aggressive
429 isolate (i.e. to which the host is less susceptible) may trigger defense responses that ultimately
430 prevent the more aggressive isolate from developing to the extent that it would have if used
431 alone for inoculation. The highest inoculum pressure was so high that this mechanism was not
432 expressed.

433 Cirrhi produced by different isolates may contain different densities of pycnidiospores or may
434 be produced later or earlier than five weeks after inoculation, and thus could bias the analysis.
435 These differences between isolates may also be affected by environmental factors. Such
436 aspects are beyond the scope of the current work but should be taken into account more
437 accurately in further studies. Moreover, other reasons than competition for resources sensu
438 stricto may explain the negative relationships between mean disease severity and the density
439 of pycnidia or cirrhi within lesions. The presence of necrotic leaf tissue and the presence of
440 pycnidia and cirrhi are the result of different developmental processes of the fungus and of
441 different stages of the plant-fungus interaction [30]. Therefore, we can imagine a case where
442 a proliferation of necrotic tissue does not lead to a proliferation of pycnidia due to the
443 triggering of defence responses or to fungal auto-inhibition.

444 * * *

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

450 *Positive density dependence of sexual reproduction* - Higher densities of *Z. tritici* lesions on
451 leaves and stems are associated with a higher probability of encounter and a higher intensity
452 of sexual reproduction. Mate limitation has been identified as the most common mechanism
453 underlying Allee effects [4]. This experimental result is supported by the positive correlations
454 previously established in natural conditions at both the field and landscape scales: ascospore
455 production is generally greatest after the most severe epidemics [21, 22].

456 *Negative density dependence of sexual reproduction* - Strong Septoria tritici blotch attacks (i.e
457 intense asexual multiplication) limit the resources available for sexual reproduction, resulting
458 in a detrimental effect. We showed that competition between the two parental isolates during
459 asexual multiplication is plausible and may lead to an imbalance between mating types.
460 However, this explanation appears to be less relevant in natural conditions, given the high
461 diversity of the pathogen population even at the leaf scale (sex ratio close to 1). As illustrated
462 by the negative impact on fruiting and sporulating capacity, competition also limits the
463 potential development of parental isolates in a similar way: host resources are overexploited
464 during the asexual phase, to the detriment of sexual reproduction.

465 The optimal equilibrium between asexual and sexual reproduction in semi-controlled
466 conditions corresponds to a disease severity of 30 to 45%. This optimum severity is lower
467 than that observed in the field on the leaves of the lower plant layers, on which severity may
468 reach very high levels (80-100%) and which senesce faster than the leaves of the upper layers.

Eriksen & Munk [16] reported that pseudothecia form only at disease severities exceeding 38%, following the coalescence of lesions, and that the highest proportion of pseudothecia occurs on leaves with a disease severity of 50 to 80%. Furthermore, the equation we fitted to these experimental data revealed no negative density-dependent effect, perhaps because the epidemiological survey stopped earlier (August) than in our own experiment conditions, suggesting that the pseudothecia may not have been mature in the upper plant layers.

* * *

As a final step in this study, we developed an overall synthetic representation of the relationship between the investments of *Z. tritici* in the two modes of reproduction at the host-tissue scale, taking into account the ecological processes discussed above. In Figure 11, we propose a theoretical relationship between asexual multiplication intensity (disease severity) and sexual reproduction intensity (ability to produce offspring). The number of fruitful encounters between parental isolates initially increases with increasing disease severity (1). During this first stage, competition between isolates may lead to mating-type disequilibrium, with an impact on sexual reproduction (2). At disease severities of more than 30-45%, a high-density pathogen population becomes disadvantageous, due to competition for host resources between the asexual and sexual modes of reproduction (3). This conceptual model is complementary to the findings of Suffert et al. [23], formalized by the title “Fashionably late partners have more fruitful encounters”. We can now extend the metaphor by providing *Z. tritici* with additional advice for even more fruitful encounters: “Do not live as a hermit but avoid crowded places!” This view was already put forward by Kokko & Rankin [1], in their study entitled “Lonely hearts or sex in the city?”

Is an optimal disease severity of 30-45% an appropriate ecological equilibrium determining the evolutionary endpoints of selection in *Z. tritici*? This theoretical question needs to be addressed in an evolutionary ecology perspective, and we can now use empirical data to try to

494 answer it. Indeed, a successful parasitic life depends on optimal exploitation of the host to
495 satisfy key functions directly involved in reproductive fitness. Theoretically, both weakly
496 aggressive isolates causing only a few small lesions, and very aggressive isolates causing
497 large numbers of large lesions, have a lower probability of transmitting a part of their genetic
498 background by sexual reproduction than isolates with intermediate levels of aggressiveness.
499 This view is complementary to the findings of Kema et al. [19], which explained the
500 maintenance of avirulent individuals in the pathogen population despite the widespread
501 deployment of resistance genes (e.g. AvrStb6 despite the extensive presence of Stb6 in the
502 wheat lines used in breeding programs worldwide [31]; Thierry Marcel, INRA BIOGER,
503 com. pers.). Sexual reproduction therefore plays a key role in both the evolution of virulence
504 and aggressiveness.
505 A previous study [23] revealed an absence of functional trade-offs between the two modes of
506 reproduction in *Z. tritici*: no adaptive compromise was established between pathogenicity and
507 transmission in analyses of pathogen life traits at the individual level. An overall
508 epidemiological trade-off was, however, established between intra- and interannual scales
509 over a larger spatiotemporal scale (field and surrounding area), probably driven by the
510 consequences of sexual reproduction for local population dynamics relating to selection and
511 counter-selection [22]. However it is not clear that the optimal equilibrium between sexual
512 and asexual reproduction corresponds to the maximal intensity of sexual reproduction, as one
513 could think; asexual reproduction may be for instance more beneficial to overall strain fitness
514 in the early stages of an epidemic, where inoculum (estimated for instance by the number of
515 infection units available per m^2 of crop) is more limited than during the spring period [32].
516 According to the findings reported here, the potential antagonism between the asexual and
517 sexual modes of reproduction and its epidemiological consequences appear to be more
518 complex, due to a dependence on scale, from host tissues up to agricultural landscape scale.

519 Sexual reproduction is clearly a key process in the eco-epidemiology of Septoria tritici blotch.
520 An understanding of its determinants may open up new perspectives for the management of
521 other foliar fungal pathogens with dual modes of reproduction.
522

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Table

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

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	×		×	×	×

[1] grain weight per spike

[2] thousand-kernel weight

[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.

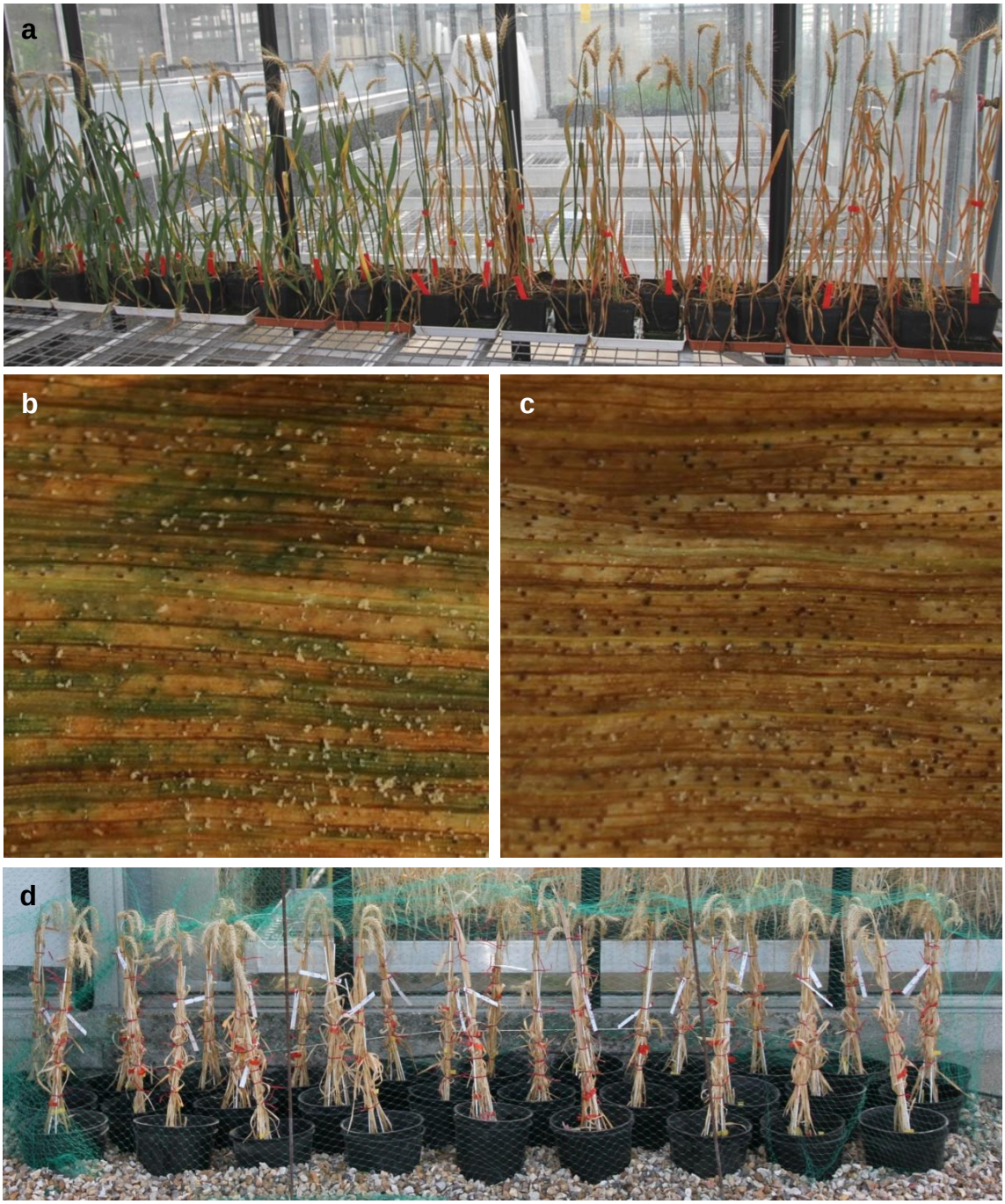


Fig.2 Visual rating scale used to quantify the intensity of *Zymoseptoria tritici* asexual multiplication (disease severity assessed as a percentage of necrotic area) on the three uppermost leaves (F1, F2 and F3) of adult wheat plants. The images show the 2-7 cm section of the flag leaves over their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

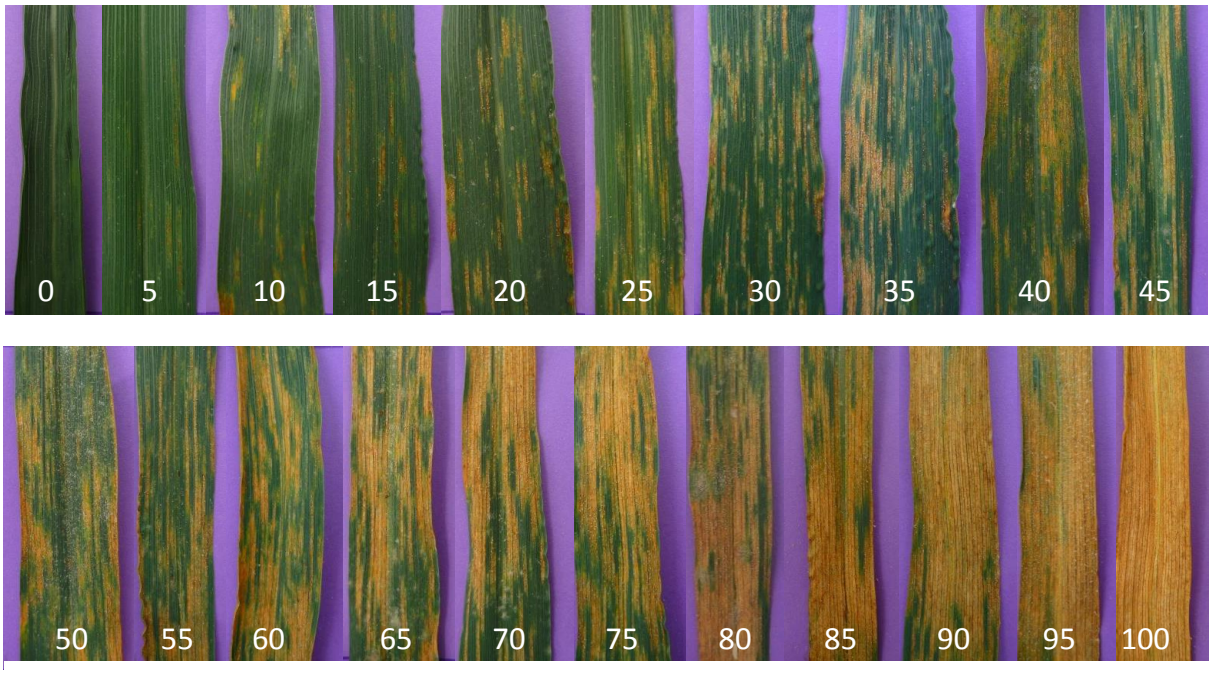


Fig.3 Relationship between the proportion of pseudothecia among the total fruiting bodies on wheat leaves in field conditions and the mean severity of Septoria tritici blotch expressed as a percentage of the leaf area covered by pycnidia or pseudothecia. An equation integrating different density-dependent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

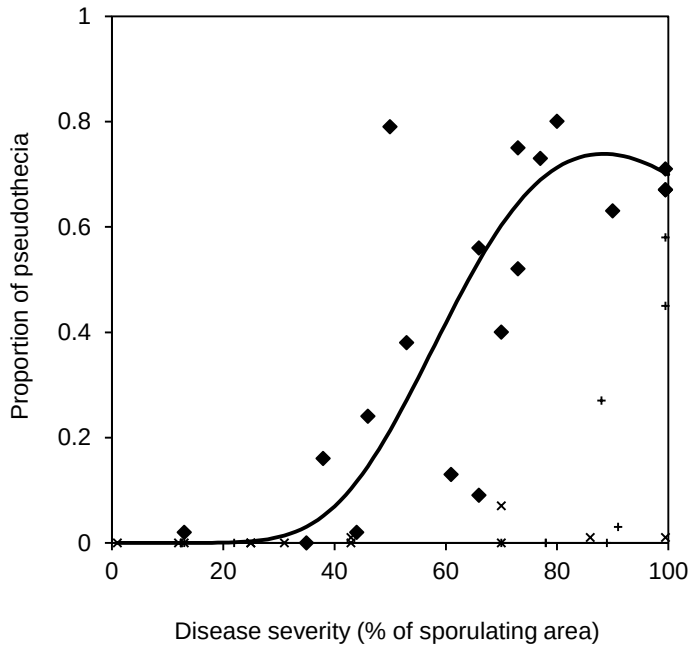


Fig.4 Impact of the vertical position of wheat tissues in standing stubble left in the field in the fall (stems and leaves, from lower to upper layers: 0-5 cm, 5-10 cm, 10-15 cm, 15-25 cm, 25-35 cm, 35-50 cm, 50-65 cm) on the intensity of *Zymoseptoria tritici* sexual reproduction expressed as the number 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.

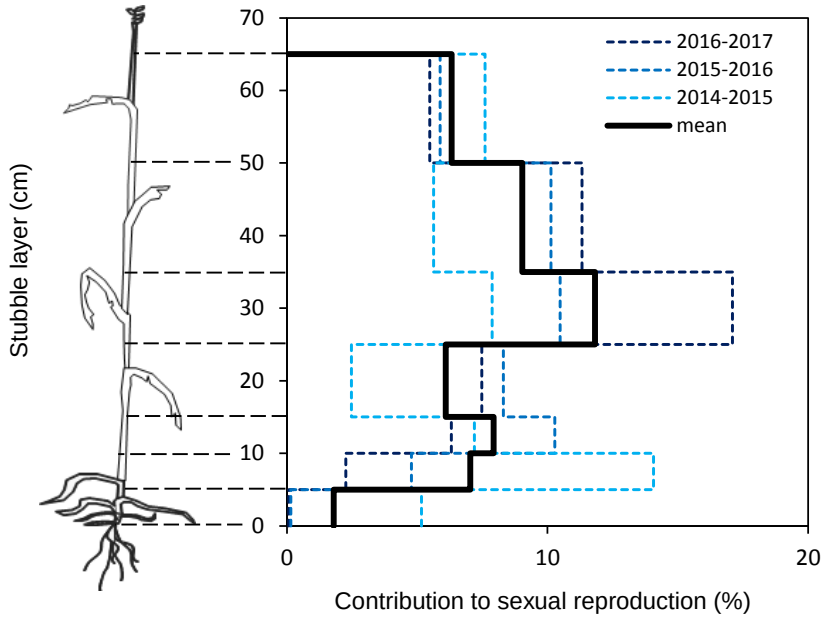


Fig.5 Relationship between *Zymoseptoria tritici* inoculum concentration (total number of conidia per mL) and mean intensity of asexual multiplication (disease severity assessed as a % of necrotic area) estimated on the three uppermost leaves of adult wheat plants. Inoculation with biparental suspensions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

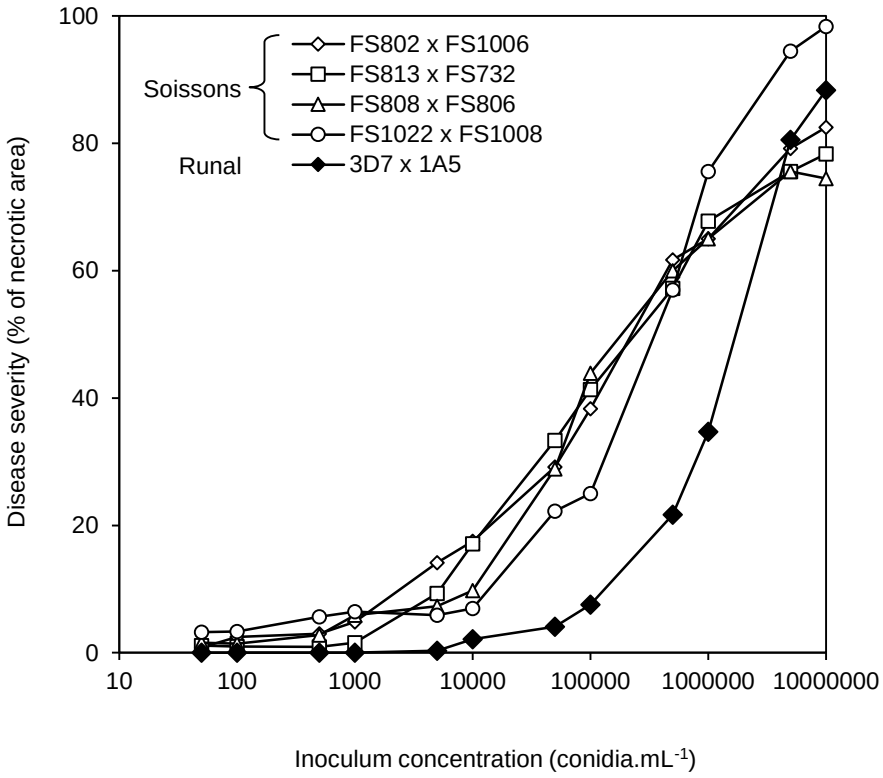


Fig.6 Relationship between the mean intensity of asexual multiplication in *Zymoseptoria tritici* (disease severity assessed as a % of necrotic area) on the three uppermost leaves of adult wheat plants cv. Soissons (black symbols) or Runal (white symbol), and (a, c, e) total grain weight per spike (GWS) 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. The Pearson correlation coefficient (R) is given for each relationship.

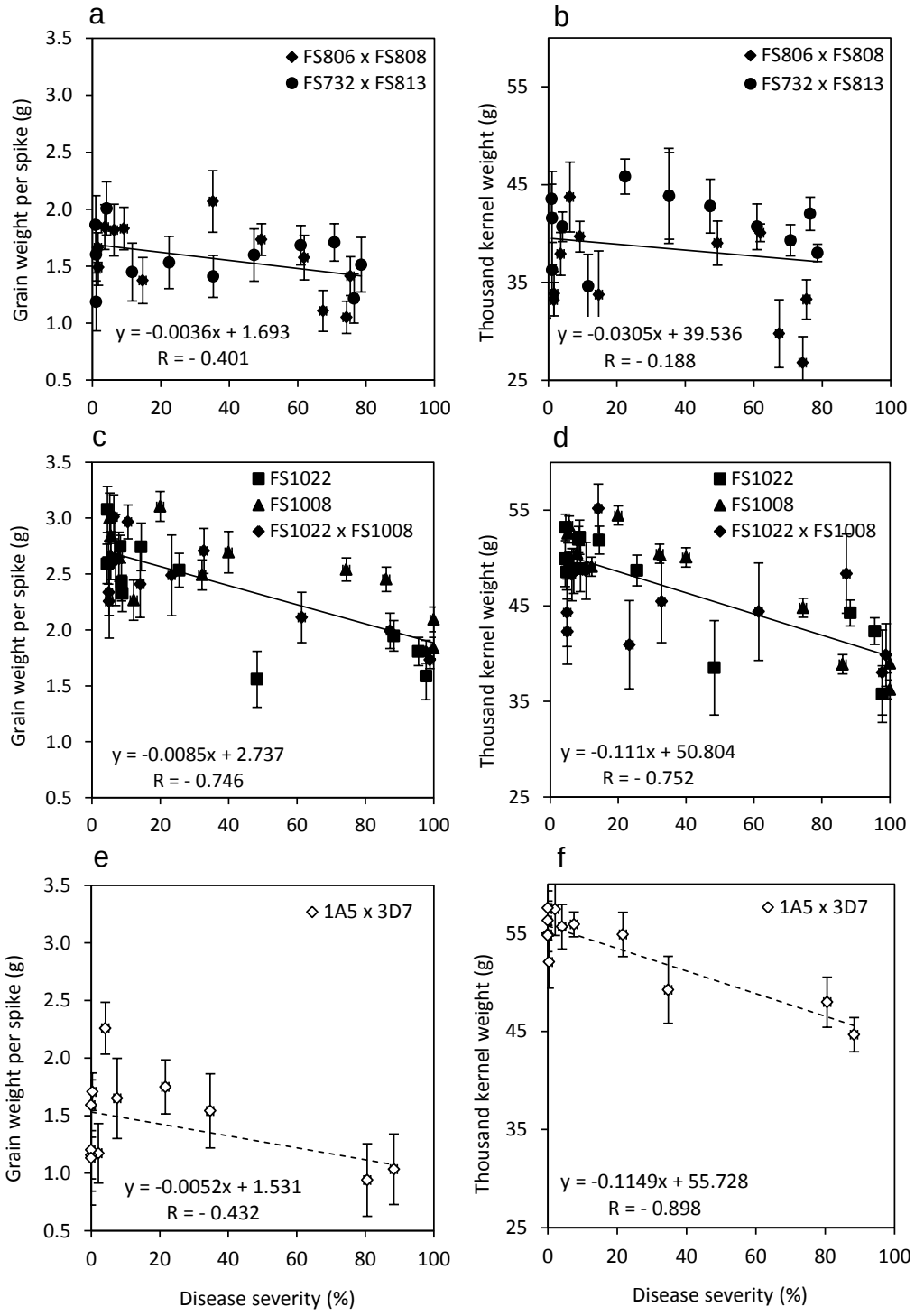


Fig.7 Relationship between the mean severity of Septoria tritici blotch (percentage of necrotic area) and (a) density of pycnidia (number of pycnidia per cm^2) or (b) percentage of pycnidia displaying exudation. Assessments were performed five weeks after the inoculation of adult wheat plants cv. Soissons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

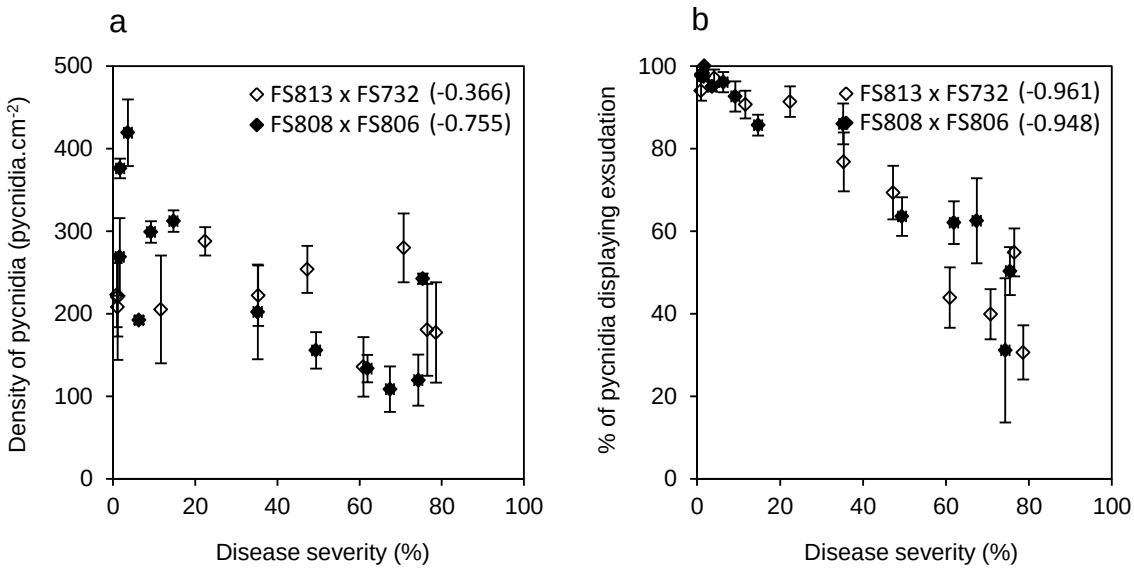


Fig.8 Relationship between inoculum concentration of *Zymoseptoria tritici* FS1008 or FS1022 isolate (number of FS1008 or FS1022 conidia per mL) in suspension alone or mixed with a second isolate present in the same amount, and the mean severity of Septoria tritici blotch (percentage of necrotic area) estimated on the three uppermost leaves of adult wheat plants cv. Soissons. ANOVA was performed (see *P*-value) at each relevant inoculum concentration (5.10^2, 5.10^3, 5.10^4, 5.10^5 and 5.10^6 conidia of each isolate per mL; see example 5.10^5 conidia of FS1008 in the scheme on the right). 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 percentage indicates that disease severity was lower after inoculation with the mixture; * indicates that the difference is significant (post hoc multiple comparison).

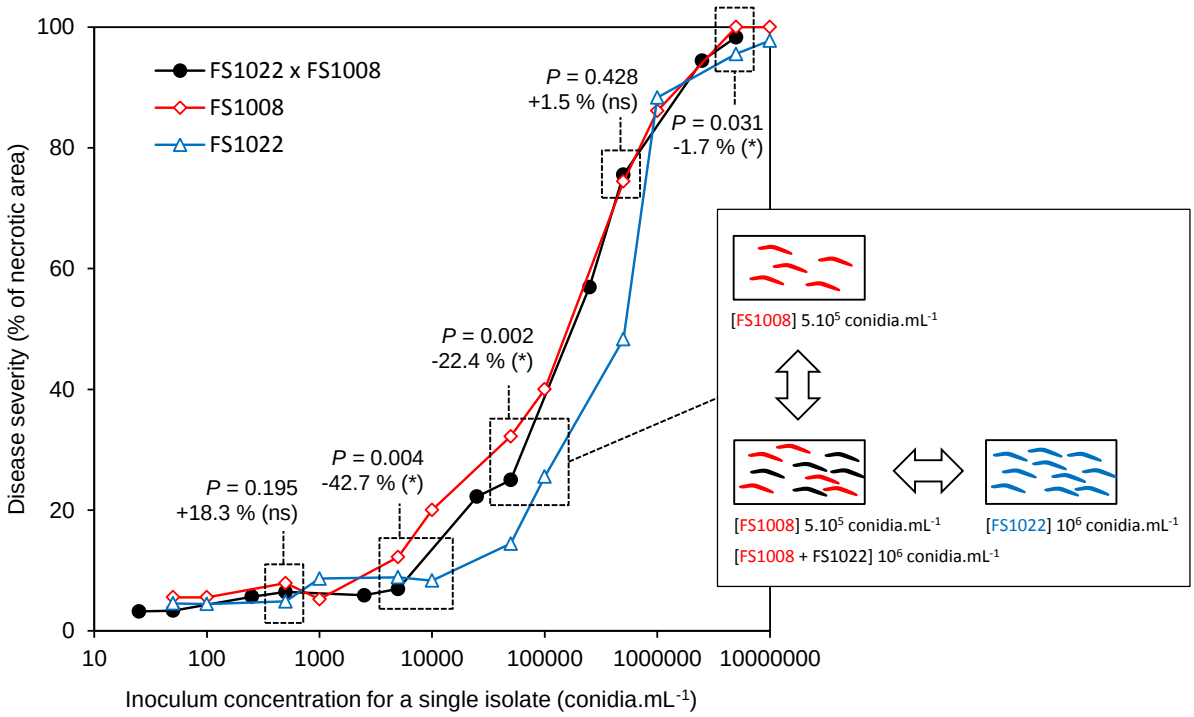


Fig.9 Impact of inoculum concentration (total number of conidia per mL) on the intensity of sexual reproduction in *Zymoseptoria tritici* (number of ascospores per g of wheat debris). Inoculation with biparental suspensions of isolates (a) FS802 × FS1006 (2015), (b) FS813 × FS732 (2016), (c) FS808 × 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). For each cross, Kruskal-Wallis test was performed and completed, when significant (see *P*-value), by a post hoc multiple comparison; letter indicates significant differences between inoculum concentrations; tests for FS802 × FS1006 (2015) were performed after exclusion of rep4 data (to avoid bias caused by the overall low amount of ascospores). The complete dataset is given in Table S1.

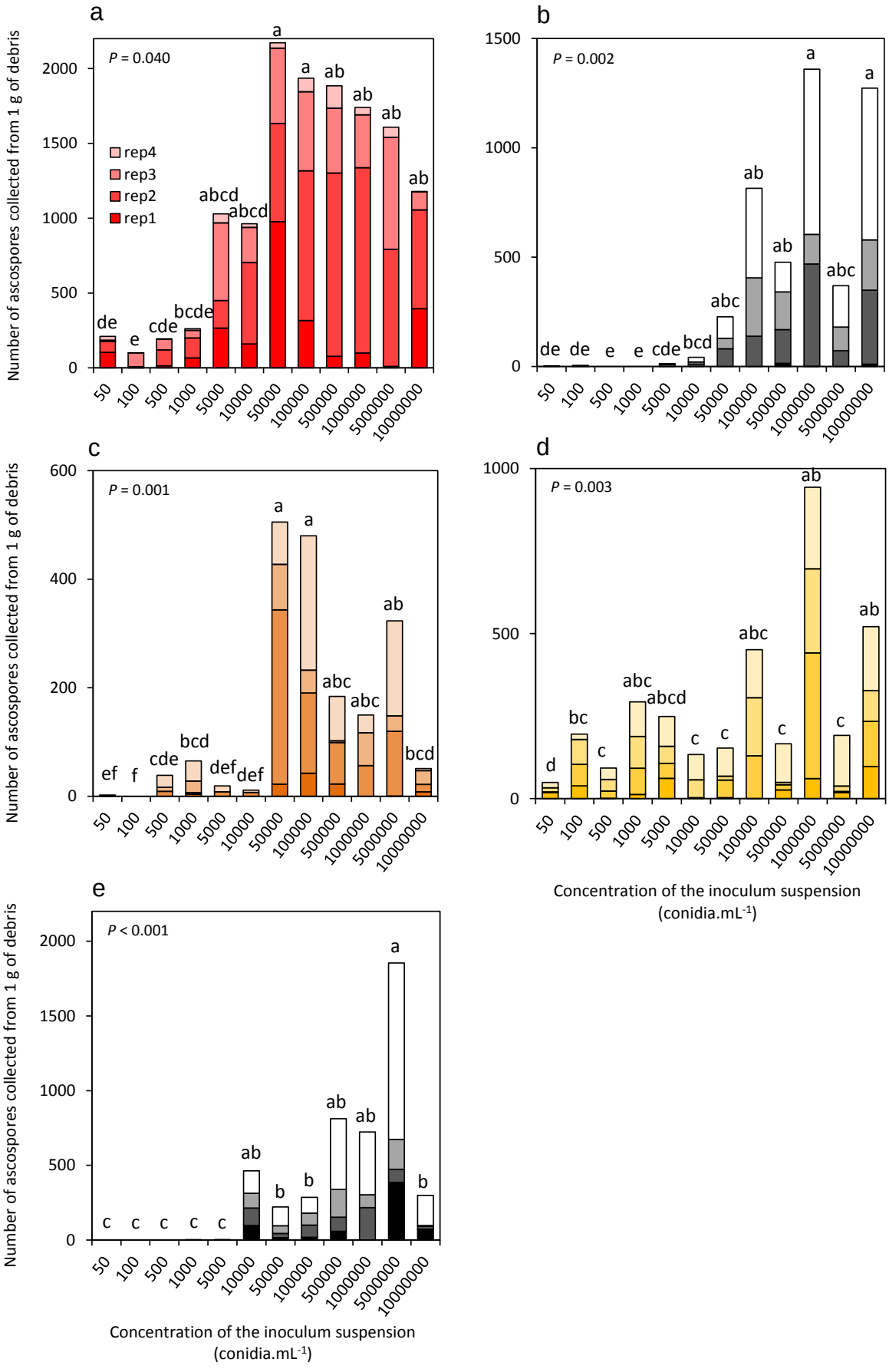


Fig.10 Empirical relationship between asexual multiplication intensity in *Zymoseptoria tritici* (disease severity assessed as a percentage of necrotic area) and sexual reproduction intensity (normalized number of ascospores for each cross expressed in %). A quadratic model (dotted lines; see eq. 2 in 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) to visualize the different relationships. Convergence was not obtained for the other two crosses (white points).

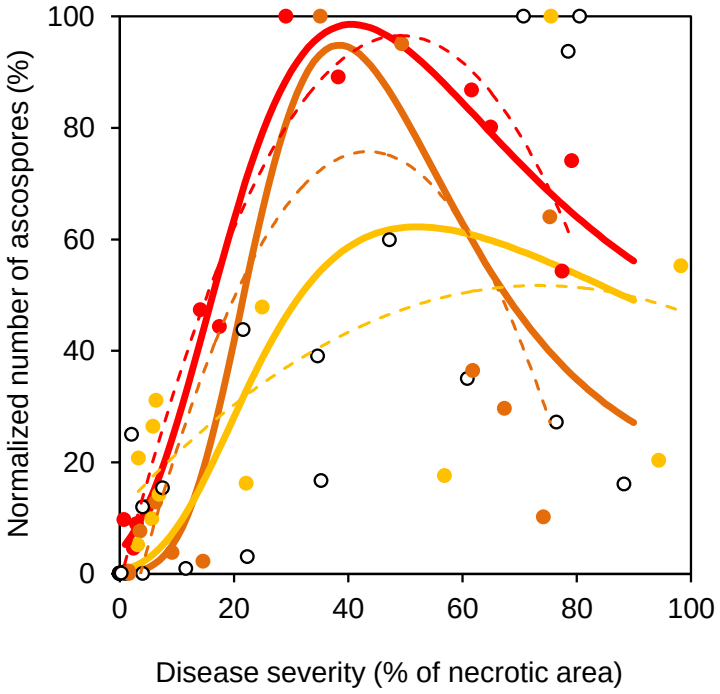
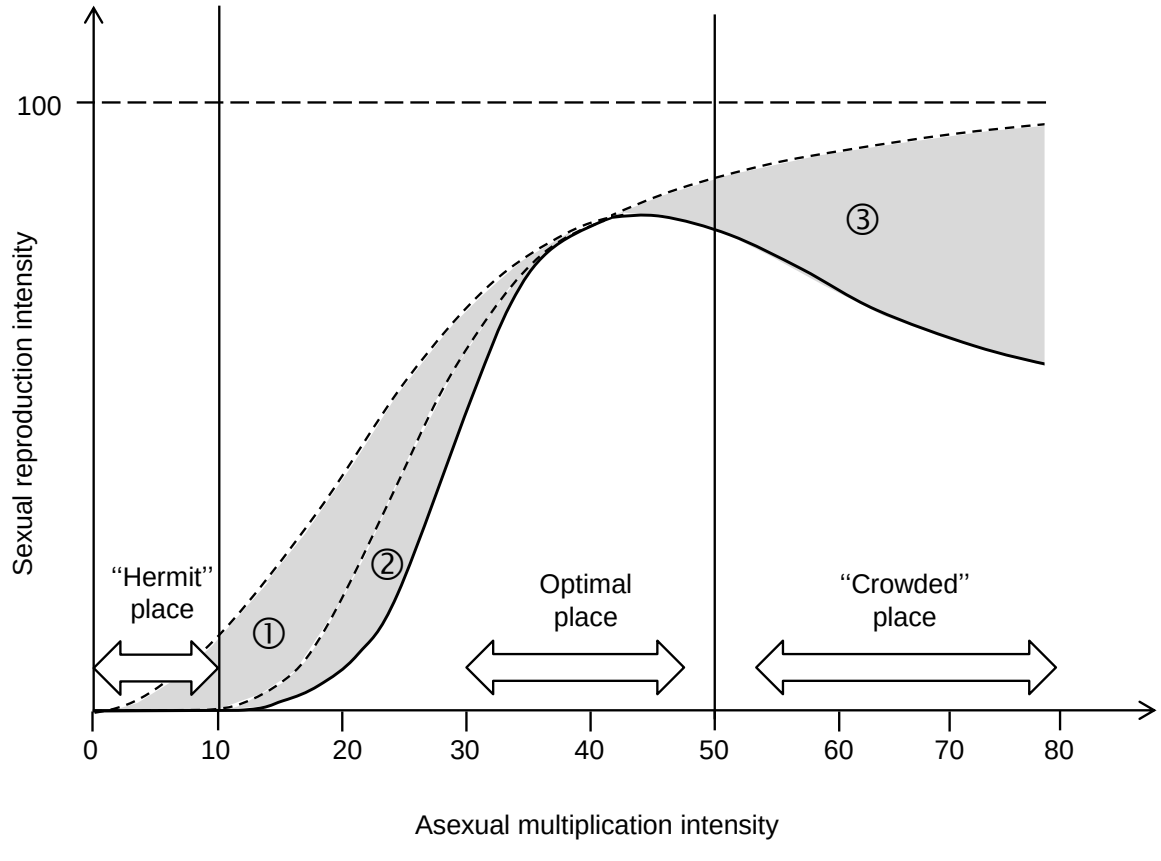


Fig.11 Theoretical relationship between asexual multiplication intensity in *Zymoseptoria tritici* (disease severity assessed as a percentage of necrotic area) and sexual reproduction intensity (normalized number of ascospores produced per g of wheat tissue), assuming that the conidia of two compatible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



Online Resource 2. Number of *Zymoseptoria tritici* ascospores collected per gram of wheat debris during four independent discharge events for the five crosses (FS802 × FS1006, FS813 × FS732, FS808 × FS806, FS1022 × FS1008, 3D7 × 1A5) performed with 12 inoculum concentrations.

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

[1] total number of conidia per mL

[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

[1] total number of conidia per mL

[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

[1] total number of conidia per mL

[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

[1] total number of conidia per mL

[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.