Measuring tolerance to a major fungal pathogen in elite wheat cultivars

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ABSTRACT

Tolerance and resistance represent two strategies that hosts evolved to protect themselves from pathogens. Tolerance alleviates the reduction in host fitness due to infection without reducing a pathogen's growth, while host resistance reduces pathogen growth. We investigated tolerance of wheat to the fungal pathogen *Zymoseptoria tritici* in 335 elite wheat cultivars. Using a novel digital phenotyping approach involving 11152 infected leaves, we discovered a new component of tolerance operating on individual leaves and revealed a substantial variation in tolerance among wheat cultivars. We also found a negative relationship between tolerance and resistance, presenting the first compelling evidence for a tradeoff between tolerance and resistance to plant pathogens. Surprisingly, the tradeoff arises due to limits in the leaf area and not due to metabolic constraints, contrary to what ecological theory suggests. Our analysis indicates that European wheat breeders may have selected for tolerance instead of resistance to an important pathogen.

INTRODUCTION

Tolerance and resistance represent two important mechanisms that plants and animals evolved to protect themselves from pathogens (Roy et al., 2000; Baucom & De Roode, 2011). Tolerance to a pathogen is usually defined as the host's ability to alleviate the reduction in its fitness due to infection without reducing the growth of the pathogen (Baucom & De Roode, 2011; Ney et al., 2013). A more tolerant host genotype will suffer a smaller loss in fitness per unit increase of pathogen population present within the host (called the pathogen burden) than a less tolerant host genotype. Hence, tolerance of a host genotype can be quantified as the reduction in host fitness per unit increase in pathogen burden. In contrast, resistance is usually measured as the host's ability to suppress the infection itself and reduce the resulting pathogen burden upon infection. The difference between tolerance and resistance is illustrated in Fig. 1.

Since the concept of tolerance was first coined more than a century ago (Cobb, 1894), numerous studies [reviewed by Pagan & Garcia-Arenal (2018)] investigated tolerance to pathogens in crop plants (Caldwell *et al.* 1958; Schafer, 1971; Newton *et al.*, 1998; Bingham *et al.*, 2009; Ney *et al.*, 2013; Newton, 2016), model plants (Kover & Schaal, 2002; Pagan et al., 2008, Shuckla et al., 2017) and wild plants (Roy et al., 2000; Inglese & Paul, 2006; Carr et al., 2006). Råberg et al. (2007) were the first to demonstrate tolerance to an infectious disease in animals. The therapeutic potential of tolerance in human medicine inspired a surge of further investigations (Medzhitov et al., 2012; Ayres & Schneider, 2012; Råberg, 2014; Soares et al., 2017). Several studies uncovered molecular mechanisms of tolerance in model animal species (Ayres & Schneider, 2008; Shinzawa et al., 2009; Richardson et al., 2010; Maze-Guilmo et al., 2014), while Blanchet et al. (2010); Jackson et al. (2014); Hayward et al. (2014); Maze-Guilmo et al. (2014) characterized tolerance to parasites in wild animal populations. Zeller & Koella (2017) used experimental evolution approach to determine how tolerance/resistance evolve in mosquito populations exposed to microsporidian parasites.

It is generally thought that since both tolerance and resistance are defense strategies that require reallocation of host resources, they should confer fitness costs to the host (Roy & Kirchner, 2000; Simms & Triplett, 1994; Brown, 2002). For this reason, a metabolic tradeoff between tolerance and resistance is expected due to a limitation in host resources. A large body of ecological theory has been developed based on this premise (van der Meijden et al., 1988; Herms & Mattson, 1992; Roy & Kirchner, 2000; Fornoni et al., 2004; Restif & Koella, 2004; Miller et al., 2005; Best et al., 2008). However, empirical evidence for a tradeoff between tolerance and resistance remains sparse. A few

studies reported a negative relationship between tolerance and resistance to herbivory (Fineblum & Rausher, 1995; Stowe, 1998; Baucom & Mauricio, 2008) and Råberg et al. (2007) presented a similar finding in mice infected with malaria. Other studies reported no correlation between tolerance and resistance in plants subjected to herbivores (Mauricio et al., 1997), in humans infected with HIV (Regoes et al., 2014) or in wild sheep infected with a parasite (Maze-Guilmo et al., 2014). Interestingly, in *Drosophila melanogaster* populations exposed to a bacterial infection, tolerance and resistance exhibited a positive correlation (Howick & Lazzaro, 2014). Likewise, no evidence for a tradeoff between tolerance and resistance was so far reported in plant pathogens.

In this study, we investigated tolerance to the fungal pathogen *Zymoseptoria tritici* in 335 elite European wheat cultivars. *Z. tritici* causes septoria tritici blotch (STB), a disease that is a major constraint on wheat production globally and the most destructive disease of wheat in Europe (Fones & Gurr, 2015). *Z. tritici* spores germinate on wheat leaves and penetrate the leaves through stomata (Kema et al., 1996). After penetration, the fungus grows for several days within leaves without producing visible symptoms. During this asymptomatic period, the pathogen invades the host mesophyll around the position of the initial penetration. After 10 to 20 days of asymptomatic growth, the fungus becomes necrotrophic and kills the invaded plant tissue forming necrotic lesions. Asexual fruiting bodies called pycnidia begin to form in the necrotic lesions soon thereafter. Spores that form in the pycnidia provide inoculum to start the next cycle of pathogen reproduction. The formation of necrotic lesions corresponds to host damage caused by the pathogen that can be quantified as the proportion of leaf area covered by lesions (PLACL). The number of pycnidia provides a measure of pathogen reproduction that can be quantified by counting the number of pycnidia present on an infected leaf, N_p.

Control of STB relies mainly on applications of fungicides and deployment of STB-resistant wheat varieties. However, populations of *Z. tritici* are extremely diverse due to a high degree of sexual reproduction and large effective population sizes. As a result, the pathogen has the capacity to rapidly adapt to both fungicides (Fraaije et al., 2005; Zhan et al., 2006) and host resistances (Cowger et al., 2000; McDonald and Mundt, 2016) as a result of strong directional selection favoring particular pathogen genotypes. In contrast, host tolerance does not impair pathogen reproduction and is not expected to impose strong directional selection. For this reason, tolerance presents a promising alternative to protect wheat yield that is not prone to pathogen adaptation.

Several previous studies investigated tolerance of wheat to STB empirically (Eyal & Ziv, 1974; Zuckerman et al., 1997; Parker et al., 2004; Foulkes et al., 2006; Collin et al., 2018). Van den

Berg et al (2017) used mathematical modeling to reveal functional traits in wheat that contribute to tolerance. These studies used wheat yield (measured as tons of grain per hectare or as the thousand kernel weight) to quantify the plant fitness (the *y*-axis in Fig. 1) and the PLACL or healthy area duration (HAD, Waggoner & Berger (1987)) to quantify the pathogen burden (the *x*-axis in Fig. 1). Accordingly, tolerance was quantified as the yield loss associated with each unit increase in PLACL or unit loss in HAD.

PLACL and HAD quantify the damage that the pathogen causes on an infected host plant. However, these quantities do not necessarily accurately reflect the size of the pathogen population present within the infected host plant (Stewart et al., 2016a; Karisto et al., 2018). For this reason, tolerance measured in these traditional ways is considered to be tolerance to the disease, which may not coincide with tolerance to the pathogen (Gaunt, 1981). The goal in this study was to characterize wheat tolerance to its pathogen, *Z. tritici*. With this in mind, we used green leaf area to quantify the plant fitness and the number of pycnidia per leaf to quantify the pathogen burden. By conducting these measurements on 11'152 individual wheat leaves belonging to 335 different cultivars (counting in total 2'187'776 individual pycnidia), we were able to identify and measure a novel component of wheat tolerance to *Z. tritici* that operates on the scale of individual leaves. We call this "leaf tolerance" as opposed to the "whole-plant tolerance" that was characterized previously. A way to estimate tolerance over a range of pathogen burdens as we describe here is to estimate range tolerance (Baucom & De Roode, 2011). The component of tolerance that we measured represents fecundity tolerance rather than mortality tolerance, because this disease does not kill its host but instead reduces its fecundity.

We used a combination of mathematical modeling and field experimentation to formulate and test several hypotheses connected to leaf tolerance of wheat to *Z. tritici*. First, based on our current understanding of the infection biology of *Z. tritici*, we formulated and tested empirically two alternative hypotheses regarding the relationship between the green leaf area and the number of pycnidia per leaf. Second, we tested the hypothesis that wheat cultivars differ in terms of their leaf tolerance. Finally, we tested the expectation of a tradeoff between tolerance and resistance and found a significant negative relationship between tolerance and resistance. Surprisingly, our analysis indicates that this negative association arises due to the limitation in the leaf area of wheat plants and not as a result of metabolic costs associated with tolerance/resistance as predicted by ecological theory.

MATERIALS AND METHODS

Here we analyzed a subset of the raw data reported in (Karisto et al., 2018). Below, we describe the main features of the experimental design that are relevant for this analysis. A comprehensive description of the experimental design can be found in (Karisto et al., 2018).

Plant materials and experimental design

In total, 335 elite European winter wheat (*Triticum aestivum*) varieties from the GABI-wheat panel (Kollers et al., 2013a,b) were evaluated in this experiment. Two biological replicates of the wheat panel were grown during the 2015-16 growing season in two complete blocks separated by approximately 100 m at the Field Phenotyping Platform site of the Eschikon Field Station of the ETH Zurich, Switzerland (coordinates 47.449°N, 8.682°E) (Kirchgessner et al., 2017). The complete blocks were composed of 18 rows and 20 columns consisting of 1.2 x 1.7 m plots, with the genotypes arranged randomly within each block. Best practices recommended for conventional, high-input wheat production were used, including applications of fertilizers and pesticides. Complete details are given in (Karisto et al., 2018).

STB inoculum, sampling of infected leaves

All STB infection was natural, with the majority of primary inoculum likely originating from airborne ascospores coming from nearby wheat fields that surround the Eschikon field site. As a result, the infections analyzed in this experiment were caused by hundreds of thousands of different pathogen strains. For this study we used leaves exhibiting obvious STB lesions that were collected on 4 July 2016 (approximate range of GS 75 [milk development] to GS 85 [dough development]). Up to 16 infected leaves were collected at random for each plot from the leaf layer below the flag leaf. The sampled leaves were placed into paper envelopes, kept on ice in the field, and stored at 4°C for 2 days before mounting onto A4 paper with printed reference marks and sample names, as described by Stewart et al. (2016b). Absorbent paper was placed between each sheet of eight mounted leaves and sheets were pressed with approximately 5 kg at 4°C for 2 to 3 days prior to scanning at 1,200 dpi with a Canon CanoScan LiDE 220 flatbed scanner.

Determination of the green leaf area and the number of pycnidia per leaf

Scanned images were analyzed with the software ImageJ (Schindelin et al., 2015) using the

macro described by Karisto et al. (2018). The maximum length of the scanned area for each leaf was 17 cm. When leaves were longer than 17 cm, bases of the leaves were placed within the scanned area, while the leaf tips extended outside the scanned area. For each leaf, the following quantities were automatically recorded from the scanned image: total leaf area (A_{tot}), necrotic and chlorotic leaf area (A_{necr}) and the number of pycnidia (N_p). Necrotic and chlorotic leaf areas were detected based on discoloration of the leaf surface and were not based on the presence of pycnidia. We then calculated the green (Healthy) leaf area as $H = A_{tot} - A_{necr}$.

Statistical analysis

Statistical analysis was conducted in the Python programming language (version 3.6.2, https://www.python.org) using the open-source packages scipy (version 0.19.1), numpy (version 1.11.1) and matplotlib (version 1.5.3; Jones et al., 2001). The Python package rpy2 (version 2.8.6, https://rpy2.bitbucket.io/) was used to access statistical routines of R (R Core Team, 2016).

To control for the effect of total leaf area on the number of pycnidia per leaf, we performed the adjustment $N_{p,i} \rightarrow (A_{tot} / A_{tot,i})\ N_{p,i}$ prior to the analysis, where $N_{p,i}$ and $A_{tot,i}$ is the number of pycnidia and the total area of an individual leaf i and A_{tot} is the mean total leaf area averaged over the whole dataset. First, we pooled together the data from leaves belonging to different cultivars and fitted the relationship between N_p and H using a linear function $H = H_0(1 - \kappa N_p)$ and an exponential function $H = H_0 exp(-\kappa N_p)$. For both functions, the slope κ can be used to quantify tolerance: small κ-values correspond to high tolerance and large κ-values correspond to low tolerance. This overall fit gave us the baseline to which we then compared the tolerance of individual wheat cultivars. Second, we estimated κ in each of the 335 wheat cultivars by fitting both the linear and the exponential functions to individual leaf data belonging to each of the cultivars. Next, we used multiple bootstrap t-tests with resampling cases (Davison and Hinkley, 2001) to compare κ-estimates in each cultivar to the baseline κ-estimate, where we used the false discovery rate correction for multiple comparisons. Fits were performed using the nonlinear ordinary least-squares optimization with the Nelder-Mead method in the lmfit package (version 0.9.7) for Python (Newville et al., 2014). We also determined the significance of the effects of the spatial block and the cultivar on tolerance: we used likelihood ratio tests to compare more complex models in which data from each cultivar/spatial block was fitted using separate κ or H_0-values to simpler models where only single κ or H_0 parameters were fitted to the whole dataset. To determine whether there is a relationship

between tolerance and resistance of wheat to *Z. tritici*, we used Spearman's rank correlation test to analyze correlations between tolerance quantified as the slope κ and resistance quantified as the average number of pycnidia per leaf (Karisto et al., 2018).

RESULTS

A novel component of tolerance

Recent studies (Karisto et al., 2018; Stewart et al., 2016a) demonstrated that factors responsible for leaf damage during the infection are largely uncoupled from the pathogen's capacity to reproduce on leaves. Based on this knowledge, we devised a simple mathematical model that describes the change in the green leaf area corresponding to an increase in the number of pycnidia on a leaf (for more details see Supplementary Appendix 1.1).

We assumed that each lesion of STB has an area a_l and contains n_p pycnidia and derived the differential equation that governs the relationship between the number of pycnidia and the green leaf area remaining on the leaf:

$$\frac{dH}{dN_p} = -a_p, \tag{2}$$

where $a_p = a_l/n_p$ is the area of the lesion that corresponds on average to a single pycnidium. If a_p depends neither on H, nor on N_p, then the solution of Eq. (5) is a linear function:

$$H(N_p) = H_0(1 - \kappa N_p), \tag{3}$$

where $\kappa = a_p/H_0$ and H_0 is the green leaf area in the absence of infection. Alternatively, if a_p is proportional to the green leaf area H, i.e. $a_p = \kappa H$, then the solution of Eq. (5) is an exponential function:

$$H(N_p) = H_0 exp(-\kappa N_p). \tag{4}$$

In both its linear and exponential versions, the model predicts that the leaf loses its green area as it carries higher numbers of pycnidia. We consider the green leaf area, H, as a quantity representing plant fitness and the number of pycnidia per leaf, N_p, as a proxy for the pathogen population present on the leaf (pathogen burden). Consequently, the slope of the decrease, κ, characterizes the tolerance of wheat to *Z. tritici*: it measures the amount by which the green leaf area decreases when a single pycnidium is added to the leaf. We call κ the intolerance parameter, as the cultivars with higher κ-values will lose their green leaf area at a higher rate than cultivars with lower κ-values when the number of pycnidia is increased.

Using this model we developed a novel way to measure tolerance of wheat to *Z. tritici* that operates on the scale of individual leaves ("leaf tolerance") as opposed to the "whole-plant tolerance" that was studied previously in this pathosystem (Eyal & Ziv, 1974; Parker et al., 2004; Foulkes et al., 2006; Collin et al., 2018). We demonstrated in Supplementary Appendix 1.2 that these two components of tolerance contribute to overall tolerance as multiplicative factors. Based on two sets of biological assumptions, we formulated two hypotheses about the shape of the relationship between the green leaf area and the number of pycnidia on the leaf represented by Eq. (3) and (4). We next tested these hypotheses using the empirical data gathered from wheat leaves naturally infected by *Z. tritici*.

Green leaf area decreases nonlinearly with the number of pycnidia

Figure 2a shows 11152 individual leaf measurements of the number of pycnidia per leaf, N_p, and the green leaf area, H. Overall, leaves lose more of their green area when they carry a larger number of pycnidia. The exponential function, Eq. (4), provided a better fit (standard error of the estimate s=472, coefficient of determination $R^2 = 0.37$) than the linear function, Eq. (3) (s=508, $R^2 = 0.28$). For this reason, we estimated the overall slope using the exponential function and obtained the best-fit parameter values: for the slope $\kappa = 0.00172$ and the intercept $H_0 = 1816$ mm^2. The spatial block had a significant effect on κ (likelihood ratio 6.9, $p = 0.008$) and H_0 (likelihood ratio 41, $p = 1.4 \times 10^{-10}$), but the cultivar had a much greater effect on κ (likelihood ratio 856, $p = 1.3 \times 10^{-47}$) and H_0 (likelihood ratio 2636, $p < 10^{-50}$).

Though the exponential function fit the combined dataset better than the linear function, the fit of the exponential function increased in the less tolerant cultivars. For example, in a more tolerant cultivar Intact (blue curve in Fig. 2c), the linear fit yields s=349, $R^2 = 0.26$, while the exponential fit yields s=345, $R^2 = 0.28$. But in a less tolerant cultivar Lynx (red curve in Fig. 2c), the linear fit yields s=430, $R^2 = 0.59$ compared to the exponential fit that gives s=415, $R^2 = 0.62$.

Ranking of cultivars according to their tolerance to *Z. tritici*

We estimated κ for each cultivar by fitting the empirical dependency of the green leaf area on the number of pycnidia with the exponential function [Eq. (4)], i.e. we obtained tolerance curves for each cultivar (Fig. 4a). The distribution of the κ-estimates is shown in Fig. 2b. Next, we ranked the cultivars according to their tolerance to *Z. tritici* (see Fig. 2c and Table S1). Smaller κ-values

corresponded to more tolerant cultivars. We also compared κ-estimates for each cultivar to the baseline value (black line in Fig. 2c). We found that 22 cultivars were significantly more tolerant than the baseline (blue squares in Fig. 2c) and 25 cultivars were significantly less tolerant than the baseline (red squares in Fig. 2c). Thus, the cultivars that we investigated in our field experiment exhibited significant differences with respect to leaf tolerance.

To determine to what extent the ranking of cultivars with respect to their κ-estimates was conserved between the two replicate blocks, we estimated the κ-values for each cultivar separately in each of the blocks. The κ-estimates exhibited a positive and significant correlation between the two replicates ($r_S = 0.18$, $p = 0.001$). In addition, we obtained similar data for a subset of 38 cultivars in 2015 (Stewart et al., 2016), which allowed us to evaluate the robustness of the outcomes. The κ-estimates exhibited a positive but a non-significant correlation between the two years ($r_S = 0.3$, $p = 0.07$).

Evidence for a tradeoff between leaf tolerance and resistance

We found that the estimates of tolerance, κ, for each of the 335 cultivars correlated negatively with the mean number of pycnidia per leaf, N_p, the measure of resistance to STB, with $r_S = -0.27$, $p = 5.8 \times 10^{-7}$ (Fig. 3). Interestingly, κ-estimates correlated positively with mean PLACL values in each cultivar ($r_S = 0.31$, $p = 8.6 \times 10^{-9}$).

Why do more tolerant cultivars carry more pycnidia on their leaves than less tolerant cultivars? A possible explanation is that since the leaf area is limited, this places a constraint on the maximum number of pycnidia that a leaf can carry. This constraint should lead to a negative relationship between N_p and κ in cases where the number of pycnidia on leaves belonging to the same cultivar approach the maximum allowed by the leaf area. A less tolerant cultivar suffers a larger necrotic area forming, on average, per pycnidium [parameter a_p in Eq. (2)]. Consequently, the maximum number of pycnidia per leaf is lower in a less tolerant cultivar than in a more tolerant cultivar. Therefore, the limitation in the leaf area should affect more strongly pathogen populations infecting less tolerant cultivars, where the green leaf area decreases more steeply with increasing numbers of pycnidia (Fig. 4a). If the limitation in the leaf area is the dominant factor responsible for the negative relationship between tolerance and resistance, then the negative correlation should be present in intolerant cultivars, but absent in tolerant cultivars.

To test this expectation, we subdivided all cultivars into two groups according to their

tolerance estimates using the baseline tolerance ($\kappa = 0.00172$) as the threshold. Next, we conducted the Spearman's correlation test in each of the groups separately. We found that intolerant cultivars exhibited a significant correlation between tolerance and resistance ($r_S = -0.34, p = 4.3 \times 10^{-6}$), while tolerant cultivars showed no significant correlation between the two traits ($r_S = -0.09$, $p = 0.27$).

Figure 4 illustrates why non-tolerant cultivars should be more strongly affected by the limitation in the leaf area than tolerant cultivars. Figure 4a shows the tolerance curves for tolerant (blue) and intolerant (red) cultivars. We determined the maximum number of pycnidia that can be reached in each cultivar by computing the number of pycnidia, N_{pm}, at which 95 % of the green leaf area is lost on average (this is given by the intersection of each tolerance curve with the horizontal line H/H_0=0.05 in Fig. 4a). Next, we computed the ranges in terms of N_{pm} corresponding to tolerant cultivars (blue-shaded area in Fig. 4b) and non-tolerant cultivars (red-shaded area in Fig. 4b). To determine the extent to which pathogen populations infecting tolerant and intolerant cultivars could be affected by the limitation in the leaf area, we compared these ranges with the overall distribution of leaves with respect to the numbers of pycnidia they carry (cf. the histogram in Fig. 4b and the blue- and red-shaded areas). While the blue-shaded area in Fig. 4b contained 38 leaves that constitute only about 0.3 % of the total number of 11152 infected leaves, the red-shaded area contained a much more substantial proportion (22 %) of the leaves (2485 leaves out 11152). Therefore, the non-tolerant cultivars contained a much greater proportion of the leaves on which pathogen populations were likely to be affected by limitations in the leaf area compared to the tolerant cultivars. Combined with the observation that only non-tolerant cultivars exhibited a negative relationship between tolerance and resistance, these data support our hypothesis that the negative relationship between tolerance and resistance arises largely due to a limitation in the leaf area.

DISCUSSION

We discovered a novel component of tolerance in wheat to *Z. tritici* that operates on the scale of individual leaves (leaf tolerance). We devised an approach to quantify leaf tolerance empirically based on automated measurements of the green leaf area and the numbers of pycnidia on individual leaves. We gathered data from 11152 individual infected leaves and characterized leaf tolerance in 335 elite European wheat cultivars. Cultivars exhibited significant differences in leaf tolerance, suggesting that this trait is at least partially under genetic control. We also found a negative

relationship between leaf tolerance and resistance to *Z. tritici*, indicating that there is a tradeoff between tolerance and resistance. Our study presents the first clear evidence for such a tradeoff in the context of plant-pathogen interactions. We discuss the consequences of this possible tradeoff for the selection of tolerance/resistance in agricultural host populations.

Surprisingly, the nature of this tradeoff turned out to be different from what we expected based on ecological theory. Our analysis shows that the tradeoff is only present in non-tolerant cultivars and that the limitation in the leaf area is the dominant factor responsible for its occurrence. Hence, the limitation in the leaf area is expected to constrain the evolution of pathogen populations towards higher reproductive fitness on non-tolerant cultivars, but the pathogen may overcome this limitation by evolving lower virulence (Anderson & May, 1982). According to our current understanding in ecological theory a metabolic tradeoff between tolerance and resistance is expected that arises due to limitation in resources available to the host. Our data does not exclude the possibility of a metabolic tradeoff, but its detection may require an even more comprehensive dataset than what we have at hand. Evidence for the metabolic tradeoff is more likely to be found in the future by considering a larger number of sufficiently tolerant cultivars, because as we demonstrated here in more tolerant cultivars the relationship between tolerance and resistance is not dominated by the limitation in the leaf area.

The dataset we used to characterize tolerance to a plant pathogen is unusually large compared to previous studies. For example, the number of different wheat genotypes used to study tolerance of wheat to STB in earlier studies ranged from 2 to 25 (Eyal & Ziv, 1974; Zuckerman et al., 1997; Parker et al., 2004; Foulkes et al., 2006; Collin et al., 2018). Råberg et al. (2007) investigated tolerance of mice to malaria infection using five mouse strains and three strains of *Plasmodium chabaudi*. Only the study of human tolerance to HIV (Regoes et al., 2014) and the study of tolerance in the wild population of Soay sheep to a gastrointestinal nematode infection (Hayward et al., 2014) had comparably large datasets that included thousands of infected individuals. Pathogens of crop plants offer several advantages when conducting experiments to understand basic eco-evolutionary principles of host-pathogen interaction. For example, both host and pathogen populations can be replicated in large-scale field studies that can be conducted across different environments (e.g. with varying levels of irrigation, fertilization, planting densities, inoculum levels, etc.) with relatively little effort. Remarkably, as we demonstrated here, both tolerance and resistance can be readily quantified from digital images of infected leaves. An experiment of a similar magnitude would be much more challenging and resource-intensive using animal or human hosts.

Our analyses and interpretations are based on two important assumptions: (i) the green area of the second leaf is a good measure of plant fitness, and; (ii) the number of pycnidia per leaf is a good measure of the size of the pathogen population on a leaf. We justify these assumptions as follows: (i) Compared to infected leaves that have a large fraction of their surface area covered by lesions, leaves with a larger green area intercept a larger fraction of the incoming radiation, which contributes directly to plant yield. Numerous field experiments demonstrated that this effect is strongest for the three upper leaves [for example, (Eyal & Ziv, 1974; King et al., 1983)] if the green leaf area is measured during the critical phase of seed development (around GS 75). In our field experiment, we could not determine yield corresponding to each individual infected leaf that we sampled. However, we measured overall yield per plot and found significant correlations between the green leaf area of second leaves (sampled at GS 75-85) and yield, measured both as tons per hectare and as thousand kernel weight (see Supplementary Appendix 1.3). Thus, we have strong evidence that the green leaf area that we measured is a substantial contributor to plant fitness.

To justify (ii) we first note that the number of pycnidia per leaf, N_p, was shown to be the main factor that determined the number of pathogen spores produced on an infected leaf (Stewart et al., 2016a). Second, a recent field experiment reported that the proportion of the leaf area covered by STB lesions was largely independent from the number of pycnidia produced on a leaf (Karisto et al., 2018). Combining these two findings leads us to conclude that the number of pycnidia per leaf is a better indicator of the pathogen population inhabiting a leaf than the area of a leaf damaged due to infection.

According to our statistical analysis, an exponential decrease better fits the empirical dependency of the green leaf area on the number of pycnidia per leaf than a linear decrease, demonstrating that leaf tolerance curves were nonlinear. This deviates from what was reported in earlier analyses of wheat tolerance to *Z. tritici*: tolerance curves were typically fitted using linear functions (Eyal & Ziv, 1974; Parker et al., 2004; Foulkes et al., 2006), with the notable exception of the study by Shaw & Royle (1989b) that used a family of nonlinear curves. It was important to establish the departure from linearity in our study for two reasons. First, it allowed a more accurate comparison of tolerance estimates in different cultivars against the baseline. Second, it provided additional insight into the biology of the infection, because the linear model and the exponential model are based on different biological assumptions.

Our analysis of the model (Supplementary Appendix 1.1) indicates that the linear function Eq. (3) approximates well the relationship between the green leaf area and the number of pycnidia on the

leaf when the number of pycnidia on the leaf is sufficiently low. This implies that the number of lesions on the leaf is also likely to be low, with the necrotic area covering only a small proportion of the total leaf area. Under this scenario, lesions develop mostly independently of each other. However, when lesions start to occupy a large proportion of the total leaf area, they become more likely to influence each other's development due to limitations in space and/or resources. Under this scenario, Eq. (3) is no longer a good approximation and the necrotic area $a_p = a_l/n_p$ that corresponds to a single pycnidium may depend on both green leaf area H and the number of pycnidia N_p already present on the leaf. Above, we considered the simplest case of this dependency when a_p is proportional to H, which resulted in the exponential solution [Eq. (4)]. This dependency may result from the lesion area a_l being proportional to the remaining green leaf area H. Biologically, this means that as more of the green leaf area becomes occupied by lesions, lesions tend to grow to a smaller size due to limitations in available green space and/or resources in the leaf. Alternatively, this dependency may arise due to the number of pycnidia per lesion n_p being inversely proportional to the remaining green leaf area H. This may occur due to an increased activation of plant defenses as more of the leaf area becomes occupied by lesions.

Breeding for resistance to STB disease is based on disease assessments that do not quantify pathogen reproduction on the leaves. The amount of disease is typically assessed visually using a categorical scale of severities corresponding to different ranges in terms of the proportion of necrotic area on the leaves (or PLACL). As a result, breeders are likely to select for cultivars with lower PLACL. But as we have shown above, cultivars with lower PLACL are on average more tolerant. Hence, by focusing on PLACL wheat breeders may have inadvertently selected for increased tolerance. Due to the tradeoff between tolerance and resistance, this simultaneously favors lower levels of STB resistance. Some support for this hypothesis is given by our preliminary analysis of the relationship between tolerance/resistance and the year of cultivar registration. In a subset of 205 out of 335 cultivars, we had information on cultivar registration years. In those cultivars, tolerance increased with the year of cultivar registration: the correlation between the intolerance parameter κ and the cultivar's registration year was negative and significant (r_S = -0.17, p = 0.02). In contrast, resistance did not exhibit a significant correlation with the cultivar's registration year (r_S = -0.06, p = 0.36). This pattern suggests that the selection practices used by plant breeders have led to wheat populations with higher tolerance but lower resistance to STB in Europe.

The method to quantify leaf tolerance presented here can potentially be used to measure tolerance to other pathogens that infect plant leaves. The necessary condition is that digital images of

infected leaves should enable quantitative measurements of both the damage to the plant induced by the pathogen and the size of the pathogen population on the leaf. This should be possible for a large number of necrotrophic pathogens that form visible fruiting bodies on the leaf surface.

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DATA ACCESSIBILITY STATEMENT

Raw data is available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.171q4

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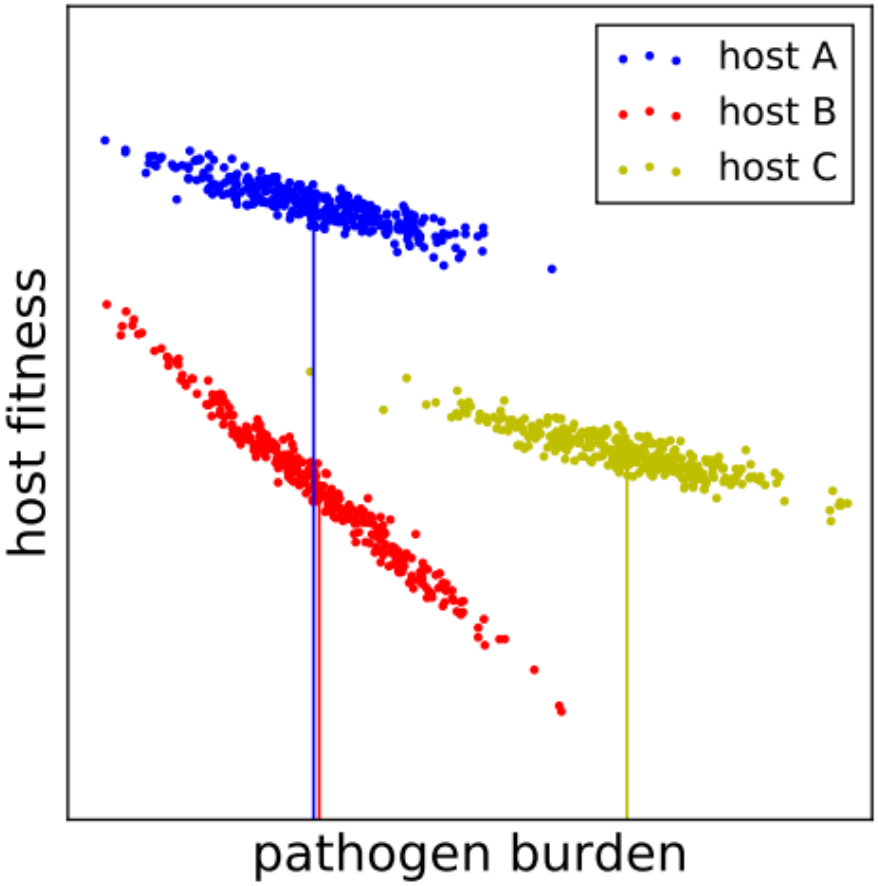

Figure 1 Differentiation and quantification of tolerance and resistance. The figure illustrates the relationships between host fitness and pathogen load for three hypothetical host genotypes: A (blue), B (red) and C (yellow). For each host genotype, tolerance is quantified as the rate at which the host loses its fitness with an increase in pathogen burden. The difference in resistance between hosts can be measured on the X-axis as the difference in the mean pathogen burden (vertical lines). The position of each genotype on the Y-axis is determined by its fitness in the absence of the pathogen and is not related to tolerance or resistance. Host genotypes A and B have the same resistance because the average pathogen burden that they carry is the same. However, genotype A is more tolerant than genotype B because it loses its fitness faster with increasing pathogen burden. This is reflected in the steeper slope in genotype A compared to genotype B. In contrast, host genotypes A and C have the same tolerance, but genotype A is more resistant than genotype C. Finally, the comparison of host genotype B and host genotype C represents a mixed case: genotype B is more resistant, but less tolerant than genotype C.

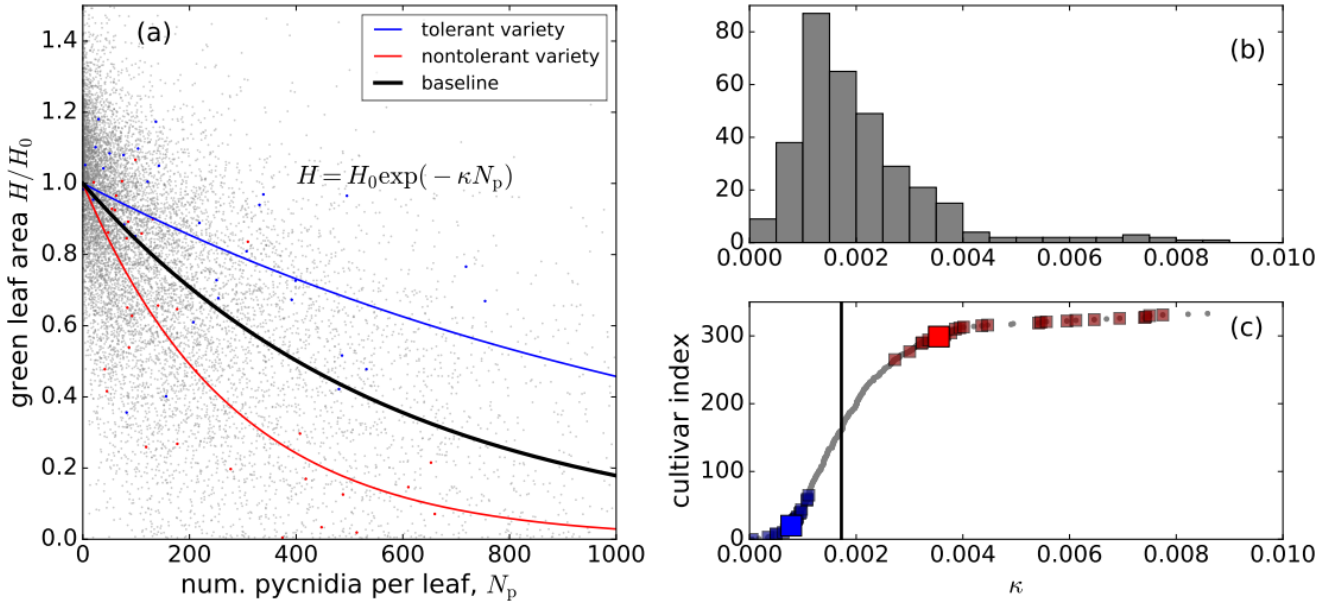

Figure 2 Tolerance of wheat to *Z. tritici* measured on the scale of individual leaves. (a) Green (Healthy) leaf area normalized by the leaf area in the absence of disease, H/H_0, is plotted versus the number of pycnidia per leaf N_p. 11152 individual leaf measurements are shown using grey points. Best fit curves based on the exponential function $H/H_0 = exp[-\kappa N_p]$ are shown for all data (black curve) and for two example cultivars, a tolerant cultivar (Intact, blue curve), and a nontolerant cultivar (Lynx, red curve). (b) The distribution of the 335 cultivars with respect to their κ-estimates. The most tolerant cultivars are those with the lowest κ-estimates at the left of the distribution. (c) Ranking of wheat cultivars according to their tolerance. κ-estimates for the 335 cultivars are shown in order of decreasing tolerance, that is increasing slope κ (grey points). Cultivars with tolerance significantly different from the baseline tolerance (black line) are marked with blue (more tolerant) and red (less tolerant), according to bootstrap t-tests with the confidence threshold of 0.05. Cultivars illustrated in panel (a) are marked using larger blue (cultivar Intact) and red (cultivar Lynx) squares.

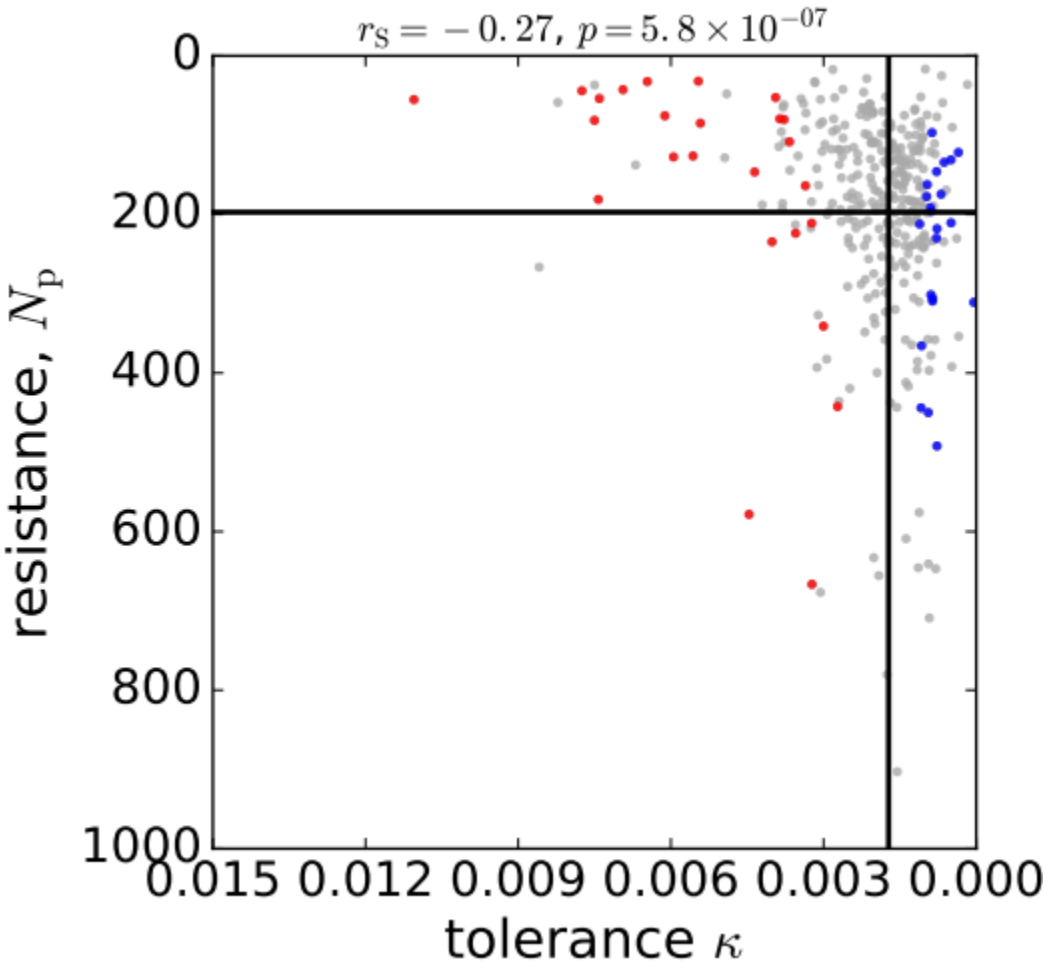

Figure 3: Correlation between leaf tolerance and resistance of wheat to STB. The measure of resistance, N_p is plotted against the measure of tolerance, κ, for each of 335 wheat cultivars (grey circles). Horizontal line shows the mean resistance and vertical line shows the baseline tolerance; blue/red circles mark the cultivars that are significantly more/less tolerant than the baseline.

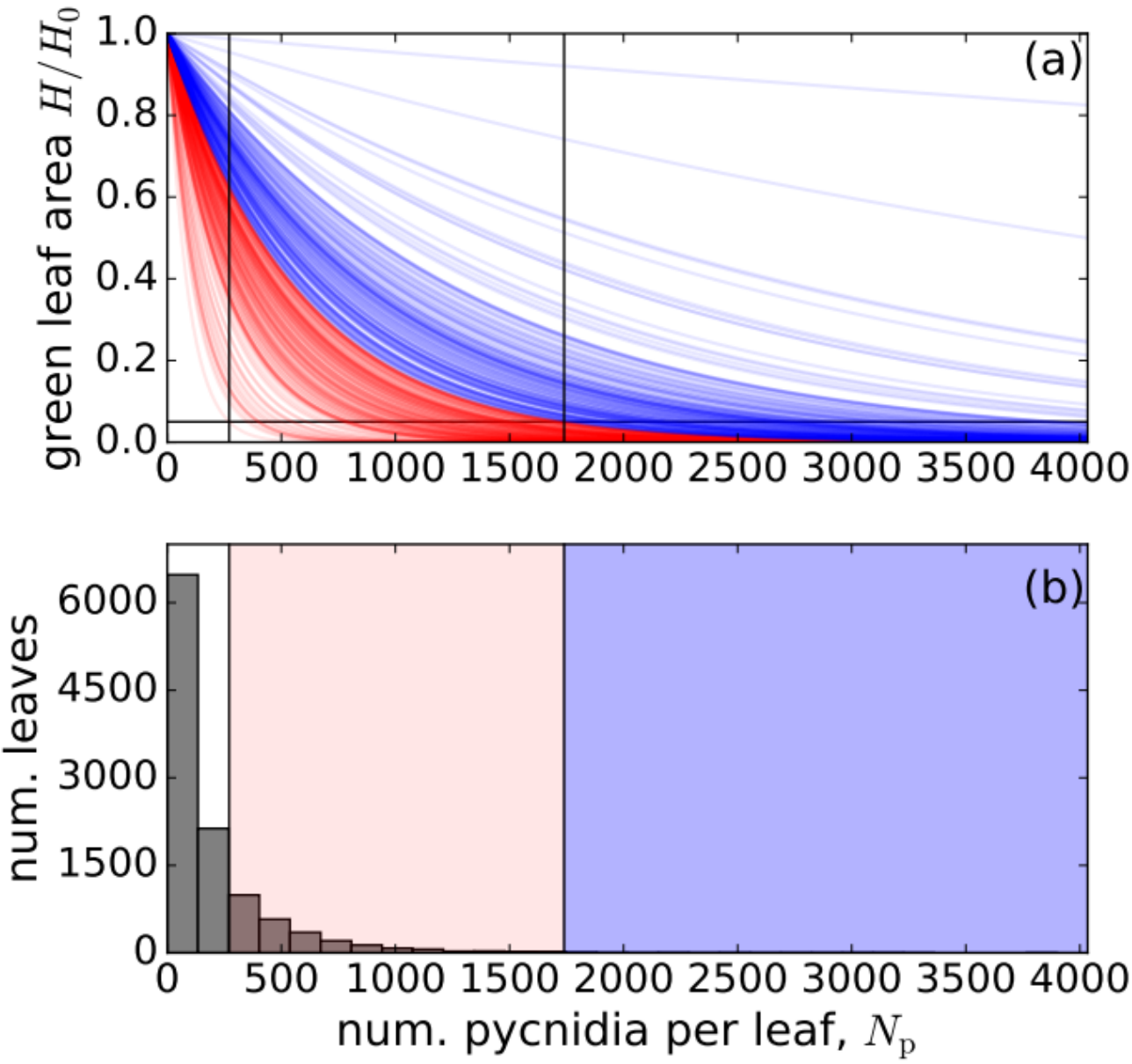

Figure 4: Limitation in the leaf area in tolerant and non-tolerant wheat cultivars. (a) Tolerance curves fitted to individual leaf data (raw data points are shown in Fig. 1a) for 335 wheat cultivars, blue curves correspond to cultivars more tolerant than the baseline (black curve) and red curves correspond to cultivars less tolerant than the baseline. The horizontal line shows the threshold H/H_0=0.05. (b) Distribution of 11152 individual infected leaves with respect to the number of pycnidia per leaf, N_p. Shaded areas illustrate the ranges of N_p-values in which the pathogen population infecting tolerant (blue) and non-tolerant (red) wheat varieties is affected by the limitation in the leaf area.