Title

Assessing phenotypic quantitative resistance of *Digitaria sanguinalis* to *Ustilago syntherismae*: from individual to population level

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Title

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Abstract

Digitaria sanguinalis can exhibit a smut caused by *Ustilago syntherismae*. In the present paper we deal with the phenotypic expression of the grass that can be observed under field conditions. Plants can be apparently healthy, completely smutted or show both tendencies, inflorescences bearing spikelets and at the same time sori (single individuals). Plasticity in fitness-related traits such as tillering pattern and the proportion of inflorescences with spikelets or completely transformed into sori at individual level was examined in distinctive individuals. In the study period 2011-2014, we observed and collected 244 individual plants (3.2% of the plants reaching the reproductive stage) with between 1 and 12 tillers. The mean number of reproductive structures per plant was 24.2 (20.4 exhibiting sori and 3.7 bearing spikelets). The spatial and temporal dynamics of the single individuals at plot scale was also analysed. We discuss the importance of individual responses from the perspective of plant resistance in the broadest sense: any system that can prevent infection or reduce the impact of fungi. Furthermore, we consider the importance of this subpopulation in disease prevalence.

Keywords: partially smutted; tillering; host plant resistance; large crabgrass; smut fungus

Introduction

Among fungi there are many species that can cause plant diseases. A central aim in plant pathology is to understand why and how pathogens damage their hosts (Pariaud et al. 2009). Pathogenic fungi can display three main strategies depending on their nutrition methods: biotrophs, necrotrophs and hemibiotrophs. In this paper we deal with biotrophic fungi that tend to cause disease on only one or a few related plant species, or in other words have a very limited host range. The two partners maintain a highly specialized relationship. Among the great diversity of existing host-pathogen interactions, we focus on smut fungi which can cause sterilization in plants.

In the pathosystem under study, *Digitaria sanguinalis* (L.) Scop. and *Ustilago syntherismae* (Schwein.) Peck are the main actors. Large crabgrass, sometimes known by other common names such as hairy crabgrass, is a summer annual species, currently distributed worldwide and usually considered as a weed. In temperate areas it only exhibits sexual reproduction, and the percentage of self-pollination is very high. As a representative of the family *Poaceae*, large crabgrass can produce tillers during the period of vegetative development and this process is strongly influenced by environmental conditions (Peters and Dunn 1971). *D. sanguinalis* features an intravaginal system of branching, and the spatial distribution of tillers follows a model described as a combination of dense and sparse branching.

The fungus (Ustilaginomycetes, Basidiomycota) can interact with the grass and cause disease. The observable characteristic is the sori that appear instead of the host's inflorescences, full of a dark powder which is the mass of ustilospores (hence the name smut) that overwinter in soil.

It is in the rhizosphere that the resistant fungal spores (ustilospores) and the initial host seedling can meet. Plants are provided with several pre-existing defences (Balmer et al. 2013). Likewise, fungal persistence relies on the identification of nearby suitable host plants and the ability to overcome host defences.

The establishment of disease by a biotrophic pathogen implies access to the interior of the plant organs to obtain the necessary nutrients. The first and very crucial step for the entry of the biotrophic fungi into the host tissues is the contact between the fungi and the plant surface (Tucker and Talbot 2001). Although the aptitude of this type of fungi to recognize the presence of the plant and vice versa by physical and chemical means is well known (Dagdas and Bozkurt 2015), it is important to consider that the encounter has a fortuitous component (being at the right place at the right time). In the interaction here presented, germination of fungal ustilospores must occur more or less at the same time as plant seedlings initiate their development after seed germination, and additionally the distance between the two organisms has to be small enough to permit physical contact.

According to Burdon and Thrall (2002), when a fungus-host plant interaction is studied it is necessary to consider all mechanisms of resistance (avoidance, tolerance). In the pathosystem under study we are interested in the disease resistance mechanisms of *D. sanguinalis*. As Burdon and Thrall (2003) summarize, there are two broad types of genetically determined resistance to infection in host plants that are called qualitative and quantitative. Niks et al. (2015) separate two different aspects of resistance and the associated terms qualitative and quantitative: the phenotypic phenomenon and the mode of inheritance. Jones and Dangl (2006) in a review allude to a complex interplay between biotrophic pathogen attack and host defence, and they refer to this relationship as a multi-phase ‘zig-zag’ process.

Tack et al. (2012) indicate that variation in host resistance has received much attention, and this could be due mostly to the importance for breeders to fight against crop diseases. But they emphasize the need to study the variation in pathogenicity, particularly in natural systems. In the pathosystem under study, the fungus can enter, depending on the environmental conditions, by overcoming the host plant resistance in the seedling stage (Mas and Verdú 2014). As Begerow et al. (2006) summarize very well, the life cycle of *Ustilago* species alternates the haploid phase with the dikaryotic parasitic mycelia, and they can only carry out the process of infection when the hypha is dikaryotic. Under laboratory conditions artificially testing infections, it has been observed that fungi enter mainly in the coleoptile and/or mesocotyl areas of the recently germinated seedling (Mas and Verdú, 2014). However, it cannot be ruled out that the entry of the fungus in field conditions might occur in young tillers (shoot infection), as happens in *Ustilago bullata* Berk. (Falloon et al. 1988). So this brings us to a well-known aspect that relates resistance with the stage of development at which the plant is infected.

Normally, plants that have been infected by a soil-borne *Ustilago* species sooner or later develop disease symptoms, for example, total sterilization. Although most smutted plants do not produce spikelets, some of them bear inflorescences with spikelets and at the same time transform inflorescences into sori; we call these distinctive partially smutted plants. This is a very interesting characteristic because each individual ensures the production of seeds and ustilospores, and this could have consequences from the point of view of disease maintenance. Some grasses can exhibit this feature, but usually they are perennial species (e.g. *Heteropogon contortus* (L.) Beauv. ex Roem. and Schult. parasitized by *Sorosporium caledonicum* (Pat.) Vánky, Fullerton 1975; *Bromus catharticus* Vahl by *U. bullata*, Falloon 1979; *Sorghum*

halepense (L.) Pers. by *Sporisorium cruentum* (J.G. Kühn) Vánky, Astiz Gassó et al. 2017).

The interaction between the smut fungus and the host plant admits different levels of study, from the molecular basis to the population ecology and genetics, and requires a significant effort to understand the coexistence of the two species evolving in space and time. Clearly, the way in which resistance is distributed in host populations and indeed the type of resistances occurring in them (whether qualitative or quantitative) will be very strongly influenced by the interplay of life history features of both host and pathogen and their interaction with the environment (Barret et al. 2008). As Thrall and Burdon (1997) argue, the relative spatial scales at which hosts and pathogens interact are crucial to understanding the evolution of resistance/virulence structure. *U. syntherismae* is a parasitic fungus that shows a transmission mode from flowers of one host to seedlings of another susceptible individual. According to Thrall and Burdon (1997), this means that in the present case its dispersal scale may be substantially smaller than that of its host.

Alexander (2010) proposed the necessity to answer two interrelated questions: 1) Is there any variation in the effects of disease on individual plants of *D. sanguinalis*? and 2) What is the variability of the *D. sanguinalis* population at phenotypic level through time and space? In a previous work we described some characteristics of the interaction of the two populations of the pathosystem (*D. sanguinalis*-*U. syntherismae*) at laboratory and field scales (Gallart et al. 2009; Mas and Verdú 2014; Verdú and Mas 2015; Mas and Verdú 2018). This paper presents results showing the phenotypic response of individual plants of *D. sanguinalis* against the possibility of infection by *U. syntherismae* and the monitoring of the dynamics of the interaction, from the perspective of host resistance and fungus virulence.

The three main objectives were: a) to study the effect of the smut pathogen on the fitness of the distinctive partially smutted individuals, in particular under two aspects: the vegetative development (tillering capacity) and, at the mature stage, how sori and panicles are distributed within the individuals; b) to evaluate the quantitative importance of inflorescences transformed into sori and inflorescences bearing spikelets; and c) to describe their spatial-temporal variation at plot scale and discuss the importance of these plants in disease prevalence.

Materials and methods

The *D. sanguinalis* plants were obtained from a study plot in a corner of a field near Barcelona belonging to the Institut de Recerca i Tecnologia Agroalimentàries experimental station (Torre Marimon, Caldes de Montbui, 41°36044″ N, 2°10017″ E). The field has an agricultural past with several crops (maize, sunflower, barley) grown under conventional tillage (Verdú and Mas 2015). In September 2004, smutted inflorescences of large crabgrass (*D. sanguinalis*) were observed for the first time.

At the beginning of each season in the four-year study period (2011-2014), quadrats of 0.25 m^{-2} (30, 26, 35 and 28 respectively) were set up to sample the plant population. Within the quadrats no seedlings that did not belong to *D. sanguinalis* were allowed to grow; the seedlings of other plant species that appeared within the quadrats were removed weekly. At the end of the season, using a Leica DISTO™ Plus laser distance meter, the precise location of the quadrats was obtained.

At the end of the season plants present in the quadrats considered within the plot were collected, counted and sorted according to their external appearance (in relation to disease status). Three types were initially considered by direct observation in the field, according to the signs of the disease perceived outwardly (or not) in the individuals. In

addition, in the laboratory, we confirmed through microscopic observation the existence of plants that have a healthy external appearance, possessing spikelets, but are nevertheless infected by the fungus (Mas and Verdú 2014). Table I summarizes the four phenotypes observed in *D. sanguinalis* individuals that we can consider, on the basis of the characteristics mentioned above. It should be noted that in addition to the four types there is another one, the group of immature plants (at the vegetative stage) at the end of the season, most of them belonging to the late cohorts. Without considering the inheritance system, according to Niks et al. (2015), a phenotypic nature of resistance has been assigned for each phenotype.

In the lab, each of the partially smutted plants (distinctive plants) was examined to determine the number of inflorescences bearing spikelets (with matured caryopses: IBS), the number of sori (ITSo), and in some cases the number of tillers (NT) produced by the individual. Histological sections were made of all stem nodes of some distinctive plants. The internodes were discarded mainly because they are fistulous, and so the hyphae were more difficult to observe there than in the nodes. The sections were made by hand under a stereomicroscope, using razor blades, and were not embedded in resin previously. The sections, between 5 µm and 20 µm thick, were cleared by immersing them in 5% NaOH at 45ºC for 2h, washed with distilled water, stained for 1 minute with 0.05% toluidine blue, washed again, and mounted in diluted polyvinyl alcohol for microscopic examination.

The proportion of distinctive (partially smutted) plants was obtained by dividing the number of distinctive plants by all the plants in the quadrat.

The analyses of the distinctive plant density (distinctive plants per m^{-2}) and the proportion of distinctive plants, as well as the three variables (IBS, ITSo and NT) related to their fitness, were performed using generalized linear models. The effect

'year', the co-variable 'total plant density' and their interaction were considered in the models. 'Total plant density' (plants per m^{-2}) was decimal logarithm transformed prior to the analyses. In addition to the interannual variation, we focused our efforts on within-population density effects because the proximity of neighbours can profoundly affect the development of individual plants. The three fitness variables mentioned were also analysed with the same models considering only the co-variable 'total plant density'.

In the case of the proportion we used a binomial distribution, while for the density and the fitness variables a negative binomial distribution was employed. Parameters were estimated using the complementary logit link function (in the case of the proportion) and the log link function (in the rest of the variables) and Type III analysis options; the dispersion parameter was estimated as the deviance divided by its degrees of freedom because of overdispersion, and all statistics were adjusted appropriately. Likelihood ratio statistics were used to compute the significance of each source of variation. The GENMOD procedure (SAS, 2013) was used to perform generalized linear models and the corresponding means comparisons. The UNIVARIATE procedure (SAS 2013) was employed to obtain the basic statistics of all variables.

The number of distinctive plants in each quadrat (number of individuals per 0.25 m^{-2}) allowed us to study at plot scale the spatial variation across time in the resistance structure of *D. sanguinalis* to the smut fungus *U. syntherismae*. Data were subjected to the bivariate interpolation method, and contour maps of distinctive plant abundance within the study plot were generated using the G3GRID and GCONTOUR procedures of SAS (SAS 2013) for each of the four years.

Results

In the four-year study period (2011-2014), a total of 244 distinctive individuals were collected, two of them in a state that did not allow their transfer to the laboratory to be analysed (they crumbled in the hand). Considering all the years, the mean annual percentage of distinctive plants with respect to the total plants was 3.0%. Without considering the possible existence of asymptomatic plants, this rate would represent a low level of qualitative resistance in the population.

All the observed individuals described in this work as distinctive plants exhibited sori (inflorescence transformed into an ustilospore mass) and panicles with spikelets. This means that in the case of individuals with only one tiller the parent shoot or primary culm branches at one of the nodes located above the base. Plants can vary considerably in appearance, not only with respect to the number of tillers, but also with respect to the distribution of inflorescences with spikelets or completely transformed into sori. In this regard, Figure 1 shows two very different patterns. There are individuals that display separate tillers that are either completely smutted or only bear spikelets (Fig. 1 B). But there are also individuals in which both sori and spikelets can be observed in one and the same tiller (Fig. 1 A).

Among the sources considered in the analyses of the number and the proportion of distinctive plants (Table II), only the co-variable 'total plant density' was significant from the statistical point of view, although not to the same degree for both response variables. In general the probability functions obtained with the model were quite similar between years. In the range of densities of total plants observed in the study (111-722 individuals per m^{-2}), the probability remained practically the same or rose very slightly as the density increased.

As Table III shows, the mean number of distinctive plants per m^{-2} observed in the field over the period 2011-2014 ranged from 4.8 to 11.1. This represents a quite low percentage (between 0.5% and 4.2%) of the total plants.

The comparison of the years depends on the mean density of total plants considered. If we use a density of 324.6 individuals per m^{-2}, the global mean density recorded in the study period, the least-square mean of 2012 differs significantly from that of the rest of the years. This is due to the particular meteorological conditions that occurred in 2012, with a prolonged dry summer period followed by the beginning of autumn with rainfall that allowed the emergence of a late cohort. Indeed, in that year the density of total plants was comparatively very high.

In the four-year study period, on average, a distinctive individual showed a number of tillers slightly higher than 5 (ranging from 1 to 12). The mean number of reproductive structures was 24.2 (20.4 exhibiting sori and 3.7 bearing spikelets). The percentage of inflorescences with spikelets with respect to the total number of inflorescences in the distinctive plants was 15.4%. In spite of its inheritance, this value would represent the level of resistance of a phenotypic nature.

The variation throughout the study period is presented in Table III. In the first three years, the values of the variables were quite similar, whereas 2014 was the year with the highest values. This is largely due to the effects of intraspecific competition, as in 2014 the density of potentially competing plants was rather lower than what was observed in the other three years.

The models obtained for predicting the fitness variables in the distinctive plants as a function of density, considering the four years together, are given in Figure 2. The numbers of tillers, inflorescences with spikelets and sori present in the distinctive plants were significantly density-mediated. In general, the higher the density of competing

plants the lower the values of these three variables. However, the number of sori (ITSo) is the variable most affected by density.

Figure 3 shows the spatial variation of the number of distinctive plants (ndp) of *D. sanguinalis* within the study plot throughout the survey period. The distribution maps obtained reveal an aggregate spatial pattern of the variable (means are always lower than variances), mainly due to environmental factors, in particular differences in the rainfall recorded each year, and soil heterogeneity within the study plot.

The maps show the existence of areas with a relatively high presence of distinctive plants.

Discussion

The distinctive plants studied represent a group of plants, from the point of view of phenotyping expression, with some level of resistance, i.e., plants with a capacity to counteract the advance of the fungus or colonization once infection has occurred. The observed relative presence of these plants in the 2011-2014 populations was low (3.0% of the total plants). Considering a longer period, the values of this percentage ranged between 0.53% and 7.34% (Gallart et al. 2009; Verdú and Mas 2015).

In the aerial part, distinctive plants may have simply a parent shoot or may have experienced tillering. The number of tillers varied from 1 to 12, with a mean value of 5.03. It should be taken into consideration that there was a certain level of intraspecific competence, depending on the particular conditions of each year. Another interesting feature is how sori and inflorescences with spikelets are distributed in the distinctive individuals. The inflorescences that produce spikelets only represent on average 15.4% of the total, which means that these plants produce more sori loaded with spores. Tillers

can carry only sori or inflorescences, or it may also be the case that a tiller exhibits both sori and inflorescences.

Peters and Dunn (1971) studied the development of tillers in *D. sanguinalis*. They observed that tiller formation began a little more than a month after emergence, depending on the tiller. Once the fourth leaf stage is attained the plant development is mainly by means of tillering. This characteristic defines the habit of the species, and depends on the space available for plants. In fact, this trait is subjected to the level of competence experienced by individuals (Fig. 2).

Thus, tillering and the distribution of sori and inflorescences show the plasticity of the plants, and these traits are very important in their interaction with *U. syntherismae*. Falloon et al. (1988) observed infection from an artificial inoculation of actively tillering plants and they produced healthy and smutted inflorescences. The behaviour of *D. sanguinalis* plants displays a dependence of genet-level fitness on ramet-level fitness as a modular organism, which can be exploited in this way by the fungus. Another interesting aspect is the possibility of the host plant being infected by more than one genotype belonging to the same pathogen (Read and Taylor 2001). One of the best studied interactions from the perspective of the existence of multiple infections is *Microbotryum violaceum* (Pers.) G. Deml and Oberw. – *Silene latifolia* Poir. (López-Villaciencio et al. 2007).

In the laboratory we have also confirmed the existence of asymptomatic plants with the presence of internal fungal hyphae (Mas and Verdú 2014). So, the effects of the disease on the individual plants can be appreciated in three forms, two of which symptomatically involve sterilization (fully or incompletely). Completely smutted plants (exhibiting only sori) have experienced fungal infection and subsequent successful colonization. They do not overcome the fungi, so they do not afford any

possibility of resisting fungal attack. Other plants, which we call distinctive because they exhibit both sori and spikelets, have been able to counteract the initial infection, but not totally. As Burdon and Thrall (2002) point out, these plants have some mechanism of resistance that partially reduces the impact of the pathogen. The resistance of *D. sanguinalis* is quantitative according to its phenotypic nature, but its inheritance could be qualitative or quantitative (Niks et al. 2015). And as Barret et al. (2008) specify, pathogen evolutionary dynamics can be strongly influenced by qualitative versus quantitative genetic control over innate resistance in host plants.

Distinctive plants represent the empirical evidence that links disease dynamics to host population genetic structure. They exhibit tolerance to the pathogen once infection has occurred, either in the seedling stage or maybe later when the plants start to develop tillers, although with respect to this second alternative we have no confirmation. But as Falloon et al. (1988) point out, the infection of tillers (even the buds involved) could be feasible. The different forms of distinctive plants, in relation to the variation in the distribution of sori and panicles on the tillers observed, reveal a certain degree of diversity in infection processes (in quality and quantity). The type of resistance expressed by the distinctive plants, appreciable from the phenotypic point of view, can be considered as partial (Niks et al. 2015). With respect to the mode of inheritance, it would seem to be a case of quantitative resistance, due to the participation of several genes, each contributing a small proportion of the resistance level (Niks et al. 2015). Without going into the issue of the type of inheritance of *D. sanguinalis*, the subpopulation formed by distinctive plants, as well as asymptomatic plants in which the effects of infection are not visible, would represent the possibility of partially neutralizing the pathogen. Distinctive plants that produce spikelets would ensure the entry of seeds into the soil bank. This offspring, considering the high degree of self-

pollination shown by the species, would very likely be susceptible to fungus infection, thus representing a chance of disease prevalence. If we raise the hypothesis that pathogen population remains without variation in the degree of virulence, without the concurrence of asymptomatic and distinctive individuals, the *D. sanguinalis* population would at any given time be formed by individuals either completely resistant or completely susceptible to fungi.

In the populations from 2011 and 2012, the mean number of inflorescences bearing spikelets per square metre formed by non-smutted plants was around 1700 and 1600 respectively, while the mean number of sori formed by entirely smutted plants was around 900 and 700 (Verdú and Mas 2015). Merging the mentioned inflorescence and sorus densities with data from distinctive plants for these two years (Table III), it becomes evident that the inflorescences bearing spikelets formed in distinctive plants were 3.8% and 0.7% of all the inflorescences formed in 2011 and 2012, but their sori represent 15% in 2011 and 8.1% in 2012. The relative contribution of the distinctive plants to the plant and fungal fitness was low in terms of proportion of inflorescences bearing spikelets and proportion of sori, but not as low for the fungal fitness as for the plant fitness.

We have in front of us a very interesting system to investigate from a co-evolutionary point of view. The life histories of the smut pathogen and the host grass are highly determinant for understanding the interaction of the two populations (Barret et al. 2008), and consequently the evolution of both host resistance and smut virulence are closely related, although the two aspects have mostly been studied independently. At this point, for obvious reasons we have undoubtedly paid special attention to the plant resistance. However, as has been verified in the laboratory, the fungus shows some variability in the degree of virulence. In an artificial infection experiment, the

ustilospores of partially smutted plants were found to be significantly less virulent than spores from completely smutted plants (Jorba et al. 2015). Therefore, there is evidence of genetic variability in the plant population, and everything points to the existence of variability in the smut pathogen population. As Susi et al. (2015) comment, co-infection is a common phenomenon in nature. Hence, the possibility of the existence of multiple infection or co-infection processes must be considered. This allows us to raise the possibility that distinctive plants are the result of this process by some pathogen strains representing several genotypes. Moreover, although there is no sign of it, the possibility that some distinctive plants arise from a later infection cannot be excluded. So the interaction turns out to be an interplay between two actors with the respective capacity to infect or colonize (i.e. the fungus) and at the same time to avoid or resist (partially or completely, i.e, the plant.

In the study period we observed a varying number of distinctive plants depending on the total population density, which in turn is related to the particular conditions of the year. The spatial distribution of these plants tended to be clumped. However, as Burdon and Thrall (2008) comment, the temporal and spatial nature of host plant and pathogen interactions is characterized by a permanent dynamic of change. Effectively, the study plot is heterogeneous from the environmental point of view (Mas and Verdú, 2018), not only with respect to the abiotic factors (especially related to soil surface characteristics, appreciable at a glance), but also in relation to the biotic factors (in particular the two interacting populations, as the number of ustilospores and spikelets are heterogeneously distributed in the study plot; Mas and Verdú, 2018). The distribution of distinctive plants and the identification of relatively important areas within the plot, although differing over time, can be useful to study the causes of the variability in this subpopulation. Figure 3 shows that, year after year, there was a single

patch of distinctive plants, with a gradient that roughly seems to have the highest densities at the bottom left-hand quarter of the plot. A study addressing the distribution and abundance of ustilospores in the soil in the winter of 2012-13 and the plant disease expression in 2013 (Mas and Verdú, 2018) shows that this area of the field was also the area with the highest ustilospore abundance in the 5 cm topsoil and also the area with the highest percentages of diseased plants, and at the same time the highest total plant densities. So, in spite of their relatively small relevance in number per surface area, the role played by the spikelets and sori formed in this area by distinctive plants could be crucial in disease prevalence or, in other words, the production of susceptible spikelets and/or low-infective ustilospores in an environment with a relatively high proportion of resistant spikelets and high-infective ustilospores permits the presence of diseased plants each year.

It must be taken into account that the first and very important step in host-pathogen interaction concerns the encounter between ustilospore and seedling, and the probability is conditioned by the amount of spores and seeds and the distribution of both. If the meeting occurs, what will happen depends on the type of ustilospore and seed involved. Resistance in *D. sanguinalis* plants can be expressed by three phenotypes: a) healthy plants that prevent fungal entry, b) asymptomatic plants, and c) distinctive plants. Very likely, the inheritance of these three types is different. In any case, only the distinctive plants are detectable at the field level. Furthermore, the distinctive plants represent a paradox. On the one hand they reduce the fitness of the pathogen population (the number of ustilospores falling into the soil will be lower than in the case of completely smutted plants). On the other hand their seeds, which are probably susceptible, assure the chance of infection for the next generation.

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FIGURE CAPTIONS

Figure 1. Appearance of partially smutted *Digitaria sanguinalis* plants (distinctive individuals) collected in the study plot (Torre Marimon, Barcelona). Both individuals A and B possess three tillers. However, in A one of the three tillers exhibits at the same time sori (2) and an inflorescence bearing spikelets; B has one completely smutted tiller and two bearing spikelets. The micrographs in the centre of the figure are from plant A; the bottom micrograph is of a longitudinal section of the stem base, and the top one is of a longitudinal section of the node that bears the upper leaf of the tiller.

Figure 2. Variation in the number of tillers (NT), number of sori (NTSo) and number of inflorescences with spikelets (IBS) of the distinctive plants of *Digitaria sanguinalis* as a function of density in a study plot (Torre Marimon, Barcelona) over the period 2011-2014. Fitted functions were drawn from the parameters estimated in the generalized linear model with 95% confidence limits.

Figure 3. Contour maps obtained by means of data interpolation showing the spatial variation of the number of distinctive plants of *Digitaria sanguinalis* per quadrat (0.25 m^{-2}) within the study plot (Torre Marimon, Barcelona) over the period 2011-2014. Legend: 0- not surveyed, 1 to 7- from lowest to highest densities, which vary each year. The steps of the grey scale represent leaps of 0.7 plants $0.25m^{-2}$ in 2012, of 0.8 plants $0.25m^{-2}$ in 2011, of 0.9 plants $0.25m^{-2}$ in 2014 and of 1.2 plants $0.25m^{-2}$ in 2013.

Table I. Phenotypic expression of *Digitaria sanguinalis* plants from the point of view of disease status that can be caused by *Ustilago syntherismae*.

Plant phenotype	External symptoms	Presence of internal hyphae	
Non-Smutted (NS)	All the inflorescences bearing spikelets	NO	Healthy plants (1)
		YES	Asymptomatic plants (2)
Entirely Smutted (ES)	All the inflorescences transformed into sori	YES	Completely smutted plants
Partially Smutted (PS)	Branch tips with inflorescences bearing spikelets and branch tips with sori	YES (at least in some tillers of the individual)	Distinctive plants (3)

(1)These plants may not have experienced any contact with the pathogenic fungi, or they may prevent fungal entry (phenotypically qualitative resistance*).

(2)Plants reduce the impact of fungi by preventing the formation of spores (phenotypically qualitative resistance*).

(3)Plants can reduce the impact of a pathogen once the infection has occurred (phenotypically quantitative resistance*) but fungi can form spores.

* According to the classification proposal by Niks et al. (2015) of the qualitative/quantitative nature of resistance to biotrophic filamentous plant pathogens.

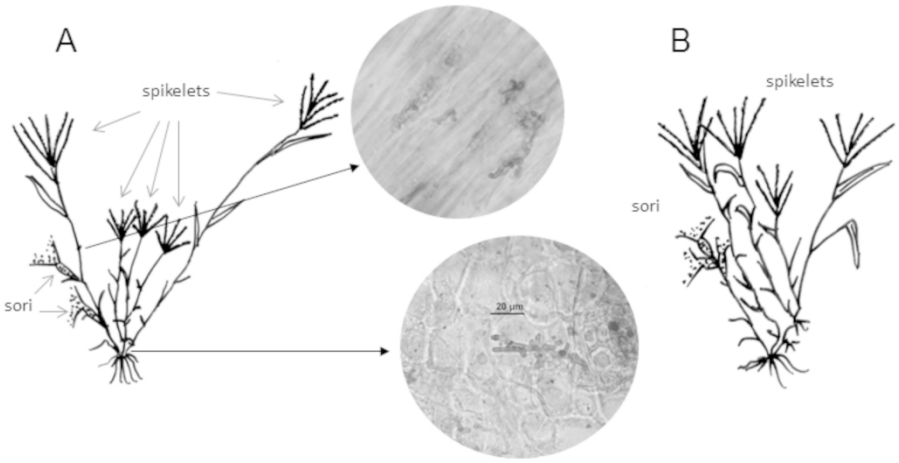
Table II. Likelihood ratio statistics of the effect 'year', the co-variable 'total plant density', and their interaction in the analysis of two variables related to the abundance of *Digitaria sanguinalis* distinctive plants (upper third) and four variables related to their morphology (lower third) in the study plot (Torre Marimon, Barcelona) over the period 2011-2014.

Variable	Effects								
	Year			Total plant density[a]			Year *Total plant density		
	df num/ df den	chi-square	*P* > chi-square	df num/ df den	chi-square	*P* > chi-square	df num/ df den	chi-square	*P* > chi-square
Distinctive plant density (pl m^{-2})	3/111	4.65	0.1991	1/111	40.69	<.0001	3/111	3.5	0.3201
Proportion of distinctive plants		6.13	0.1053		5.72	0.0168		5.34	0.1486
Number of inflorescences (pl^{-1})	3/234	1.45	0.693	1/234	6.31	0.012	3/234	0.07	0.9956
N. of inflorescences bearing spikelets (pl^{-1})		3.89	0.2732		2.41	0.1206		3.76	0.2886
N. of inflorescences transformed into sori (pl^{-1})		0.4	0.9392		4.57	0.0325		0.1	0.9917
Number of tillers (pl^{-1})		0.68	0.879		0.02	0.9023		0.53	0.912

[a]Total plant density was decimal logarithm transformed.

Table III. Annual variation of two variables related to the abundance of *Digitaria sanguinalis* distinctive plants (upper third) and four variables related to their morphology (lower third) in the study plot (Torre Marimon, Barcelona) over the period 2011-2014. SD, standard error or the mean. n, number of observations.

Variable	2011			2012			2013			2014		
	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	n
Distinctive plant density (pl m^{-2})	10.7	1.5	30	4.8	1.6	26	11.1	2.5	35	5.1	1.6	28
Percentage of distinctive plants (%)	3.6	0.5	30	0.5	0.2	26	4.2	0.8	35	3.3	0.9	28
Number of inflorescences (pl^{-1})	13.2	1.3	80	15	2.5	30	15.5	1.1	96	79.3	8.8	36
N. of inflorescences bearing spikelets (pl^{-1})	3.9	0.4	80	2.4	0.4	30	3.6	0.4	96	5.1	1.6	36
N. of inflorescences transformed into sori (pl^{-1})	9.3	1.3	80	12.6	2.5	30	11.9	1.1	96	74.3	9	36
Number of tillers (pl^{-1})	4.6	0.6	10	3.8	0.3	30	4.6	0.3	37	7	0.5	28
Total plant density (pl m-2)	295.5	31.7	30	722.5	75.5	26	225	26.1	35	111	12.8	28



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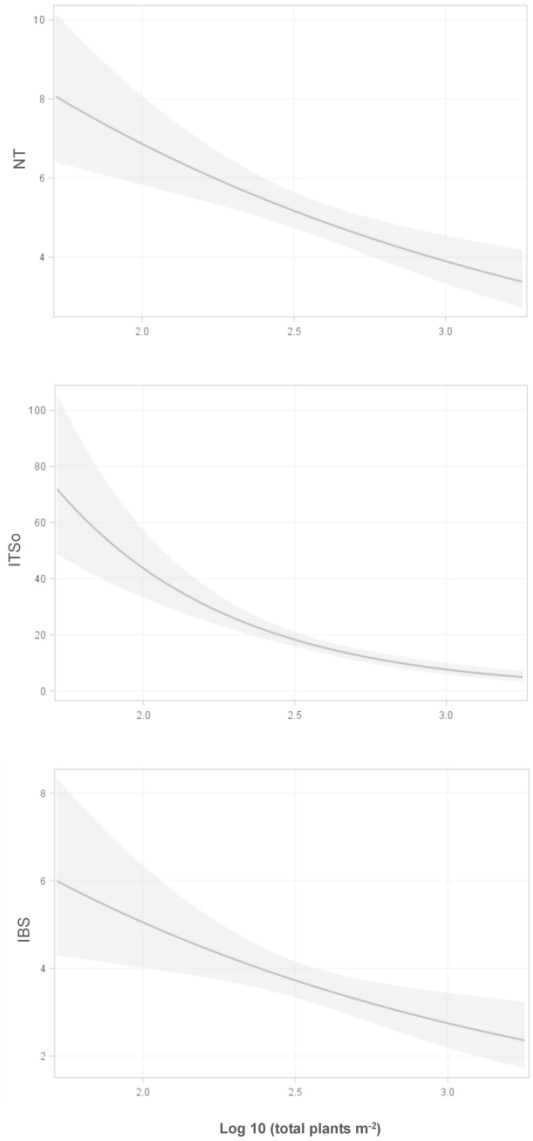

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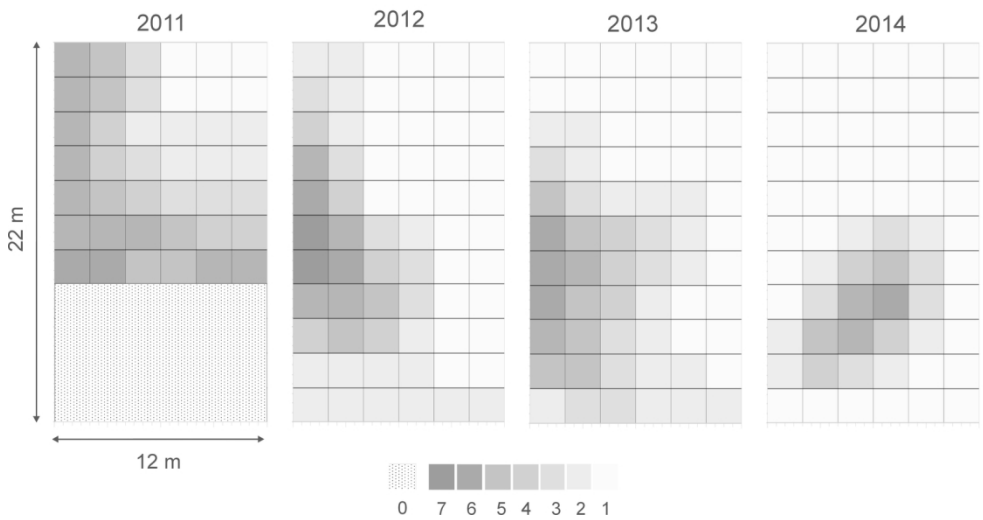
262x526mm (300 x 300 DPI)



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