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Role of rain-splash in the progress of *Septoria* leaf blotch within a winter wheat variety mixture

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Summary

The efficiency of variety mixtures for controlling foliar fungal diseases dispersed by rain-splash is assessed by quantifying physical and genetic barriers against *Mycosphaerella graminicola (Septoria tritici)*. The mixture of a susceptible wheat variety with a partially resistant wheat variety, as well as the mixture with barley, did reduce the disease of the susceptible variety in pure stands by 14% and 56%, respectively. The progress of *Septoria* disease depends on the number of infectious cycles in relation to rainfall occurrence and characteristics. Two effects have been identified. First, the "umbrella" effect of totally or moderately resistant varieties occurs when raindrops were intercepted. This effect is decreasing with the increase of kinetic energy of rain. Second, the "barrier" effect corresponds to the interception of sporecarrying droplets by the partially or totally resistant plants. The comparison of experimental results with those of a preliminary model of splash-dispersal in a variety mixture shows similar trends.

Key words: wheat, variety mixtures, rain-splash dispersal, Mycosphaerella graminicola

Introduction

Septoria leaf blotch caused by Mycosphaerella graminicola is the most damaging fungal foliar disease of wheat in Europe. In this paper the opportunity to limit disease progress through the use of mixture of winter wheat varieties is investigated. Such an approach has been identified to be successful for wind-dispersed diseases such as brown and yellow rusts and powdery mildew on cereals when using a mixture of two varieties containing at least one highly resistant variety; (Finckh et al., 2000).

Because the length scale of spore dispersal by rain-splash and the spatial characteristics of a wheat variety mixture canopy are of similar orders of magnitude, the use of variety mixtures could be of interest for splash-dispersed foliar diseases (Huber *et al.*, 2006). Few other research works have reported that variety mixture could be effective for reducing the amount of diseases (in the case of *Rhynchosporium secalis* of winter barley) when it is dispersed by rain–splash (e.g. Newton *et al.*, 1997). For the case of *Septoria* leaf blotch, effects of the mixture for reducing the disease amount has been shown to be moderate (Cowger & Mundt, 2002).but significant (Mille *et al.*, 2006) Thus, for *Septoria* leaf blotch, the research challenge is two-fold: (i) from the epidemiological point of view this pathogen is known to be typically splash-dispersed with pycniospores being carried by droplets produced by raindrop impact on lesions, (ii) from the plant protection point of view the lack of fully resistant wheat cultivars and the loss of efficiency of fungicides, favours ecological

approaches to disease control such as mixtures of varieties or species.

Experimental and modelling results which examine two situations of mixed canopy, a mixture of moderately resistant and susceptible wheat varieties and a combination of wheat and barley varieties, are presented.

Materials and Methods

Field experimentations

Compounds of the cultivar mixtures were Koreli, partially resistant wheat (PR) against *Septoria* leaf blotch, and a susceptible (S) wheat variety Sogood. A mixture with the susceptible wheat variety Sogood and barley has been produced to test the case of a totally resistant variety. Barley was used in the mixture because of the lack of a totally resistant wheat cultivar.

Field experiments were split in two parts, one was treated with fungicide and the other was without fungicide treament. Therefore four conditions of mixtures were studied: varieties Koreli (PR) and Sogood (S) in pure stands, a mixture of Sogood (S) and Koreli (PR) (1:3) and a mixture Sogood (S) – barley (1:3). The mixture proportions (in grains) were ¹/₄ for the susceptible variety and ³/₄ for the resistant variety. Each of these four mixtures was repeated in four plots. Seeds were sown with a density of 250 grains m⁻².

Septoria leaf blotch disease was assessed by evaluating the percentage of foliar area affected by the disease, counting the pycnidia on the leaf, and evaluating the surface of senescent leaves, on a sample of 20 cultivars (5 plants per plots) once a week and making sure to measure at least 5 plants by variety in the mixture. The foliar senescence was measured in both treated and untreated plots, in order to distinguish natural and disease-induced senescence dynamics. Therefore, two aspects of resistance are taken into account, the first one is the genetic plant resistance and the second one is the physical barrier to the splash droplet transfer created by both resistant and susceptible plants. Disease progress is analyzed in relation to plant occurrence in space and rain conditions over time.

Measurements of rain were of two kinds, a classical tipping bucket rain gauge (0.2 mm in precision) for the rain rate and a optical single-beam disdrometer (CETP-Precis mécanique, Paris). The probability density functions (Fig. 1) of drop diameters and velocities (Ulbrich, 1983) for the computation of integral rain parameters such as kinetic energy (Uijlenhoet & Stricker, 1999).

Modelling approaches

The mechanistic model of water transfer by rain splash in a canopy consists of several components: a set of raindrops interception by organs of the canopy, splash droplet production from impacting raindrops (Yang *et al.*, 1992), droplet motion in the free canopy space (Macdonald & McCartney, 1988), and droplet interception by plant elements. Forcing variables are rain characteristics in terms of drop size and velocity distribution depending on the type and intensity of rain (Ulbrich, 1983) and a simplified canopy structure (around 1 m^2) described in three dimensions, consisting of a single stem described with a cylinder, and five levels of rectangular leaves.

This model is based on experimental results obtained using single incident drops. Numerical integration techniques rely on Monte Carlo methods (Binder & Heermann, 1992) and also on the explicit calculation of droplet trajectories. Modelling techniques and validations are detailed elsewhere (Saint-Jean *et al.*, 2004). The model has been adapted here to take into account three aspects. First, elements of the canopy structure are characterized by a level of resistance. Second, the water transfer has been modified to take into account the transport of the disease (spores). To do so, droplets have been affected with a potential of infection depending on the resistance of the host. Droplets generated from totally resistant plants have no potential for infection, and droplets generated from nil to moderately resistant plants have a potential of infection linked to the level of resistance (0 to 1). Third, multiple rain occurrences have been taken into account in the model for simulating several cycles of the disease progression. Therefore, the final stage of the potential diseases progression are injected in to the initial state of a next run of the model, and so on.

Results

Disease progress

The weekly evolution of the percentage of disease foliar area summed over the whole plant of the several mixtures is plotted on Fig. 1. During two periods in the first 9 weeks, the level of disease is maintained approximately constant with non-significant differences in the four types of mixture. The emergence of new green and healthy foliar surfaces during this period compensates for the increase of surface diseased at the leaf scale. In the last 4 weeks of the growing season, the number of leaves remains constant, *i.e.* no new leaves are emerging and the amount of diseased surfaces are increasing in all conditions of mixtures. The less the amount of resistant compound in the mixture, the more diseased the surfaces and the steeper the disease progression.

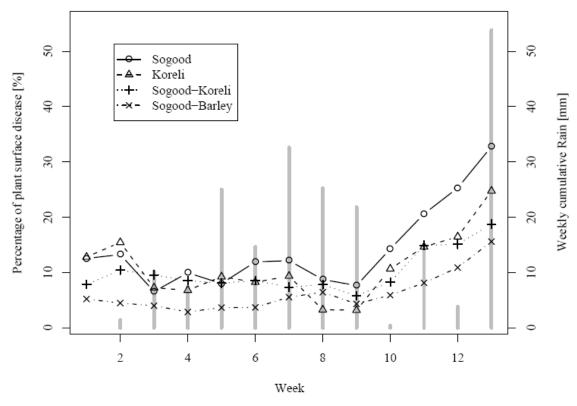


Fig. 1 Time change of the diseased plant surface (left legend), mean over at least five plants on four plots of different mixtures: Sogood (S) pure stand, Koreli (PR) pure stand and mixtures Sogood (S) –Koreli (PR) and Sogood (S) – Barley (R). Weekly cumulative rain rate (vertical bars) over time (right legend) shifted by approximately 2 weeks to account for the latency period of the pathogen. First week is March 17^{th} 2008

In order to compare the differences between the amount of diseases over the whole season on the several mixtures, the area under the disease progress curve (AUDPC) was calculated (Jeger, 2004) in terms of percentage of foliar surface diseased (Fig. 1):

$$\text{AUDPC} = \int_{t_0}^{t_f} Y_t \, dt \quad ,$$

with Y_t the percentage of the diseased surface, and t_0 and t_f , the times of first and last measurements. The AUDPC is 1158 (Sogood (S)), 904 (Koreli (PR)), 840 (Sogood (S) – Koreli (PR)) and 504 (Sogood (S) – Barley (R)). From the Tukey HSD (Honestly Significant Difference)

test, it has been shown that Sogood (S) and (Sogood (S) – Barley (R)) were significantly different from the other mixture. Koreli (PR) plots and Sogood (S) – Koreli (PR) plots were non-significantly different in terms of diseased surface over the whole seasons (*p*-value <0.01).

On other hand, comparing the disease incidence reduction of the susceptible cultivar, in the condition of mixture and in pure stand, it was found that disease incidence of Sogood (S), with Koreli (PR) and with Barley (R) was been reduced by 14% and 56%, respectively. Also, it is important that the disease incidence has also been reduced on the partially resistant cultivar Koreli (PR) in the mixture compared to the pure stand.

Comparison of the canopy green surface

In order to evaluate the potential of the mixtures, the progression of green and healthy surface during the last period of the growing season is compared with the fungicide-treated plots. In Fig. 2, the progression in time of the green foliar surface is plotted. The treated plots clearly show greener and healthier surfaces, whereas the susceptible plants in the pure stand and untreated plots show the worst cases. In between, the benefit of the mixture is clearly seen. Indeed, in the mixture susceptible-moderately resistant plot, a completed healthy leaf has been preserved compared to those in the pure stand and one and half healthy leaves have been preserved in the mixture susceptible-totally resistant plot.

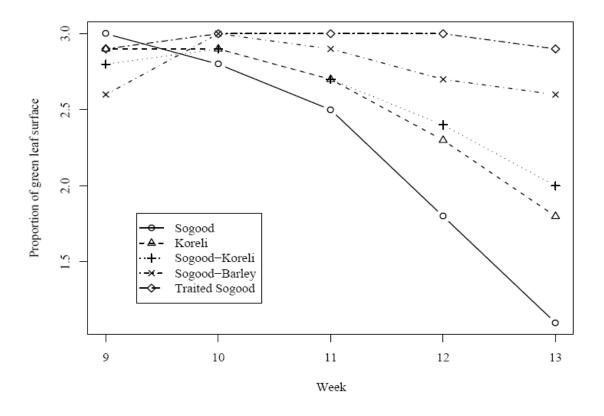


Fig. 2. Time change of the quantity of green (or healthy) leaves. A value of 1 corresponds to 1 green leaf of different mixtures: Sogood (S) pure, Koreli (PR) pure and mixtures Sogood (S) –Koreli (PR) and Sogood (S) – Barley (R). Week 9 is May 14^{th} 2008 (emission of flag leaf).

Numerical simulations

With the help of numerical simulations, the possibility of simulating the transfer of fungal spores by rain splash within a mixture of cultivars was simulated. Fig. 3 illustrates the dispersal potential

of a splash-dispersed disease after 5 rain events. As a first approach, each of the five successive rain events was considered to be equal with a rain rate of 10 mm h^{-1} and of the kind of a classical shower (Ulbrich, 1983). The latent period of the pathogen was not considered to be limiting and the canopy architecture was assumed to be constant over time.

It can be shown that the disease progression, initially on the first row, slows down in the case of the mixture. Also, the plant surface diseased percentage is kept below 20%, and the last rows are preserved from any disease. In comparison to the susceptible monocrop, the first four rows (at 80 cm from the initial source) of plants are at least at a level of surface diseased of 30-40 %, and the last row starts to show one or two leaves affected by the disease.

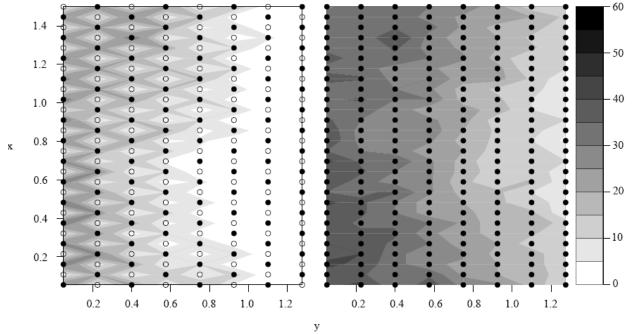


Fig. 3. Comparison of the spatial spread of a potential of disease within two canopies of a size of 1.3 x 1.5 m, the key level scales 0 (lighter gray) to 60 % (darker gray) of percentage of surface diseased on the plant. Initial inoculum was on the first leaves of the first row of plants (*i.e.* on the left side of sub figures). On the right side potential of dispersion of a canopy composed of pure stand (white dots are position of plants), on the left side, the potential of dispersion the bi-cultivar of equal proportion (black dots are position of susceptible plants and white are position of resistant plants).

Discussion

In the variety mixture (1/4 moderately susceptible + 3/4 moderately resistant), preliminary results show that disease progress was reduced by a delay of one leaf compared to the susceptible variety in the pure stand. These results should be put in the context of the crop yield assement, because this is extremely dependent on the healthiness of the last leaves. The benefit was higher in the bicrop mixture and was confirmed by the use of a physical 3D model of spore transfer by rain-splash (Saint-Jean *et al.*, 2004).

Progression of the disease has been compared to characteristic of the rain (Fig. 1). By the use of a disdrometer, the energetic parameters of the population of raindrops, such as their kinetic energy, was computed. Preliminary results points out that the creation of new pycnidia on a leaf is correlated with rain events of drops with high kinetic energy. Along with the genetic barrier created by the different resistances, a physical barrier, named an "umbrella", is created, preventing raindrops reaching lesions. The kinetic energy of raindrop expresses here the possibility of the raindrop to penetrate the canopy. The "umbrella" effect of totally or moderately resistant varieties occurs when raindrops were intercepted. This effect decreases with increasing of kinetic energy of rain.

Surface disease progression was controlled and maintained in the case of the mixture compared to

the pure stand and also fungicide-treated crop. Preliminary results show the possibility of disease management by the use of a mixture variety with low input of fungicide for the case a splash dispersed disease. It was also noticed that the architecture and development of the plants within the mixture was changed because of inter-competition between varieties or species competition for environmental resources. Therefore, the concept of the mixture in terms of proportion and in terms of agronomical concordance must be applied with real care.

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