AFPP – 5TH INTERNATIONAL CONFERENCE ON ALTERNATIVE METHODS OF CROP PROTECTION
LILLE – 11th, 12th & 13th OF MARCH 2015

ELICITOR SCREENING TO PROTECT WINTER WHEAT AGAINST ZYMOSEPTORIA TRITICI BLOTCH

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ABSTRACT

Plant protection strategies are strongly focused today on the development of alternative methods in order to complete or replace conventional chemical inputs. Elicitors of biological origin are increasingly considered as a promising tool as biocontrol agents. They offer the possibility, through the induction of plant natural defences, to preventively protect plants against a large spectra of diseases. Multiple elicitors have already been identified since their first discovery in the late 70s, but few research has been focused on crop pathogens although such diseases can strongly impact both yield and grain quality. We describe hereby a method of elicitor screening to protect wheat against *Zymoseptoria tritici*. Focus is made on the importance of a good disease infection protocol in order to achieve screening tests.

Keywords: Elicitors, Wheat, *Zymoseptoria tritici*, Screening, Methodology.

RÉSUMÉ

Les stratégies de protection des plantes sont fortement concentrées à l'heure actuelle sur le développement de méthodes alternatives afin de compléter ou remplacer les produits chimiques conventionnels. Les éliciteurs d'origine biologique sont de plus en plus considérés comme un des outils prometteurs en tant qu'agents de biocontrole. Ils offrent la possibilité, par la stimulation des défenses des plantes, de protéger celles-ci à titre préventif contre un large spectre de maladies. Un grand nombre d'éliciteurs ont déjà été identifiés depuis leur découverte à la fin des années 1970, mais peu de recherche s'est concentrée sur les pathogènes des cultures céréalières alors que ceux-ci peuvent fortement affecter leur rendement et la qualité des grains. Nous décrivons ici une méthode de criblage de molécules élicitrices pour lutter contre *Zymoseptoria tritici* sur blé. Une attention toute particulière est portée sur l'importance du développement d'un protocole de reproduction de symptômes de la maladie de la septoriose.

Mots-clés : Eliciteurs, Blé, *Zymoseptoria tritici*, Screening, Méthodologie.

INTRODUCTION

Plants are constantly exposed to a wide range of biotic and abiotic stresses in their environment, and the inherent risk of productivity and quality loss has made it crucial to develop effective plant protection strategies (Balmer *et al.*, 2013). Up to now, chemical fungicide treatments are the most efficient disease control methods. However, the related environmental and health issues caused by these chemicals, along with the growing awarness of society of the importance of agricultural sustainability, make it urgent to develop safer methods for pathogen control. Accordingly, major advancements were made these past decades in disease management research to develop efficient biological control methods.

Elicitors are plant immunity-triggering compounds which are currently considered as one of the most promising tools in agriculture for the induction of plant resistance to various diseases, thereby contributing to the reduction of chemical inputs in agroecosystems (Mejía-Teniente *et al.*, 2010).
Although numerous elicitor products are already available on the market, it appears that a variable efficiency in the field, along with an uneasy integration in the current legislation and agricultural strategies, make this new biocontrol agent difficult to use (Walters *et al.*, 2013). Besides these limitations, few elicitor treatments have yet been efficiently and specifically designed to protect crop plants such as wheat, which is grown and consumed worldwide. Hence, there is a strong need to better understand the mechanisms of induced resistance in plants and develop elicitor use in agriculture for various pathosystems.

Following this approach, the objective of our project is to develop a method based on eliciting agents to protect wheat against *Zymoseptoria tritici*, a hemibiotrophic fungi which can impact wheat yield up to 30-50% (Goodwin, 2007). Several products showing potential elicitor properties in other studies have been chosen for screening tests under greenhouse conditions. An evaluation of the best conditions for the development of *Zymoseptoria tritici* disease symptoms (fungal isolate, plant development stage, environmental conditions) was first realized. Such study is of crucial importance, prior to the screening experiments of potential elicitors, to ensure the good comparison between treated plants and a control showing a standard level of infection. Finally, an elicitor screening protocol was developed and is hereby described.

MATERIAL AND METHODS

INOCULUM PREPARATION

The Z*ymoseptoria tritici* isolate (TO1187) is kindly provided by SIAH A. (ISA Lille, France). The isolate is grown on potato dextrose agar (PDA) medium (SIGMA) for 7 days at 20 °C and with a photoperiod of 16/8-h day/night cycle. Inocula are prepared by washing cultures with 10 mL sterile water and adjusting the spore suspension to 10^6 spores.mL^{-1}.

PLANT GROWTH CONDITIONS AND DISEASE INFECTION TEST

Wheat grains of the susceptible cv. Avatar were sown in 18-cm-diameter pots. Five pots of 8 grains were used as replicates for each bag application period and inoculation modality. The pots were placed in the greenhouse at 20 °C (± 2 °C) with a photoperiod of 16/8-h day/night cycle and supplementary illumination.
After 3 weeks (wheat plants at 3-4 leaf stage), plants of each pot were inoculated using a hand sprayer until runoff with a 20 mL spore suspension (10^6 spores.mL^{-1}), amended with 0.05% of polyoxyethylenesorbitan monolaurate (TWEEN 20®, SIGMA) surfactant. The control plants were treated with sterile water containing 0.05% Tween 20® alone.

Plants were covered with transparent plastic bags for 48 hours or 96 hours immediately after inoculation in order to test which water-saturated conditions are favorable to disease infection. Plants were stored in a growth chamber at 20 °C (± 2 °C) with a 16/8-h photoperiod.
The disease severity was scored every 2 days from 14 to 26 days post-inoculation (d.p.i) by assessing the percentage of third leaf area covered with lesions (chlorosis and necrosis) and the pycnidium density.
Comparison between leaf area bearing lesions and pycnidia for the two bag coverage time scales were carried out using the ANOVA test at a significance level of *P=0.05* (XLSTAT 2014).

SCREENING METHODOLOGY

Wheat grains of the susceptible cv. Avatar are sown in 18-cm-diameter pots. Five pots of 8 grains are used as replicates for each treatment. The pots are placed in the greenhouse at 20 °C (± 2 °C) with a photoperiod of 16/8-h day/night cycle and supplementary illumination.
After 3 weeks (wheat plants at 3-4 leaf stage), plants of each pot are treated using a hand sprayer with a 20 mL solution of the various "elicitor" products, tested respectively at three different concentrations. Two categories of control plants are used, one using sterile water only, and one using benzo-(1,2,3)-thiadiazole-7-carbothionic acid S-methyl ester (BION®, SYNGENTA).

Five days after plant treatment, plants of each pot are inoculated using a hand sprayer with a 20mL spore suspension (10^6 spores.mL^{-1}), amended with 0.05% of polyoxyethylenesorbitan monolaurate (TWEEN 20®, SIGMA) surfactant.
Plants are covered with transparent plastic bags for 3 days immediately after inoculation in order to ensure water-saturated conditions, and stored in a growth chamber at 20 °C (± 2 °C) with a 16/8-h photoperiod.
The disease severity is scored every 2 days from 14 to 26 days post-inoculation (d.p.i) by assessing the percentage of third leaf area covered with lesions (chlorosis and necrosis) and the pycnidium density.

RESULTS

The assessment of *Zymoseptoria tritici* colonization of wheat leaves was undertaken in the first step of this project and results are shown in Figures 1 and 2. Plants were inoculated by leaf spraying with the fungal pathogen (Isolate TO1187) or sprayed with water only (Control). Different water-saturated conditions were tested by covering plants with plastic bags over different time scales: a 48 hour plant coverage or a 96 hour plant coverage. Figure 1 shows that a 96 hour bag application enabled inoculated plants to develop symptomatic lesions up to 25% on the third leaf surface after 26 d.p.i, compared to 5% only for plants covered for 48 hours. Similarly, the pycnidium density within the symptomatic lesions reached up to 60% after 26 d.p.i for a 96 hour coverage, against 10% for a 48 hour coverage (Figure 2).

Figure 1 : Symptomatic foliar lesions (necrosis and chlorosis), scored every 2 days on the third wheat leaf from 14 to 26 days post-inoculation (d.p.i). Plants were inoculated with a *Zymoseptoria tritici* strain (isolate TO1187) or with water alone (Control). Plants were covered with plastic bags for 48 hours (48h) or 96 hours (96h) immediately after inoculation. Means tagged with the same letter are not significantly different using the Tukey Test (*P= 0.05*).

Lésions foliaires symptomatiques (nécroses et chloroses) mesurées tous les deux jours sur la troisième feuille de blé entre les jours 14 et 26 post-inoculation (d.p.i). Les plantes ont été inoculées avec une souche de *Zymoseptoria tritici* (Isolate TO1187) ou avec de l'eau seulement (Contrôle). Les plantes ont été couvertes d'un sac plastique durant 48 heures (48h) ou 96 heures (96h) immédiatement après inoculation. Les moyennes présentant la même lettre ne sont pas significativement différentes suivant le test de Tukey (*P=0.05*).

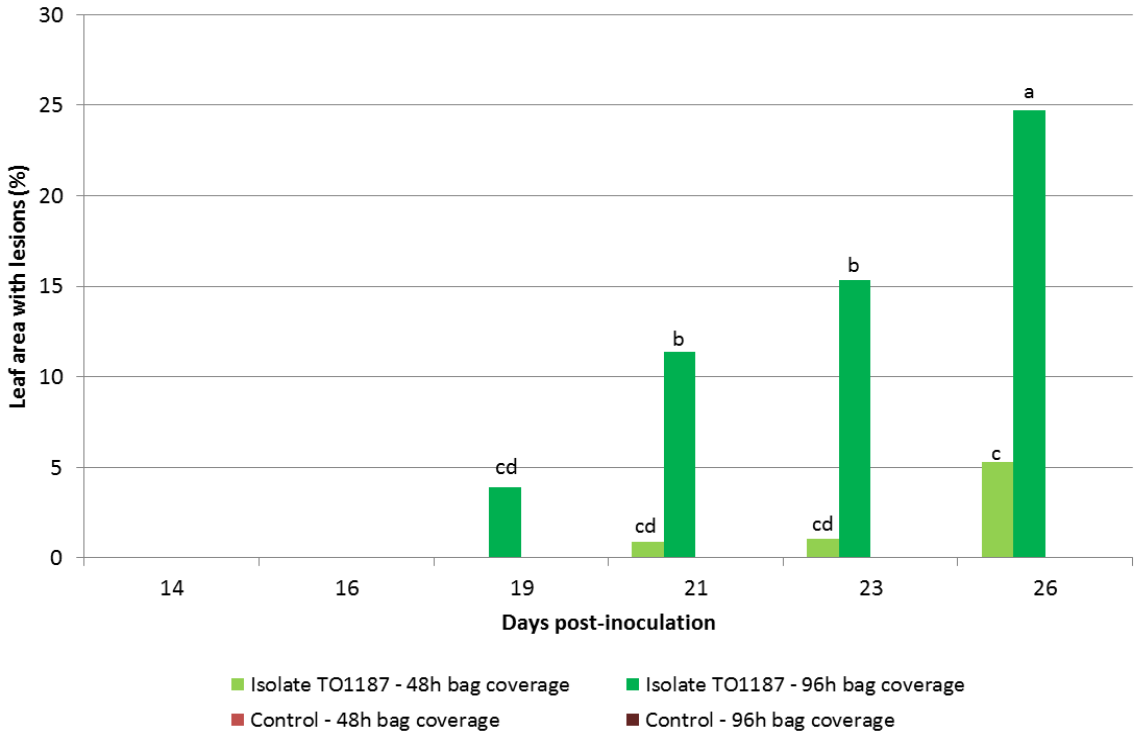
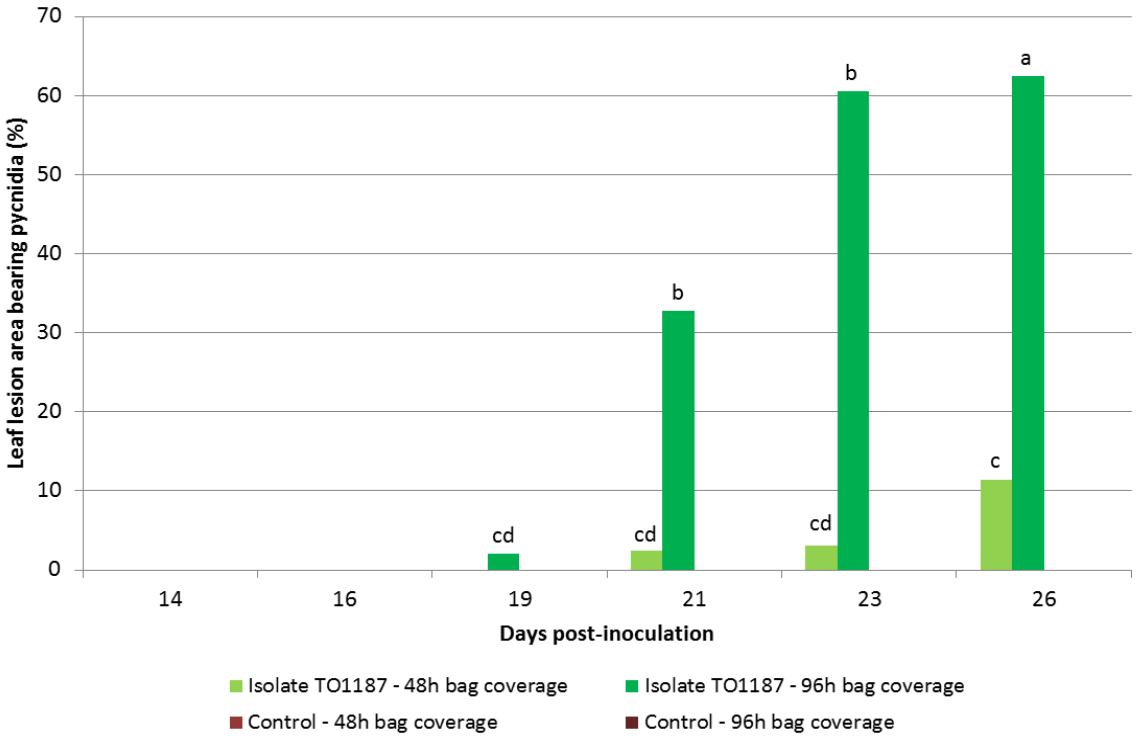


Figure 2 : Pycnidum density within the symptomatic lesion area, scored every 2 days on the third wheat leaf from 14 to 26 days post-inoculation (d.p.i). Plants were inoculated with a *Zymoseptoria tritici* strain (isolate TO1187) or with water alone (Control). Plants were covered with plastic bags for 48 hours (48h) or 96 hours (96h) immediately after inoculation. Means tagged with the same letter are not significantly different using the Tukey Test (*P= 0.05*).

Densité de pycnides au sein des lésions symptomatiques, mesurée tous les deux jours sur la troisième feuille de blé entre les jours 14 et 26 post-inoculation (d.p.i). Les plantes ont été inoculées avec une souche de *Zymoseptoria tritici* (Isolate TO1187) ou avec de l'eau seulement (Contrôle). Les plantes ont été couvertes d'un sac plastique durant 48 heures (48h) ou 96 heures (96h) immédiatement après inoculation. Les moyennes présentant la même lettre ne sont pas significativement différentes suivant le test de Tukey (*P=0.05*)



DISCUSSION

The requirement of high humidity conditions for 3 days after inoculation to encourage *Zymoseptoria tritici* infection is a well-known fact. To this end, plants are generaly enclosed in small chambers made out of attached plastic sheets to ensure high relative humidity (Goodwin, 2007). However, the temperature in such chambers can become too high for good pathogen infection. Symptoms become usually visible at least 14 days after inoculation, and full symptom expression may require 26 days or more to be obvious. In this study, we used plastic bags to cover the inoculated plants for 2 or 4 days. After 26 d.p.i, leaf lesions and pycnidium density were significantly more abundant on plants which were covered for 4 days immediately after inoculation. The cinetic of leaf lesion and pycnidia emergence clearly shows an increase of disease symptom development with time. It appears that day 26 after inoculation is most appropriate to measure clear differences between modalities. *Zymoseptoria tritici* infection reached up to 25% of the wheat leaf area, with the production of asexual spores within pycnidia.

CONCLUSION

The results of the disease infection test provides good tools to ensure the effective infection of wheat plants with the pathogen *Zymoseptoria tritici*. Parameters such as temperature, relative humidity, plant development stage and fungal isolate remain of major importance to ensure the development of evident symptoms 3 weeks after plant inoculation. The coverage of plants with plastic bags for 4 days immediately after infection ensures good infection levels (25% symptomatic lesions on wheat leaves).
The following step in this project is henceforth the carrying out of elicitor screening tests. A first discrimination of elicitors products considering their dose-efficiency to reduce disease severity should be realized. After further screening of all the products in hand, a determination of the elicitation pathways of the most efficient molecules tested will be undertaken. We finally intend to study the influence of various factors (i.e Temperature, relative humidity, plant development stage) on the elicitation potential and develop a formulation to be tested under field conditions.

ACKNOWLEDGEMENTS

We thank Dr A.SIAH and Mr.F.Dresen for their valuable suggestions during this study. This research was supported by the AgricultureIsLife platform of the University of Liege, Gembloux, Belgium.

REFERENCES

Balmer D., Planchamp C., Mauch-Mani B., 2013. On the move: induced resistance in monocots. *Journal of Experimental Botany*, 65, 5, 1249-1261.

Goodwin S.B., 2007. Back to basics and beyond: increasing the level of resistance to Septoria tritici blotch in wheat. *Australian Plant Pathology*, 36, 6, 532-538.

Mejía-Teniente L., Torres-Pacheco I., González-Chavira M.M., Ocampo-Velazquez R.V., Herrera-Ruiz G., Chapa-Oliver A.M., Guevara-González R.G., 2010. Use of elicitors as an approach for sustainable agriculture. *African Journal of Biotechnology*, 9, 9155-9162.

Walters D.R., Ratsep J., Havis N.D., 2013. Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany*, 64, 1263-1280.