O.44 - Sustainable management of rapeseed blackleg by modelling and monitoring of a pilot production area

Boillot M.[1], Sauzet G.[2], Lô-Pelzer E.[3], Penaud A.[1], Pic E.[1], Carpezat J.[1], Pinochet X.[1], Aubertot J-N.[4], Bousset L.[5], Jeuffroy, M.H.[3]

[1] CETIOM, Centre de Grignon, BP04, 78850 Thiverval-Grignon, France
[2] CETIOM, Le domaine du Chaumoy, 18570 Le Subdray, France
[3] INRA Grignon, UMR d'agronomie, 78850 Thiverval-Grignon, France
[4] INRA Toulouse, UMR AGIR, 31326 Castanet-Tolosan, France
[5] INRA Rennes, UMR Bio3P, 35653 Le Rheu, France

Contact: boillot@cetiom.fr

Abstract

Since their introduction in 2004, commercial hybrids containing the new resistance gene Rlm7 gained market share, reaching 30% of the oilseed rape sown areas in the Centre region of France. In order to prevent the breakdown of the specific resistance, CETIOM set up a bio-vigilance programme in the Centre of France, in a small region where the risk of phoma stem canker is high and where oilseed rape fields represent 25% to 30% of the cropping area.

Cultural practices, leaf spots, phoma stem canker severity and fungus pathotypes are monitored. A simulation model, called SIPPOM, was developed to help ranking of control strategies of phoma stem canker on oilseed rape. This paper presents the results of a survey in commercial fields of winter oilseed rape for four cropping seasons and presents how the overall behaviour of SIPPOM will be assessed using data collected by the bio-vigilance programme.

Phoma stem canker, caused by the *Leptospheria maculans/L biglobosa* complex species, is one of the most important oilseed rape diseases in France and in Europe. Chemical applications, agronomic practices and plant genetic resistances are several means of controlling the disease, but none of them are completely successful. Nowadays, the most effective method is the use of plant genetic resistances. Winter oilseed rape cultivars have two types of resistance to phoma stem canker: either specific or quantitative. Quantitative resistance, which relies on complex mechanisms, is often associated with major genes in cultivars. Nevertheless, selection of specific resistance is easier and its efficiency is immediate although single major gene resistance had already been overcome in the past within a few years of commercialisation. The latest major gene introduced in oilseed rape is named Rlm7. Since their introduction in 2004, commercial hybrids containing Rlm7 have gained market share, reaching 30% of the oilseed rape sown areas in the Centre of France.

After the crisis of the Rlm1's gene breakdown at the end of the 1990s, the French research organisations aimed at preventing similar events. They organised themselves to design strategies to avoid such phenomena. For a few years, CETIOM has been promoting a turnover strategy in the use of varieties based on a fine characterisation of both quantitative and specific resistances (Pinochet et al., 2004). It has also encouraged stubble breaking and ploughing to limit primary inoculum production. However, CETIOM needed to develop more powerful tools to demonstrate the interests of the recommendations at the production scale. When Rlm7 appeared, two directions were taken. First, CETIOM started to develop a bio-vigilance approach in the Centre of France, in a small region where the risk of phoma stem canker is high and where oilseed rape fields represent 25% to 30% of the cropping area.

At the same time, as part of a PhD thesis, a simulation model was developed to represent the dynamics of the size and the genetic structure of *L. maculans* populations under the influence of various cropping systems.

This paper aims at presenting the current advancement of the CETIOM approach and the methods developed to improve potential resistance breakdown prevention.

Epidemiological and agronomic monitoring

Material and methods

The pilot production area is located between St-Florent-sur-Cher and Issoudun, in the Centre of France, 250 kilometres south of Paris. Each year, between 20 to 30 fields (ca. 400 ha) are closely monitored during the whole cropping season - at the emergence stage, at midNovember, at the end of winter, at flowering and at crop maturity. These observations are completed by farmers' surveys on their cultural practices. Fields sown with Rlm7 varieties have been preferentially monitored. Sampling and agronomic observations methods are the ones described in a previous article (Pinochet et al., 2005). Leaf spots are recorded in autumn between October and November, on each field, on samplings of 4*25 plants. The final disease index G2 (Aubertot et al., 2004) is established using observations on 8*5 plants.

Results

During the past four years, the climatic situation has been mild, thus low disease pressure was noted. Ascospore releases occurred late - main peaks were not detected before mid-October - which limited the contamination of oilseed rape seedlings and young plants.

Leaf spots, which appeared 2 or 3 weeks after the first main ascospores peak, were numerous - up to 270 spots/m² - on classical genotypes having no efficient specific resistance. On the contrary, on Rlm7 varieties, the number of leaf spots almost never reached more than 50 spots/m². In 2007-2008, the number of leaf spots was negligible.

Discussion

The main objective of the pilot production area monitoring was to identify a bio-vigilance indicator likely to detect the beginning of a specific resistance breakdown. The number of leaf spots per surface unit, which depends on the green biomass before winter, seemed to be a promising criterion. Fifty spots/m² for a green biomass under 2000 g/m² could be a reliable threshold for Rlm7 genotypes under which the resistance breakdown has not occurred. Yet, additional data are necessary to improve the accuracy of the criterion. In fact, the number of leaf spots per surface unit and its period of apparition highly depend on the climatic scenario.

For the past four years, annual epidemic scenarios have been quite alike. More contrasted situations are needed. Moreover, the detection of the right symptoms is not as simple as it could appear. Mistakes can easily be made with other pathogen spots such as *L.Biglobosa* or *Pseudocercosporella capsella*. Lastly, the specificity of the Avrlm7 phenotype hardens the task. In fact, a great part of the leaf spots collected on Rlm7 varieties are avirulent whereas they were expected to be virulent, in accordance with the gene-for-gene relationship. The mechanisms controlling the expression of this interaction are still misunderstood.

These difficulties do not question the bio-vigilance indicator, but they point out that this sole criterion is not sufficient to detect and prevent the beginning of a resistance breakdown. In addition to agronomic observations, genetic analyses are a complementary way to monitor the dynamic of *L.maculans* populations.

Genetic analyses: a tool to monitor the pathogen population dynamics

Material and methods

Each year, twenty leaves per field, each one with at least one spot, are collected and sent to CETIOM's laboratory in Grignon for genetic analyses. A first PCR procedure sorts out Tox+ from Tox° (*L.biglobosa*). Further analyses are conducted for Tox+ isolates. Virulence pattern for the gene A/avrlm 7 is obtained using Williams tests, which provide results within one month.

In 2004-2005, 94 strains were sampled, 58 in 2005-2006 (many strains died before pathogen isolation), 287 in 2006-2007 and 229 in 2007-2008. Pathogens collected on Rlm7 varieties were immediately isolated. The other leaf spots were frozen. They were defrosted to be analysed during the autumn 2007.

Results

The proportion of virulent isolates avrlm7 depends on the year under consideration. ar. Figure 1 presents the percentage of avrlm7 isolates among *L.maculans* strains (without *L.biglobosa* strains) sampled on Rlm7 varieties a given cropping season.

(The total number of analysed *L.maculans* isolates sampled from Rlm7 varieties are 20, 35, 135, 79 for the 2004-05, 2005-06, 2006-07, 2007-08 cropping seasons respectively.)

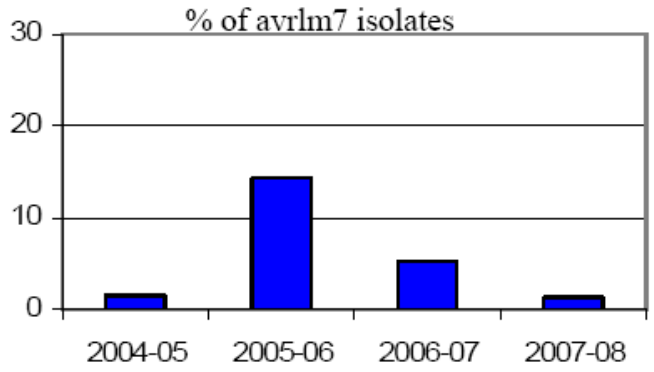

Figure 1. Percentage of avrlm7 isolates among *L. maculans* strains.

Discussion

Genetic analyses of sampled leaf spots are reliable and are a way to evaluate, for a given year, the proportion of virulent strains in a pathogen population. However, the result remains valid only for the sampled area. It cannot be extrapolated to the entire region. Full-scale monitoring on all French territory is therefore excluded as long as genetic analyses methods remain so tedious to apply. Recently, CETIOM's laboratory validated a new multiplex PCR procedure to analyse virulence patterns of the genes A/avrlm1 and 4, based on a method developed by the PMDV Unit, INRA Versailles. The technique is faster since results are obtained within 3 days. New molecular markers for A/avrlm7 alleles are also under development at the PMDV Unit. A PCR procedure would facilitate the enlargement of monitoring to larger areas. Nevertheless, it would remain a tedious and time-consuming method. Sustainable management of specific resistances requires monitoring of pathogen populations to detect as early as possible a resistance breakdown, but it is also requires anticipation and the design of strategies to prevent resistance breakdown. However, it is highly difficult to test disease management strategies using traditional field experiments. Modelling is a good tool to handle complex systems at multiple-years and regional scales.

SIPPOM, a Simulator for Integrated Pathogen POpulation Management

Structure of SIPPOM

SIPPOM (Lô-Pelzer *et al.*, 2008) simulates the dynamics of the size and the genetic structure of *L. maculans* populations under the influence of various cropping systems. It also calculates an economic result and an environmental criterion associated to the simulated strategies directly as a function of the cropping systems being considered. In comparison with field experiments, more complex scenarios, with a great number of parameters, can be assessed thanks to modelling. SIPPOM is not a real-time simulator but it is a powerful tool to rank control strategies according to various criteria.

Using SIPPOM

CETIOM is currently testing the global behaviour of SIPPOM. For this purpose, agronomic and epidemiological data collected in a small area (17 km²) included in the pilot production area in the Centre of France are used. All the winter oilseed rape fields of the past four years had been identified in the monitored sector. The study aims at comparing the collected data with simulations of SIPPOM to test the overall behaviour of the model. In addition, CETIOM will test different strategies minimizing the risk of phoma stem canker. The effects of several factors will be evaluated, such as the interaction between sowing date and climatic conditions, or the distance between infected stubble and new oilseed rape fields. As a last action, strategies that minimise the risk of resistance breakdown (cultivar deployment strategies, ratio of Rlm7 varieties, specific ploughing of fields with Rlm7 cultivars) will be assessed. Combined with an experimental approach, modelling with SIPPOM opens the way to innovative phoma stem canker sustainable management strategies.

References

Aubertot JN, Schott JJ, Penaud A, Brun H, Doré T, 2004. Methods for sampling and assessment in relation to the spatial pattern of phoma stem canker (*Leptosphaeria maculans*) in oilseed rape. European Journal of Plant Pathology. 110: 183-192.

Lô-Pelzer E, Aubertot JN, Bousset L, Salam MU, Jeuffroy MH. 2008. SIPPOM-WOSR: Simulator for Integrated Pathogen POpulation Management for blackleg on canola. ENDURE International Conference. 12-15 October. La Grande Motte, France.

Pinochet X, Balesdent MH, Salvi F, Mestries E, Rouxel T. 2004. Identification of specific plant resistance factors to Phoma (Leptosphaeria maculans) among winter oilseed rape varieties: interest for variety testing and for the promotion of a first step of a durable management of resistances. International Organisation for Biological Control WPSR Bulletin 27/101-108

Pinochet X, Penaud A, Sauzet G, Lodé M, Carpezat J, Pic E, Mestrie E. 2007. Introduction of a new specific resistance against *L.maculans* in oilseed rape commercial varieties in France: Monitoring of introduction and control of virulence behaviour to try to avoid resistance breakdown in a pilot production area in the central part of France. Proceedings of the 12th International Rapeseed Congress, China.