# Evaluation of a diagnostic test for Sclerotinia on oilseed rape at flowering

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## ABSTRACT

If oilseed rape receives a preventive fungicide spraying each season, severe attacks are rare. In order to propose a new method for disease management, CETIOM is adapting and evaluating the petal test used in Canada. If it had been used on experimentation networks, without strong loss risk, ¾ needless sprayings would have been avoided in 2000, ½ in 2001 and ¾ in 2002. Although the method could be made lighter, it will probably not be used in France at field scale in many situations. Three other possible use conditions appear : on field networks with extrapolation to other fields, on a few selected fields only, or to acquire experimental references.

**Key words** : Decision support tool – *Sclerotinia* – risk – co-conception – diagnostic test

## INTRODUCTION

*Sclerotinia sclerotiorum* is a fungus which attacks many crop species. For more than 25 years, the oilseed rape protection has been based on a preventive fungicide spraying, despite the rareness of heavy attacks. This situation warrants the development of a new method for disease management, all the more because a resistance to fungicides has appeared (Penaud *et al*., 2003). A petal test has been developed in Canada in order to improve the decision for fungicide spraying. It consists in evaluating the quantity of *Sclerotinia* present on rapeseed flowers at the beginning of flowering. The target of our work is to evaluate this kind of petal test in French conditions and adapt it if needed. Indeed, using a petal test would be less profitable for a French farmer than for a Canadian one because of the smaller size of fields in France. In addition to this, the presence of *Botrytis* in France (very close to *Sclerotinia* and absent in Canada) makes it more difficult to identify *Sclerotinia* colonies on Petri plates. Validation of the tool in French conditions includes two levels : an operational validation linked to ergonomy optimisation, and a technical validation of the tool's reliability.

## MATERIALS AND METHODS

The Canadian kit's method (Morrall & Thomson, 1991) consists in collecting flowering shoots at F1 stage (50% of plants with at least one opened flower) and picking off with forceps 160 petals to be plated on Petri dishes in sterile conditions. These are incubated four days at 20°C. The number of *Sclerotinia* colonies is scored to determine the percentage of infested petals. CETIOM adapted the tool for French conditions (Poisson & Penaud, 2000), where *Botrytis* disturbs *Sclerotinia* identification, using Steadman's *et al.* (1994) semi-selective medium.

The petal test using this method (with Steadman's medium) was tested in 2000 and 2001 on respectively 23 et 52 oilseed rape fields. Two other methods where tested on 9 plots in 2001 : collecting whole flowers and, in the field, placing them 4 by 4 with forceps in 9-cm-diam Petri dishes (method 2), or placing them individually in 5,5-cm-diam Petri dishes with gloved fingers or by cutting the flower's peduncle between the plate's edges (method 3). Both these methods, together with the first one (method 1) where tested at the same time on each plot. In 2002, the evaluation of the petal test was carried out on 44 fields by placing one whole flower per Petri plate straight in the field (method 3). Every year, experimentations are carried out not only by CETIOM experimenters but also by advising structures. Designs consist in a couple of strips : the first one untreated and the other treated at G1 stage (early petal fall). Plant attack rates are measured at G4-G5 stage on each strip. The experimenters' remarks about the feasibility in using the kit are collected after each season.

## RESULTS

From results obtained in 2000 (figure 1) a fungicide spraying threshold of 50% infested petals at early bloom was proposed. Indeed, none of the 13 fields infested on less than 50% of petals were dramatically attacked (harmfulness threshold chosen at 20% attacked plants). In 2001, attacks were not very severe. 3 fields with more than 20% attacked plants showed more than 50% infested petals at early bloom. Cases of overestimated attack risk are explained by an unfavourable climate after F1 stage : fungus potential present on petals can't develop.

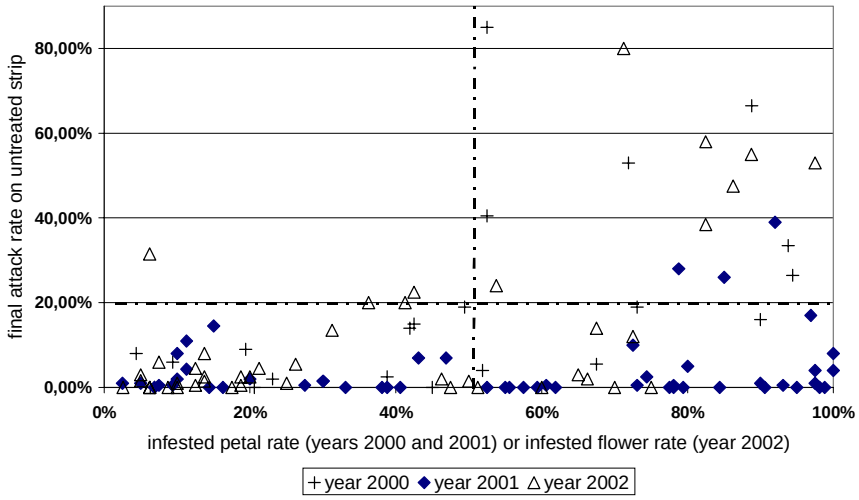


Fig.1. Attack rate according to contamination level at early bloom

Since 2000, CETIOM's experimenters came across obstacles in using the kit. Advisers consulted in 2001 about this same petal test also emphasized these difficulties. In 2000, CETIOM's experimenters had suggested ameliorations which led to suggest two new methods tested on 9 plots in 2001 (methods 2 and 3). Experimenters were in favour of method 3 : less necessary logistic than for the initial method (entirely done in the field), easily used by a single person, faster (on average 1h15 versus 3h for the initial method), and easier handlings in the field (smaller sized dishes and cutting off much less meticulous than for the initial method). But they maintained a wish for less dishes and a more functional dish-closing system. Comparison between method 3 and the initial one on 9 plots in 2001 and 9 plots in 2002 led in a given year to similar field classification for contamination rate at early bloom (figure 2). On the other hand, on a same field, the infested flower rate tends to overtake the infested petal rate. This isn't surprising as an infested flower can be the source of 1, 2, 3 or 4 infested petals. Therefore in theory we have :

$$\text{infested petal rate} \leq \text{infested flower rate} \leq 1-(1-\text{infested petal rate})^4$$

Results obtained in 2002 verify this relation. This would urge to put up the threshold, but until a large enough set of data is available, method 3 is evaluated for a 50% infested flowers' threshold.

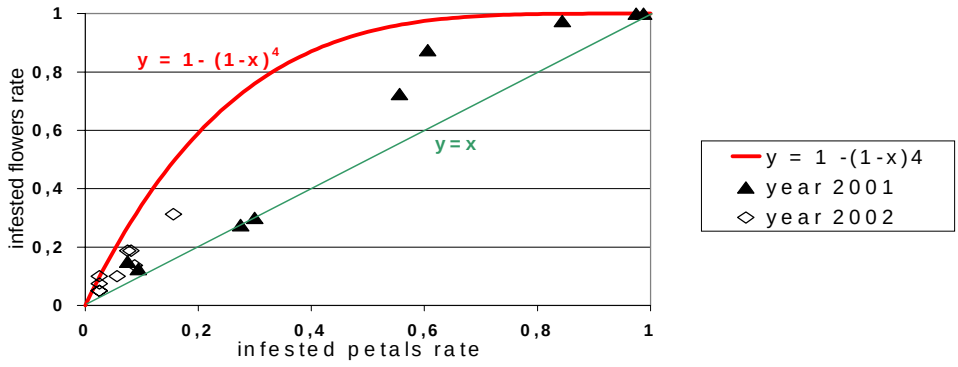


Fig.2. Infested flower rate according to infested petal rate

In 2002, method 3 was tested on 44 fields. 11 of them showed more than 20% attacked plants (figure 1), among which 4 showed less than 50% infested flowers at bloom. Three of them were attacked on 20% or 23% of the plants (very close to the harmfulness threshold), and on the 4th one the experimenter had difficulties identifying symptoms (so a doubt subsist about the actual attack rate). Among the 33 fields weakly attacked, 24 showed less than 50% infested flowers at early bloom.

According to CETIOM's experimenters, the main advisers who had already used the initial method in 2001 prefer the new one, faster and easier. But some of them consider it less comfortable to be in the field rather than in a laboratory, and think it difficult to proceed alone because of the required skill. Others preferred using forceps to cut off flowers, whereas this was not suggested from the CETIOM's experimenters point of view.

## DISCUSSION

The evaluation of the petal test for three years with the 50% infested petals or flowers threshold has shown its interest. Indeed, if the kit was used to manage fungicide spraying around test networks, without making farmers run a risk, on average 60% of needless sprayings would have been avoided for the 3 years. Because spraying occurs from the fall of the first petals, when the climate can prevent later fungus expression, an exact identification of fungicide-needing fields without overestimating the risk will remain difficult. In addition to the kit, the use of climatic models for information about risk beyond F1 stage should nevertheless lead to better predictions.

Getting the potential petal test users to work on its evaluation in the field allowed many fields to be tested, and comments to be gathered about feasibility. This second point led to work on an easier method. We succeeded in improving ways of sampling and plating flowers. But some aspects seem difficult to improve, especially the dish-closing system, the dish incubation temperature, and the reading. If the tool is developed, a help-service for reading should probably be put into place. As to the possibility of sampling less flowers, a study is on hand. The views can be different and sometimes diverging, according to which person is consulted (experimenter or potential user). This shows the interest of associating with users, to know their needs and constraints as precisely as possible.

Exchanges with users showed that unlike in Canada, the kit will not be used at field scale by many farmers in France. Three main other uses stand out : on field networks, which means learning to structure them and to extrapolate results ; on a few fields only selected with simple inquiries about their situation and needing a precise diagnostic ; or finally as an experimenter's tool to acquire references. Therefore the singularity of the kit in French conditions would be its use in development networks.

## ACKNOWLEDGEMENTS

For their essential help we wish to thank : Marianne Cerf, Catherine Pasquier and Jean-Marc Meynard from INRA, our advising structure partners who took part in the experimentation networks, CETIOM's experimenters, and CETIOM's pathology laboratory workers.

## REFERENCES

**Morrall R.A.A., Thomson J.R.**, 1991. Petal test manual for *Sclerotinia* in canola. *University of Saskatchewan.*

**Penaud A., Huguet B., Wilson V. and P. Leroux**, 2003. Fungicide resistance of *Sclerotinia sclerotiorum* in French oilseed rape crops. *Proc. 11th Int. Rapeseed Congr., Copenhagen.*

**Poisson B., Penaud A.**, 2000. Détection de *Sclerotinia sclerotiorum* sur les pétales de colza. *AFPP – Sixième conférence internationale sur les maladies des plantes.*

**Steadman J.R., Marcinkowska J., Rutledge S**., 1994. A semi-selective medium for isolation of *Sclerotinia sclerotiorum*. *Can. J. Plant Pathol. 10, 159-165.*