# Effects of a cultivar mixture on scab control in apple orchards

L. Brun<sup>1</sup>, A. Lemarquand<sup>2</sup>, G. Orain<sup>2</sup>, C. Gros<sup>1</sup>, F. Combe<sup>1</sup>, F. Didelot<sup>3</sup>, C-E. Parveaud<sup>4</sup>, C. Gomez<sup>4</sup>, L. Parisi<sup>5</sup>

# Abstract

The impact on apple scab of a cultivar mixture including the cvs Melrouge (low susceptibility to scab) and Pitchounette (Rvi6 resistant) was assessed in two experimental orchards: an organic apple orchard located in Rhône Valley (Drôme, France) and an IPM orchard in Loire Valley (Maine-et-Loire, France). Each cultivar represented 50 % of the trees mixed within the row. In the organic orchard, the resistance of Rvi6 gene was effective since the orchard plantation in 2004. In this context, the cultivar mixture showed a good efficiency to decrease the scab incidence on leaves and fruits of Melrouge, during two consecutive years. In the IPM orchard, Pitchounette resistance was overcome in 2008, and the level of disease on Melrouge was very important in 2009; no significant difference between the pure stand and the mixture was observed for scab incidence on fruit at picking in this context. The cultivar mixture enhanced scab control as long as Rvi6 resistance was not overcome and the level of disease not too high.

Keywords: cultivar mixture, apple scab, Venturia inaequalis, organic apple production

## Introduction

Standard strategies aimed to control apple scab [caused by *Venturia inaequalis* (Cooke) G. Wint.] are based on intensive fungicide applications in orchards, mainly planted with susceptible cultivars.

The plantation of scab resistant cultivars could be a good way to decrease the treatments pressure in apple orchards. However, the main resistance gene, named *Rvi6*, present in the majority of resistant cultivars, is overcome in several European countries (Bus *et al.*, 2011; Parisi *et al.*, 2006; Didelot *et al.*, 2009). When the virulent strains are present, the orchards planted with some *Riv6* resistant cultivars have to be treated intensively (Caffier *et al.*, 2010; Didelot *et al.*, 2009). In this context, partial resistance to scab could be a good alternative to reduce spraying program in orchards (Brun *et al.*, 2008) and could be also a good strategy to provide durable resistance (McDonald & Linde, 2002). However, this resistance is not able to supply a complete protection. Thus, the implementation of strategies is needed to associate different cultural practices and low-fungicides applications (Brun *et al.*, 2010; Didelot *et al.*, 2010; Didelot *et al.*, 2010).

Many studies have shown the efficiency of cultivars mixtures for reducing epidemics of aerial pathogens, mainly in cereal crop (Mundt, 2002). In apple, planting cultivar mixtures is a cultural practice that can reduce the epidemics of the fungus *Venturia inaequalis* (Bousset *et al.*, 1997; Didelot *et al.*, 2007; Parisi *et al.*, 2013). Working with a mixture of a scab susceptible cultivar and an *Rvi6* resistant one (not overcome), Didelot *et al.* (2007) showed that cultivar mixture effect was increased by moderate chemical treatments.

<sup>&</sup>lt;sup>1</sup> INRA - UERI, Domaine de Gotheron, 26320 Saint-Marcel-lès-Valence, France, Ibrun@avignon.inra.fr

<sup>&</sup>lt;sup>2</sup> INRA - UE Horticole, 42 rue Georges Morel, 49071 Beaucouzé cedex, France

<sup>&</sup>lt;sup>3</sup> INRA - IRHS, 42 rue Georges Morel, 49071 Beaucouzé cedex, France

<sup>&</sup>lt;sup>4</sup> GRAB, Domaine de Gotheron, 26320 Saint-Marcel-lès-Valence, France

<sup>&</sup>lt;sup>5</sup> INRA - UR Pathologie Végétale, CS 60094, 84143 Montfavet Cedex, France

Moreover, the association of an *Rvi6* resistant cultivar and a susceptible one is not recommended if the resistance is overcome, as it could favour the pathogen diversity (Trapman, 2006). Thus, it seems more interesting to promote cultivar mixtures between low susceptible and/or resistant cultivars.

The aim of this study is to test the efficiency against scab of a mixture between a lowsusceptible and a resistant cultivar.

## **Materials and Methods**

## Experimental orchards:

The first experimental orchard was located in the middle Rhone Valley, at Gotheron (Saint-Marcel-lès-Valence, Drôme, France), in a continental area under Mediterranean summer influences. This orchard was managed following the French rules of organic production. The second experimental orchard was located in Loire Valley, at La Rétuzière (Champigné, Maine-et-Loire, France), under an oceanic climate. This second orchard was managed following regional integrated management rules.

These orchards, planted in 2004, included 2 cultivars grafted on rootstock Pajam<sup>®</sup> 2 (4160) Cepiland. The first, Melrouge, is a mutant of Melrose, old cultivar with a low susceptibility to scab. Melrouge was planted in pure stands and in a within row mixture with Pitchounette (Figure 1). Pitchounette (3318) is a cultivar resistant to scab (*Rvi6* gene). The distance between the trees was 1.70 m and 4 m between the rows. In each experimental orchard, treatments were replicated 3 times according to a block design. Each block included a pure stand of Melrouge (Pure) and a plot with the cultivar mixture. To limit the interactions between plots, each plot was surrounded by an edge of evergreen shrubs. The results presented in this paper are based on the analysis of the 2008 and 2009 datasets.





#### Estimation of scab infection risks:

On each experimental site, the climatic data were recorded by a meteorological station from the INRA national network "Agroclim", located on grassland at about 1000 m from the orchard. Based on these data, the climatic risks were evaluated based on the "Light", "Moderate" and "Severe" scab infection risks defined by Mills & Laplante (1951), and the very light scab infection risk known as the "Angers" risk (Olivier, 1986).

#### Scab assessment:

No treatment against scab was done in 2008 and 2009 to permit the disease development for the experimental purpose.

The scab development was evaluated by several scoring on leaves in May and June and fruits at picking. On leaves, 80 shoots per plot were scored (2 shoots per tree) and when scab was observed, the number of lesions was assessed. For each plot, shoot scab severity was defined as the mean number of scab lesions per shoot. At picking, all the fruits per tree were evaluated (only 20 fruits per tree in 2009 on Gotheron site). For each plot, fruit scab incidence was defined as the percentage of fruit scabbed.

### Statistical analysis:

Shoot scab severity and fruit scab incidence were subjected to an analysis of variance (ANOVA) to evaluate differences between the Pure stand and Cultivar mixture. ANOVA were computed using Statgraphics plus 5.1 software (Manugistics, Rockville, MD, USA). The level of significance was set at 5 % for all the statistical tests. When residual standard deviation increased with the increment of predicted values, data were log transformed before ANOVA (Dagnélie, 1975). Mean comparisons were conducted using the Newman-Keuls test.

# Results

At Gotheron, scab epidemiology in 2008 and 2009 was different: 2008 was a favourable year with 20 infection risks compared to 2009 with only 7 infection risks. At La Rétuzière, 2008 and 2009 were two favourable years with 23 and 18 infection risks respectively (Table 1).

|              |      | Scab infection risks |       |          |        |       |  |  |
|--------------|------|----------------------|-------|----------|--------|-------|--|--|
|              |      | Angers               | Light | Moderate | Severe | Total |  |  |
| Gotheron     | 2008 | 1                    | 11    | 2        | 6      | 20    |  |  |
|              | 2009 | 0                    | 4     | 3        | 0      | 7     |  |  |
| La Rétuzière | 2008 | 4                    | 7     | 7        | 5      | 23    |  |  |
|              | 2009 | 1                    | 5     | 5        | 7      | 18    |  |  |

Table 1: Scab infection risks from bud break to the end of June in the 2 experimental sites.

At Gotheron, in 2008, despite the 20 climatic risks recorded, the disease level on Melrouge was not high because it was only the second year of development of disease in this orchard and the primary inoculum was not very high (data not shown). On June, only 0.3-1.1 lesions per shoot were observed, and at picking, 9.3-14.9 % of the fruits were scabbed in cultivar mixture and pure stand, respectively (Table 2; Figure 2). In 2008, the severity of the disease on shoots was significantly higher on the pure stand than in the cultivar mixture. In 2009, with a high primary inoculum, the level of disease was important: at the end of June, 18-24 lesions per shoot were counted, and at picking, 76.2-82.2 % of the fruits were scabbed in cultivar mixture and pure stand, respectively (Figure 2; Table 2). Significant difference for disease severity on shoot was only observed in May (Figure 2). The incidence of the disease on fruits at picking was significantly higher on pure stand than on cultivar mixture (Table 2). In the Gotheron orchard, Pitchounette resistance was not overcome during all the period of the experiment.

At La Rétuzière, in 2008, despite a low primary inoculum (data not shown), the incidence on Melrouge at picking was high with 60.5-73.0 % of scabbed fruits in cultivar mixture and pure stand, respectively. Scab was detected on Pitchounette for the first time in this orchard the 18 June 2008, and 0.1 % of Pitchounette apples were scabbed at picking (Table 2). In 2008, the severity of the disease on shoots was significantly higher on the pure stand than in the cultivar mixture (Figure 2). In 2009, the level of disease on Melrouge was very important: in June, 65.4-62.6 lesions per shoot were counted, and at picking, 96.0-98.3 % of the fruits were scabbed in cultivar mixture and pure stand, respectively (Figure 2; Table 2). Significant difference for disease severity on shoot was only observed in May (Figure 2). At picking, 9.2 % of Pitchounette apples were scabbed (Table 2).

|                  | Percentage of scabbed fruits at picking |                     |                     |              |  |  |
|------------------|---|---------------------|---------------------|--------------|--|--|
|                  | Gotheron - 2008                         |                     | La Rétuzière - 2008 |              |  |  |
|                  | Melrouge                                | Pitchounette        | Melrouge            | Pitchounette |  |  |
| Pure stand       | 14.9                                    | -                   | 73.0                | -            |  |  |
| Cultivar mixture | 9.3                                     | 0.0                 | 60.5                | 0.1          |  |  |
| P-value          | 0.0666                                  |                     | 0.0663              |              |  |  |
|                  | Perce                                   | entage of scabbed f | ruits at picking    |              |  |  |
|                  | Gotheron -2009                          |                     | La Rétuzière - 2009 |              |  |  |
|                  | Melrouge                                | Pitchounette        | Melrouge            | Pitchounette |  |  |
| Pure stand       | 82.2                                    | -                   | 98.3                | -            |  |  |
| Cultivar mixture | 76.2                                    | 0.0                 | 96.0                | 9.2          |  |  |
| P-value          | 0.0458                                  |                     | 0.4769              |              |  |  |





Figure 2: Shoot scab severity on Melrouge in 2008 (A, B) and 2009 (C, D) in the organic orchard (A, C) and in the IPM orchard (B, D). For each date, values followed by different letters are significantly different (P = 0.05).

#### Discussion

This experiment, made in two different French regions, permitted the evaluation of mixture efficiency in different conditions. These differences concerned the climatic conditions: the climate of Loire Valley is most favourable to apple scab, but also the presence of strains virulent to the *Rvi6* gene, detected only at La Retuzière. It is interesting to note that cv Melrouge in the mixture was significantly less scabbed on shoots in 2008 in the two locations, whatever the final level of disease (higher at La Retuzière), but not above 5 scab lesions per shoot. The results of 2009 are a good illustration of the limits of the mixture:

when the disease is too high (Gotheron, scab on shoots the 22/06), or/and when Pitchounette is overcome and do not play a physical barrier effect (La Retuzière, scab on shoots the 11/06), the mixture is not efficient to limit the disease. This result is encouraging, because there is no comparison between the high level of disease reached in these experimental fields and the situation in a commercial orchard. Moreover, in the Gotheron experiment (organic orchard), the mixture showed no negative effect on powdery mildew and rosy aphid (Parisi *et al.*, 2013). In the contrary, a positive effect of the mixture associated with sanitation was showed in 2009 for powdery mildew. Cultivar mixture cannot be considered as a stand-alone strategie and must be associated with other practices: sanitation, reduced spraying program. It could be particularly interesting in organic orchard for scab control, due to the difficulty to control this disease in absence of curative fungicides compatible with organic regulation.

Another interesting question is: does the mixture favour the overcoming of monogenic resistance genes included in the mixture? We can underline that in Gotheron, the cv Pitchounette is currently not overcome in the mixture with Melrouge, unlike cv Ariane (*Rvi6* gene), overcome since 2012 in pure stands (unpublished results). When overcome, the *Rvi6* resistant cultivars can be different in their susceptibility to disease, following the genetic background of each cultivar (presence of other resistance factors). Ariane is known to be susceptible to *Venturia* strains virulent to *Rvi6* gene (Caffier *et al.*, 2010), Pitchounette was less susceptible in greenhouse experiments (F. Laurens, personal communication). The overcoming of the resistance of a cultivar depends on many factors: quantity of compatible inoculum, climatic conditions, cultural practices (spraying program, sanitation), susceptibility of the cultivar. The mixture of cultivar is one of the components constituting the cultural practices, and its relative importance between all these factors is not known.

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