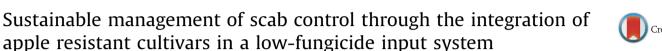
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ABSTRACT

Evaluation of the sustainability of disease control strategies through experimental field studies is poorly documented. Plant genetic resistance to pathogens offers an interesting alternative to the use of pesticides, but pathogen populations are able to adapt, thus frequently resulting in the breakdown of the resistance. Partial resistance is considered to provide more durable resistance than major genes. However, partial resistance does not confer complete protection and its efficiency can also decrease. Developing appropriate strategies which integrate resistant cultivars into crop systems is therefore needed to increase the efficiency and durability of the resistance, whatever the kind of resistance. The aim of this study was to evaluate the relevance of the association of control methods in terms of increasing the efficiency and durability of two kinds of resistances: (i) partial resistance in the apple cultivar Reine des Reinettes and (ii) major resistance (Rvi6) in the apple cultivar Ariane, when planted in a region where the climatic conditions are very favourable to the disease. It was found that the removal of leaf litter in autumn together with spraying of fungicides in the case of moderate or high risks of scab infection resulted in a sustainable control of scab on Reine des Reinettes over a five-year period and delayed the breakdown of the major resistance Rvi6 of Ariane by virulent isolates.

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1. Introduction

The development of integrated disease management offers an interesting possibility to reduce the use of pesticides, but needs to be sustainable to counteract the rapid evolution of pathogen populations. Indeed, pathogen populations are able to adapt; this results in the inefficiency of fungicide (Beckerman et al., 2015) or plant resistance (McDonald and Linde, 2002). The durability of a plant resistance can be characterized either by the time of appearance of virulent isolates or by the time of invasion of virulent isolates leading to loss of yield (van den Bosch and Gilligan, 2003). Two types of plant resistance are generally recognized: qualitative resistance conditioned by a single gene and quantitative resistance conditioned by multiple genes of partial effect, with intermediate types between these extremes (Poland et al., 2009). Partial resistance is considered to provide more durable resistance than qualitative resistance (McDonald and

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Linde, 2002). However, partial resistance does not confer complete protection and its efficiency can also decrease (Delmotte et al., 2014). Developing appropriate strategies integrating resistant cultivars into crop systems are therefore needed to reduce the inoculum size of pathogen populations, in order to both increase the efficiency of disease control and reduce the adaptation ability of pathogens to overcome plant resistance. Strategies integrating resistant cultivars with sanitation and reduced fungicide treatments have been proposed for Leptosphaeria maculans on canola, Ascochyta rabei on chickpea, Rhynchosporium secalis on barley and Puccinia asparagi on asparagus (Aubertot et al., 2006; Gan et al., 2006; Johnson, 2012; Zhan et al., 2008), but need to be tested experimentally (Gladders et al., 2006; Mundt, 2014).

Apple scab is a worldwide disease affecting apple (Malus \times domestica) and is caused by the ascomycete Venturia inaequalis (MacHardy, 1996). When scab lesions are present on fruits, a part of the production is not marketable. Moreover, once scab is present in the orchard, the control of the disease can be difficult for several years. The majority of commercial apple cultivars are susceptible to scab, which requires an intensive use of fungicides for scab control. For example, in France, the treatment frequency index for the

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protection of apple orchards against pests and diseases was 28.1 in 1997 and 35.4 in 2007, and fungicide treatments against apple scab represented 72 to 80% of the treatments applied in orchards (Sauphanor et al., 2009). This situation of high selection pressure of fungicides can lead to fungicide resistance in *V. inaequalis* populations (Hildebrand et al., 1988), and even resistance to multiple fungicides (Chapman et al., 2011). Despite the availability of meteorological data and weather warning systems in apple production areas, the number of fungicide applications is one of the major goals of research programmes in most countries with a large apple production, and is beginning to be the case for instance in the USA (Beckerman et al., 2015).

A reduction in the number of fungicide treatments is possible but depends on the protection system (conventional, organic farming, low-input) and on the disease susceptibility level of the variety (Holb et al., 2005; Simon et al., 2011). The use of resistant cultivars is a potential alternative to reduce chemical treatments (Brun et al., 2008); many resistance factors to scab have been identified in apple, either major genes or quantitative trait loci (Bus et al., 2011; Gessler et al., 2006; Soufflet-Freslon et al., 2008). However, cases of decrease in resistance efficiency over time have been reported (Bus et al., 2011; Caffier et al., 2014). The major resistance gene Rvi6 (=Vf) was the main resistance gene used in apple breeding programmes (Gessler et al., 2006) and has been deployed in several commercial apple cultivars. But this resistance has been overcome since the 90s in Europe (Parisi et al., 1993). In France, *Rvi6* has been mainly used in the northwest of the country. The first virulent isolates on Rvi6 were observed in 1995 on the cultivar Judeline (Parisi et al., 2000), and developed widely in the 2000s (Guerin and Le Cam, 2004). The first Rvi6 cultivars represented a very low acreage and were planted without any recommendation concerning scab control. Since 2002, the % of acreage planted with Rvi6 cultivars has increased in the different areas of apple production in France using the cultivar Ariane (Laurens et al., 2005). Recommendations to destroy leaf litter in autumn and to apply fungicides in the case of high scab risks are given to apple growers in order to increase the durability of resistance genes in regions where virulent isolates are not yet present (or present at low frequency). Cultivars carrying partial resistance to scab are also available, but few orchards in France are currently planted with partially resistant cultivars as most of them are old cultivars, lacking agronomic traits adapted to modern agriculture. Preserving for as long as possible the efficiency of both already employed and future resistances is of great importance.

Low-fungicide input apple systems involve the association of different scab control methods with partial effects, like sanitation and fungicide sprayings based on the risk of scab infections (Simon et al., 2011). V. inaequalis overwinters on leaf litter as pseudothecia that release ascospores, which are the primary inoculum for the following year (MacHardy, 1996). Sanitation can be used to significantly decrease primary inoculum in spring. The destruction of leaf litter though shredding, urea application, or leaf litter removal from the alleys combined with leaf ploughing within rows can reduce ascospore release by 50-95% (Gomez et al., 2007; Sutton et al., 2000; Vincent et al., 2004). Treatments with fungal antagonists together with leaf shredding and/or urea application can reduce ascospores by up to 96% (Carisse et al., 2000; Vincent et al., 2004). The epidemiology of V. inaequalis during primary infections (until the end of ascospore release) and during secondary infections (in summer) is well known (Carisse et al., 2009; Holb et al., 2003; MacHardy, 1996) and warning systems based on meteorological data are used to apply fungicides against scab at the correct time (MacHardy, 1996), with the possibility to choose a level of infection risk depending on the scab susceptibility level of the apple cultivar (Olivier, 1986).

In the present study, we make the hypothesis that integration of resistant cultivars in low-input systems, which combine several partial methods of control, could be a sustainable strategy for an efficient and durable control of scab disease. To test this hypothesis, we conducted an experiment over a five-year period with the two resistant apple cultivars Ariane and Reine des Reinettes in combination with sanitation and a reduced number of fungicide treatments. Ariane is a cultivar used in France since 2002 (Laurens et al., 2005) with a production of 24.000 ton of apple in 2014 (Association Nationale Pommes Poires, 2014). It carries the major scab gene Rvi6. Reine des Reinettes is an old French cultivar with a production of 15,000 ton of apples in 2014 (Association Nationale Pommes Poires, 2014). It was chosen because of its low susceptibility to scab on leaves and fruits, evaluated without fungicide application (Brun et al., 2008). There is no report of resistance erosion for Reine des Reinettes, whereas it is known that the breakdown of Ariane resistance results in a high scab susceptibility (Crété et al., 2013). Each year, we evaluated the efficiency of the scab control at two key points of the epidemiological cycle of the disease: at the end of the primary infection on the leaves and at harvest on the fruits.

2. Material and methods

2.1. The experimental orchard

The cultivars Ariane and Reine des Reinettes were grafted on Pajam[®] 2 (4160) Cepiland rootstock and planted during winter 1998/1999 in an experimental orchard situated in the Loire Valley (France, GPS coordinates $47^{\circ}40'27.568''N$, $000^{\circ}36'30.221''W$), in a region where inoculum virulent to *Rvi6* was present. This orchard comprised 6 plots of 810 m^2 for each cultivar (6 rows of 13 trees, with 4 m between the rows and a tree-to-tree distance of 1.25 m) surrounded by a hedge planted with different non-host species for scab. The plots were spatially grouped in three blocks. Before 2004, no fungicide against scab had been applied on these plots, except on Reine des Reinettes in 2001 and 2002 (Brun et al., 2008). Scab was present each year on Reine des Reinettes, whereas no scab was observed on Ariane. In 2004, scab was observed for the first time on two Ariane trees planted in the surrounding area, 14m away from the experimental orchard (Crété et al., 2013).

2.2. Management strategy

Since 2005, two modalities have been tested for each cultivar. Three of the six plots (called 'managed plots' in the paper) for each cultivar were managed through sanitation by leaf removal and through chemical treatments in the case of moderate or severe risks for scab infection. The choice of this strategy was in agreement with both the recommendations given for a partially resistant cultivar for increasing the efficiency of scab control, and the recommendations given for an *Rvi6* cultivar for increasing the durability of scab control.

On the three other plots (called 'control plots' in the paper) for each cultivar, there was no scab control, i.e. no sanitation and no treatment against scab. However, to avoid the disease getting out of control and to prevent a large number of isolates dispersing out of the experimental orchard, we decided to modify the strategy applied on the control plots if there was more than 30% of scabbed fruits at harvest, depending on the susceptibility level of the cultivar: we applied sanitation on control plots of Reine des Reinettes (low level of susceptibility), and both sanitation and chemical treatments to protect all scab risks on control plots of Ariane (very susceptible in case of breakdown). Download English Version:

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