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Sustainable management of scab control through the integration of apple resistant cultivars in a low-fungicide input system

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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.

Keywords. *Malus x domestica*; Resistance; Cultural practices; *Venturia inaequalis*; Chemical control; Durability

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 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 x 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 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 treatments to control the disease remains high.

Reducing 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 through 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 tons 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 tons 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°40'27.568''N, 000°36'30.221''W), in a region where inoculum virulent to *Rvi6* was present. This orchard comprised 6 plots of 810 m² 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).

2.3. Sanitation and chemical treatments

In the managed plots, leaf litter was grinded and removed once or twice every autumn after leaf fall using a shredder Rotoram FS 175 (Chabas, Charleval, France). Leaves were sucked in by the turbine and collected in a truck.

Climatic risks for scab development were evaluated using Pulsowin 3.1 software (Pulsonic, Orsay, France) and based on hourly climatic data provided by an automatic weather station (Pulsiane, Pulsonic, Orsay, France) located 650 m from the orchard. Four levels of risk were considered (Olivier, 1986): very low (A, i.e. “Angers”), low (L), moderate (M) and severe (S). Ascospore release was also estimated by Pulsowin 3.1 software. The experimental orchard was managed following the French Integrated Fruit Production rules, except for scab and powdery mildew chemical control. For powdery mildew (*Podosphaera leucotricha*) control, only the bupirimate (a specific fungicide for powdery mildew) was used. For scab control, the fungicide treatment was based on Olivier's (1986) model of treatment threshold validated and modified by Lefeuvre (1995). The strategy of Olivier is based on stop or curative treatments, which can induce the emergence of resistance to fungicides in the *V. inaequalis* populations. To reduce this risk, we chose a mixed strategy:

- preventive treatments were made before the start of the wet period at the origin of the contamination but only if a rain event associated with a high ascospore load was predicted
- stop or curative treatments were undertaken up to 24, 36, 48 or 96 hours after the start of the wet period, depending on the active ingredient used. Stop treatments with multisite fungicides were preferred to curative treatments with unisite fungicides.

These treatments were applied between the bud break of each cultivar (green tip stage in apple phenological classification) and the end of ascospore release predicted by the software Pulsowin. The applications were done at recommended application rates using a tractor with a pneumatic atomizer KWH in 2006 and 2007 or a jet atomizer worn S21 from 2008 to 2010, with a spray volume of 400 l/ha (Table 1).

2.4. Leaf litter density and potential ascospore dose assessments

In October after the harvest, the number of leaves and number of scab lesions per leaf was counted on 25 to 42 shoots per plot. The leaf area of 280 leaves was measured by an Area Meter (Delta-T Devices, England) and estimated as 19.6 and 22.1 cm² for Reine des Reinettes and Ariane, respectively. The lesion density (LD) was evaluated as the number of lesions per cm² of leaf area. In March, before bud break, the orchard leaf litter density was evaluated around two trees per plot selected at random in the four inner rows. The presence/absence of leaves was evaluated every 30 cm along four transects of 5.5 m between each selected tree and four trees located in the adjacent rows, in order to assess the proportion of orchard floor covered by leaf litter, i.e. leaf litter density (LLD). There was no overlapping of the different transects. The potential ascospore dose (PAD) was then calculated each spring for each plot according to Gadoury and MacHardy (1986), and expressed as a potential number of ascospores per square meter of orchard floor.

$$PAD = LLD \times LD \times PD \times AD \times n, \quad (1)$$

in which LD (lesion density) and LLD (leaf litter density) were estimated as explained above; PD (pseudothecial density) was estimated according to Gadoury and MacHardy

(1986) based on the value of 21.6 pseudothecia per fertile lesion, with two different equations depending on the % of scabbed leaves (I):

$$I < 10\%: PD = (2.67 - 0.0496 \times I + 0.29 \times I^2) \times 21.6 \text{ (Gadoury and MacHardy, 1986)} \quad (2)$$

$$I > 10\%: PD = (9.6447 \times \ln I - 4.6545) \times 21.6, \text{ adapted from Gadoury and MacHardy (1986)} \quad (3)$$

AD (ascus density) was set at 122 asci per ascocarp, according to Gadoury and MacHardy (1986); and n, the number of ascospores per ascus, equals 8.

2.5. Disease assessments

Scab disease was assessed for 5 years from 2006 until 2010. The 78 trees of each plot were observed in June or July at the end of the primary infection phase, as the % of infected leaves on the whole tree. The scoring scale was an ordinal scale adapted from Lateur and Populer (1994), with nine levels of disease incidence, described as intervals around the following values of scabbed leaves percentages: 0% (score 1), 1% (score 2), 3% (score 3), 12.5% (score 4), 25% (score 5), 37.5% (score 6), 50% (score 7), 75% (score 8) and 90% (score 9). Two annotators, one standing on each side of the tree, looked for the presence of sporulating scab lesions on the upper surface of all the leaves that could be observed during a period of 2 minutes and agreed on a common score for each tree. At harvest, fruits were sampled randomly on the 4 inner rows of each plot (approximately 26 kg of apples per row) and the % of scabbed fruits (at least one typical scabbed lesion observed) was evaluated.

2.6. Reference plot

In the same experimental Unit, 800m from the experimental orchard, a plot of 2095 m² (4 rows of 202 trees, with 4 m between the rows and a tree-to-tree distance of 1.25 m) planted with the scab susceptible cultivar Mitchgla, a mutant of Gala, was managed following the French Integrated Fruit Production rules. The leaf litter was grinded each autumn. All scab risks were protected by preventive, stop or curative treatments from the green tip stage of Mitchgla until 3 days to 3 weeks after the last forecasted ascospore ejection if no or few lesions were detected in the orchard. However, in 2008 and 2009 the last fungicide sprays against scab were done on the first of August and the 31th of July, respectively. At harvest, an estimation of the % of scabbed fruits was carried out by counting the number of scabbed fruits from a sample of 330 kg.

2.7. Statistical analyses

For each year and each cultivar, we analysed five variables: the leaf litter density in March, the potential ascospore dose in March, the number of scabbed trees at the end of ascospore release, the % of scabbed leaves on the scabbed trees at the end of ascospore release and the number of scabbed fruits at harvest. For each variable, we tested two factors and their interactions:

- Treatment, with two modalities (control plots and managed plots),
- Block, with three blocks (one control plot and one managed plot in each block).

For the leaf litter density, the number of scabbed trees and the number of scabbed fruits, we used a logistic regression (link function = logit). However, when the binomial model was unsuitable, a Poisson regression with a logarithmic function was used. For the potential ascospore dose (logarithm of the variable) and the % of scabbed leaves, we carried out an analysis of variance (ANOVA).

When the conditions of application of linear or generalized linear models were not respected, the data were tested by the non-parametric Kruskal-Wallis test with the factor Treatment.

All statistical analyses were performed using R version 3.0.1 (R Development Core Team, 2013).

3 Results

3.1. Predicted infection risks

The number of risks for scab infection varied from 6 to 19 according to the year (Table 2). The maximum number of risks was recorded in 2008, whereas the maximum number of severe risks was recorded in 2009.

As a treatment can (unintentionally) cover a risk under the threshold, 1 to 6 risks remained unprotected each year, mainly very low and low risks as expected by the protocol, with generally low ascospore projections except on 12/04/2007, 04/05/2008 and 27/04/2009 (Table 2). However, in 2007, two moderate risks were unprotected due to a malfunction of a leaf wetness sensor which underestimated the length of wetness. One of these risks (20 May) was not accompanied by high ascospore release, but for the other risk (7 May), 10.3% of the ascospores were ejected.

3.2. Number of fungicide treatments for scab control and active ingredients used

On the managed plots, 3 to 9 fungicides, depending on the year, were applied to protect moderate and severe risks (Table 3), whereas 11 to 24 fungicides were necessary on the reference plot of the susceptible cultivar Mitchgla. For the 5 years of experimentation, a total of 32 treatments were applied to protect the managed plots of Reine des Reinettes and Ariane, compared to 86 treatments for the reference plot of Mitchgla (Table 3). This represents a reduction of 62.8% of the number of fungicide treatments for scab control, with at least 50% of reduction for each year. The number of stop or curative treatments was the same in the managed and the reference plots, with 26 and 28 treatments, respectively. The number of treatments with unisite fungicide was also similar in the managed and reference plots, with 22 and 29 treatments respectively (Table 1).

3.3. Efficiency of leaf removal

The efficiency of leaf removal was similar on the two cultivars, but depended greatly on the year, from 43% in 2007 to 90% in 2006 (Table 4).

3.4. Disease

In the control plots of Reine des Reinettes, the % of scabbed trees increased from 37% in 2006 to 99% in 2009 (mean of the three plots), the % of scabbed fruits from 2 to 85%, and the PAD was multiplied by 40 (Fig. 1). These results can be explained by a high number of severe risks in 2009 (5 severe risks) following 2 years of high number of total risks (17-18 risks in 2007-2008). As the disease amount was above the defined threshold in 2009, we applied sanitation in autumn 2009. In 2010, which presented lower scab risks than in the previous years (including only one severe risk), the disease

was low in terms of % of scabbed leaves and % of scabbed fruits, even if the % of scabbed trees was still high.

In the managed plots of Reine des Reinettes, the disease remained low (between 3 and 17% of scabbed trees, and between 0.2 and 1.5 % of scabbed fruits), even in 2007 when two moderate risks were unprotected, showing the stability of the disease control (Fig. 1).

In the control plots of Ariane, apple scab was observed for the first time in 2006 (i.e. 2 years after the first report of apple scab on Ariane in the surrounding areas). Then the disease increased quickly to reach 96% of scabbed trees, with 7% of scabbed leaves and 35% of scabbed fruits in 2008 (Fig. 1). As the disease amount was above the defined threshold in 2008, we applied sanitation the following autumn and treated the control plots with fungicides to cover all scab risks in 2009 and 2010. As a consequence, the disease in the control plots was near to zero in 2009 and 2010, even if the PAD in 2010 was still high.

In the managed plots of Ariane, the first scab symptoms were observed in 2007 (one year after the control plots) and remained at a low level until 2009, although climatic conditions were very favourable to scab development. In 2010 (two years after the control plots), however, the disease increased greatly with up to 99% of scabbed trees with 21% of scabbed leaves and 20% of scabbed fruits (Fig. 1), although the climatic conditions were not very favourable to scab (8 scab risks, including only three moderate or severe risks), but the PAD had increased greatly in 2009 and 2010.

In the reference plot of the cultivar Mitchgla, no scabbed fruit was observed except in 2009 (2.3% of scabbed fruits).

4. Discussion

The evaluation of the sustainability of disease control strategies through experimental field studies is poorly documented. Here, in a 5-year study in apple orchards, we demonstrated the interest of integrating a resistant cultivar in a low-fungicide input system based on sanitation and a reduced number of fungicide treatments in order to improve the efficiency of a partial resistance and delay the monogenic resistance breakdown by virulent isolates of *V. inaequalis*.

4.1. Sustainability of disease control

The association of sanitation with a reduced number of fungicide treatments resulted in good efficiency of the scab control on the partially resistant cultivar Reine des Reinettes: in the managed plots, scab remained at a low level (<2%) and the potential ascospore dose remained stable from one year to another. Our results are consistent with those of Simon et al. (2011) obtained on Melrose, another old apple cultivar with partial resistance. This supports the idea that the lack of protection of some very low or low risks with fungicides does not represent a great risk when associated with sanitation and a good level of partial resistance. Even a lack of protection of some moderate risks did not induce any failure of scab control, and sanitation alone without any fungicide treatment resulted in a good control of the disease in a situation where the potential ascospore dose was high. These results are in agreement with Brun et al. (2008) who observed a very low amount of disease in the absence of fungicide treatments on Reine

des Reinettes in years with low scab risks. This demonstrates that our strategy based on sanitation and partial resistance gives some leeway if it is not possible to treat a risk by fungicides (due to climatic conditions, for example). Our experimental conditions on Reine des Reinettes were more severe than in a commercial orchard, because the control plots had a high inoculum and were in the same experimental orchard as the managed plots. Even if the plots were surrounded by tree hedges, we cannot exclude that a small amount of ascospores was able to be dispersed over the 15 m of distance between the plots, and that some dead leaves moved from one plot to another, as demonstrated by Brun et al. (2012). Furthermore, no chemical application was applied after the end of ascospores ejection, and ascospores from untreated plots may have lightly contaminated fruits in treated plots. This underlines the solidity of the results obtained in the present study.

Moreover, this strategy proved to be efficient in gaining two years in terms of durability of the *Rvi6* major gene in the cultivar Ariane, considering the time of first significant yield losses. This value is underestimated, as this experiment was carried out in severe conditions for scab, with a higher amount of primary inoculum than usually found in commercial orchards, and with a failure of the strategy in 2007, which may have promoted the increase of inoculum in the subsequent years in the managed plots. As virulent isolates were already present in the surroundings of our experimental orchard (Cr  t   et al., 2013), we can expect a higher gain in durability if such a strategy is applied as soon as the resistance gene is deployed. This experimentally confirms that a reduction in inoculum size and a reduced infection rate due to sanitation and fungicide treatments slowed down the increase in frequency of virulent isolates. Our results are in agreement with other studies (Aubertot et al., 2006; Gan et al., 2006; Johnson, 2012;

Zhan et al., 2008), but here we experimentally obtained a value of the gain of such a strategy.

In the present study, we tested two contrasting cultivars, Reine des Reinettes with partial resistance and no reported resistance erosion, and Ariane with major resistance and reported resistance breakdown resulting in a very high susceptibility to virulent isolates. Our results with two extreme types of resistance offer interesting perspectives to obtain higher resistance efficiency and longer delay of resistance breakdown for cultivars pyramiding major genes with quantitative resistance (Brun et al., 2010; Quenouille et al., 2013). Such a strategy should also be helpful in the case of erosion of quantitative resistance over time (Caffier et al., 2014).

4.2. Reduction of fungicides and implementation of a strategy based on an association of control methods on a commercial scale

This study shows that apple scab can be effectively managed with a reduction of 60% of fungicides and provides a rare example of practical and significant reduction of fungicide applications in apple orchards.

The strategy of Olivier (1986) is based on stop or curative treatments, which can induce the emergence of resistance to fungicides in the *V. inaequalis* populations (Beckerman et al., 2015; Chapman et al., 2011). To reduce this risk, we chose a mixed strategy with both preventive and stop or curative treatments. Moreover, whenever possible, multisite fungicides were used. For the applications made with unisite fungicides, we applied the rules of anti-resistance strategies (Brent and Hollomon, 2007) based on a limited number of fungicide applications per year (for example, no more than 3 applications of demethylation inhibitor fungicides per year). Such a strategy is expected

to limit the emergence of resistance to fungicides. The good efficiency of fungicide treatments based on major scab infection risks requires technical expertise for the choice of the fungicides and their applications at appropriate dates. Most apple growers benefit from the technical support of advisors, useful during the several critical steps of apple cultivation.

Sanitation is already recommended in all orchards, whatever the protection system for scab control (conventional, organic farming, low-input), as it reduces the inoculum present in the orchard (Gomez et al., 2007; Sutton et al., 2000; Vincent et al., 2004). In Europe, its application is variable according to location, and can reach up to 70% of the apple orchards. The main obstacle to its application is labour, availability of suitable agricultural machines, and unfavourable (too wet) meteorological conditions after leaf fall.

The choice of resistant cultivars is a key point for an efficient scab control in a low input-system. We need modern cultivars adapted to low-input strategies, in the pedoclimatic conditions of the main production regions (Parisi et al., 2014). Most of the cultivars carrying partial resistance to scab that are available today are old cultivars, lacking agronomic traits adapted to modern agriculture; and most of the cultivars carrying major resistance genes rely on the *Rvi6* major gene. As a consequence, there is a need to increase our knowledge of partial resistance to scab (Hampson, 2013), and there is a need to develop cultivars with new resistance factors or combinations of resistance factors (Bus et al., 2011; Patocchi et al., 2009). Our work highlights the urgent need of breeding programmes aimed at selecting cultivars adapted to low-input systems (Warlop et al., 2010).

5. Conclusions

The association of scab resistant cultivars with culture practices and chemical methods of disease control contributed to an increase in the efficiency and durability of scab control. Indeed, the number of treatments against scab was reduced by more than half compared to the number of fungicide treatments applied in the same region on susceptible cultivars. Alternating different resistant cultivars in rotation is not possible for a perennial crop, but the combination of integrating resistant cultivars in space (Didelot et al., 2007; Sapoukhina et al., 2009) along with cultivation and chemical practices, offers interesting tools for the health and sustainability of apple production in the future. Here, we demonstrate that apple scab can be controlled with reduced fungicide inputs in a French region with climatic conditions favourable to scab. Such a strategy could be applied to major apple production regions worldwide.

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451 Table 1. Description of the fungicide treatments (ingredient and number) applied for scab control from 2006 and 2010 in the experimental
452 managed plots of apple cultivars Ariane and Reine des Reinettes, and on the reference plot of apple cultivar Mitchgla

Ingredient	Mode of action	Experimental managed plots ^a		Reference plot ^b	
		preventive treatment	stop or curative treatment	preventive treatment	stop or curative treatment
Captan	multisite	0	4	15	8
Copper hydroxide	multisite	not used	not used	1	0
Dithianon	multisite	2	4	8	8
Mancozeb	multisite	not used	not used	6	0
Sulphur	multisite	not used	not used	4	0
Thiram	multisite	not used	not used	6	0
Zineb	multisite	not used	not used	1	0
Cyprodinil	unisite	3	2	8	1
Difenoconazole	unisite	0	6	0	4
Dodine	unisite	1	5	3	4
Fenbuconazole	unisite	0	3	0	1
Fluquinconazole + Pyrimethanil	unisites	not used	not used	5	0
Hexaconazole	unisite	0	1	0	1
Tolyfluanid	unisite	0	1	1	1

453

454 ^a Application of fungicides to protect moderate and severe scab infection risks

455 ^b Application of fungicides to protect all scab infection risks

456

457

Table 2. Periods of primary scab infection, number of scab infection risks and description of the scab infection risks not protected in the experimental managed plots of apple cultivars Ariane and Reine des Reinettes from 2006 to 2010

Year	Date of green tip stage		End of ascospore ejections ^a	Number of scab risks in each level ^b					Date and % of ejected ascospores for unprotected risks in the managed plots				
	Ariane	Reine des Reinettes		A	L	M	S	total number	A	L	M	S	total number
2006	25 March	3 April	14 June	3	2	1 (+1 ^c)	1 (+1 ^c)	7 (+2 ^c)	18/05: 0 19/05:1.2 20/05:0.6	0	0	0	3
2007	19 March	23 March	31 May	3	5	8	1	17	12/04:18.1 25/05: 1.5	08/05: 0 12/05: 0.3	07/05: 10.3 20/05: 0.4	0	6
2008	14 March	17 March	26 May	3	7	6	2(+1 ^c)	18 (+1 ^c)	23/03: 0.4 04/05: 8.4 22/05: 0.3	13/04: 1.6	0	0	4
2009	24 March	24 March	19 May	0	4	2	5	11	0	27/04: 6.1 ^d	0	0	1 ^d
2010	26 March	31 March	8 June	2	1(+2 ^c)	2	1	6 (+2 ^c)	26/05: 0 28/03: 0.6 ^e	27/03: 0.6 ^e	0	0	1 (+2 ^e)

^a End of ascospore ejections predicted by the software Pulsowin

^b Number of infection periods in each risk level between the green tip stage and the end of ascospore ejections. The risks are described following Olivier (1986): A Angers, L low, M moderate, S severe

^c Into brackets additional risks concerning only Ariane due to the earlier green tip stage date

465 ^d Untreated risk concerning only Reine des Reinettes due to missing fungicide treatments

466 ^e Untreated risks concerning only Ariane due to the earlier green tip stage date

467

468 Table 3. Number of fungicide treatments for scab control on the experimental managed plots of apple cultivars Ariane and Reine des
 469 Reinettes, and on the reference plot of cultivar Mitchgla from 2006 to 2010

Year	number of treatments in the managed plots ^a			number of treatments in the reference plot ^b		
	preventive	stop or curative	total	preventive	stop or curative	total
2006	2	3	5	6	5	11
2007	0	7	7	9	5	14
2008	2	7	9	14	10	24
2009	1	7 (-2) ^c	8 (-2) ^c	14	7	21
2010	1	2	3	15	1	16

470

471 ^a Application of fungicides to protect moderate and severe scab infection risks

472 ^b Application of fungicides to protect all scab infection risks

473 ^c Two treatments were missing in the plots of Reine des Reinettes

474 Table 4. Leaf litter density (% of orchard floor covered by leaf litter) in the experimental control and managed plots of apple cultivars
 475 Ariane and Reine des Reinettes, evaluated each year in March

Year	Ariane			Reine des Reinettes		
	control plots	managed plots		control plots	managed plots	
2006	39 ^a	12	* ^c	59	6	*
2007	45	25	*	45	26	*
2008	42	16	*	51	8	*
2009	15^b	14	NS	44	15	*
2010	15	16	NS	8	11	NS

476

477 ^a in italic, no removal of leaf litter

478 ^b in bold, removal of leaf litter

479 ^c *significant difference between control and managed plots (p<0.05), NS non-significant difference

Figure captions

Fig. 1 – Evolution over a five-year period of the potential ascospore dose (PAD in Log of number of ascospores per square meter of orchard floor) assessed in March, the % of scabbed trees and disease severity score assessed in June or July (end of primary infections), and the % of scabbed fruits at harvest for two apple cultivars Reine des Reinettes (a, b, c, d) and Ariane (e, f, g, h) in managed plots (broken line) and control plots (solid line); each curve corresponds to one of the three plots for each modality and cultivar. Managed plots = sanitation + fungicides treatments for moderate and severe scab risks (blue line); control plots = no sanitation + no fungicide treatment (red line), sanitation alone (green line for Reine des Reinettes) or sanitation + fungicide treatments for all scab risks (green line for Ariane) applied because more than 30% of fruits were scabbed in the previous year. Missing data in 2006 for severity note of Reine des Reinettes and % of scabbed fruits of both cultivars. * significant difference between control and managed plots ($p < 0.05$).

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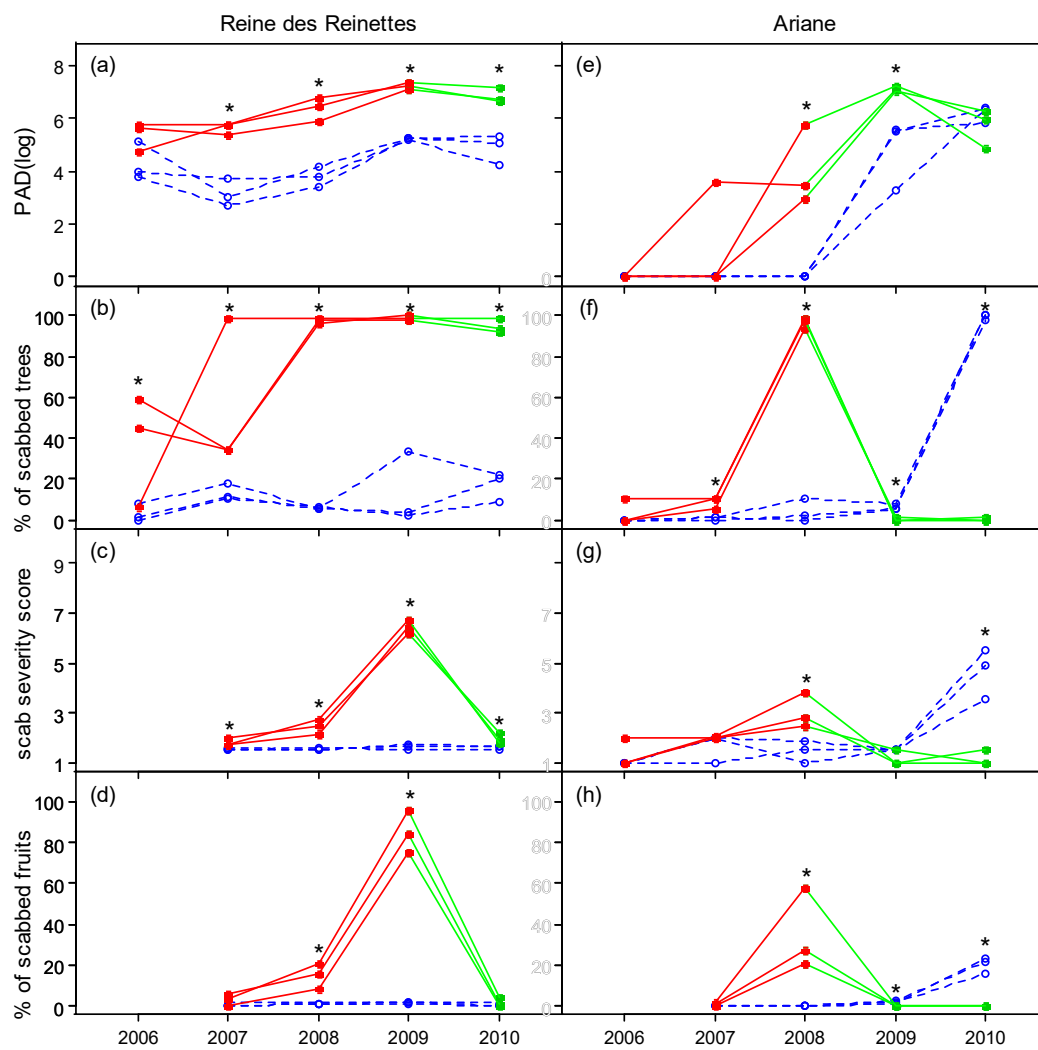


Figure 1