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

3

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15

16 **Abstract**

17 Evaluation of the sustainability of disease control strategies through experimental field  
18 studies is poorly documented. Plant genetic resistance to pathogens offers an interesting  
19 alternative to the use of pesticides, but pathogen populations are able to adapt, thus  
20 frequently resulting in the breakdown of the resistance. Partial resistance is considered  
21 to provide more durable resistance than major genes. However, partial resistance does  
22 not confer complete protection and its efficiency can also decrease. Developing  
23 appropriate strategies which integrate resistant cultivars into crop systems is therefore  
24 needed to increase the efficiency and durability of the resistance, whatever the kind of

25 resistance. The aim of this study was to evaluate the relevance of the association of  
26 control methods in terms of increasing the efficiency and durability of two kinds of  
27 resistances: (i) partial resistance in the apple cultivar Reine des Reinettes and (ii) major  
28 resistance (*Rvi6*) in the apple cultivar Ariane, when planted in a region where the  
29 climatic conditions are very favourable to the disease. It was found that the removal of  
30 leaf litter in autumn together with spraying of fungicides in the case of moderate or high  
31 risks of scab infection resulted in a sustainable control of scab on Reine des Reinettes  
32 over a five-year period and delayed the breakdown of the major resistance *Rvi6* of  
33 Ariane by virulent isolates.

34

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

37

## 38 **1. Introduction**

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

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

60 Apple scab is a worldwide disease affecting apple (*Malus x domestica*) and is caused by  
61 the ascomycete *Venturia inaequalis* (MacHardy, 1996). When scab lesions are present  
62 on fruits, a part of the production is not marketable. Moreover, once scab is present in  
63 the orchard, the control of the disease can be difficult for several years. The majority of  
64 commercial apple cultivars are susceptible to scab, which requires an intensive use of  
65 fungicides for scab control. For example, in France, the treatment frequency index for  
66 the protection of apple orchards against pests and diseases was 28.1 in 1997 and 35.4 in  
67 2007, and fungicide treatments against apple scab represented 72 to 80% of the  
68 treatments applied in orchards (Sauphanor et al., 2009). This situation of high selection  
69 pressure of fungicides can lead to fungicide resistance in *V. inaequalis* populations  
70 (Hildebrand et al., 1988), and even resistance to multiple fungicides (Chapman et al.,  
71 2011). Despite the availability of meteorological data and weather warning systems in  
72 apple production areas, the number of treatments to control the disease remains high.

73 Reducing the number of fungicide applications is one of the major goals of research  
74 programmes in most countries with a large apple production, and is beginning to be the  
75 case for instance in the USA (Beckerman et al., 2015).

76 A reduction in the number of fungicide treatments is possible but depends on the  
77 protection system (conventional, organic farming, low-input) and on the disease  
78 susceptibility level of the variety (Holb et al., 2005; Simon et al., 2011). The use of  
79 resistant cultivars is a potential alternative to reduce chemical treatments (Brun et al.,  
80 2008); many resistance factors to scab have been identified in apple, either major genes  
81 or quantitative trait loci (Bus et al., 2011; Gessler et al., 2006; Soufflet-Freslon et al.,  
82 2008). However, cases of decrease in resistance efficiency over time have been reported  
83 (Bus et al., 2011; Caffier et al., 2014). The major resistance gene *Rvi6* (=Vf) was the  
84 main resistance gene used in apple breeding programmes (Gessler et al., 2006) and has  
85 been deployed in several commercial apple cultivars. But this resistance has been  
86 overcome since the 90s in Europe (Parisi et al., 1993). In France, *Rvi6* has been mainly  
87 used in the northwest of the country. The first virulent isolates on *Rvi6* were observed in  
88 1995 on the cultivar Judeline (Parisi et al., 2000), and developed widely in the 2000s  
89 (Guerin and Le Cam, 2004). The first *Rvi6* cultivars represented a very low acreage and  
90 were planted without any recommendation concerning scab control. Since 2002, the %  
91 of acreage planted with *Rvi6* cultivars has increased in the different areas of apple  
92 production in France using the cultivar Ariane (Laurens et al., 2005). Recommendations  
93 to destroy leaf litter in autumn and to apply fungicides in the case of high scab risks are  
94 given to apple growers in order to increase the durability of resistance genes in regions  
95 where virulent isolates are not yet present (or present at low frequency). Cultivars  
96 carrying partial resistance to scab are also available, but few orchards in France are

97 currently planted with partially resistant cultivars as most of them are old cultivars,  
98 lacking agronomic traits adapted to modern agriculture. Preserving for as long as  
99 possible the efficiency of both already employed and future resistances is of great  
100 importance.

101 Low-fungicide input apple systems involve the association of different scab control  
102 methods with partial effects, like sanitation and fungicide sprayings based on the risk of  
103 scab infections (Simon et al., 2011). *V. inaequalis* overwinters on leaf litter as  
104 pseudothecia that release ascospores, which are the primary inoculum for the following  
105 year (MacHardy, 1996). Sanitation can be used to significantly decrease primary  
106 inoculum in spring. The destruction of leaf litter through shredding, urea application, or  
107 leaf litter removal from the alleys combined with leaf ploughing within rows can reduce  
108 ascospore release by 50-95% (Gomez et al., 2007; Sutton et al., 2000; Vincent et al.,  
109 2004). Treatments with fungal antagonists together with leaf shredding and/or urea  
110 application can reduce ascospores by up to 96% (Carisse et al., 2000; Vincent et al.,  
111 2004). The epidemiology of *V. inaequalis* during primary infections (until the end of  
112 ascospore release) and during secondary infections (in summer) is well known (Carisse  
113 et al., 2009; Holb et al., 2003; MacHardy, 1996) and warning systems based on  
114 meteorological data are used to apply fungicides against scab at the correct time  
115 (MacHardy, 1996), with the possibility to choose a level of infection risk depending on  
116 the scab susceptibility level of the apple cultivar (Olivier, 1986).

117 In the present study, we make the hypothesis that integration of resistant cultivars in  
118 low-input systems, which combine several partial methods of control, could be a  
119 sustainable strategy for an efficient and durable control of scab disease. To test this  
120 hypothesis, we conducted an experiment over a five-year period with the two resistant

121 apple cultivars Ariane and Reine des Reinettes in combination with sanitation and a  
122 reduced number of fungicide treatments. Ariane is a cultivar used in France since 2002  
123 (Laurens et al., 2005) with a production of 24,000 tons of apple in 2014 (Association  
124 Nationale Pommes Poires, 2014). It carries the major scab gene *Rvi6*. Reine des  
125 Reinettes is an old French cultivar with a production of 15,000 tons of apples in 2014  
126 (Association Nationale Pommes Poires, 2014). It was chosen because of its low  
127 susceptibility to scab on leaves and fruits, evaluated without fungicide application (Brun  
128 et al., 2008). There is no report of resistance erosion for Reine des Reinettes, whereas it  
129 is known that the breakdown of Ariane resistance results in a high scab susceptibility  
130 (Crété et al., 2013). Each year, we evaluated the efficiency of the scab control at two  
131 key points of the epidemiological cycle of the disease: at the end of the primary  
132 infection on the leaves and at harvest on the fruits.

133

## 134 **2. Material and methods**

135

### 136 ***2.1. The experimental orchard***

137 The cultivars Ariane and Reine des Reinettes were grafted on Pajam<sup>®</sup> 2 (4160) Cepiland  
138 rootstock and planted during winter 1998/1999 in an experimental orchard situated in  
139 the Loire Valley (France, GPS coordinates 47°40'27.568''N, 000°36'30.221''W), in a  
140 region where inoculum virulent to *Rvi6* was present. This orchard comprised 6 plots of  
141 810 m<sup>2</sup> for each cultivar (6 rows of 13 trees, with 4 m between the rows and a tree-to-  
142 tree distance of 1.25 m) surrounded by a hedge planted with different non-host species  
143 for scab. The plots were spatially grouped in three blocks. Before 2004, no fungicide  
144 against scab had been applied on these plots, except on Reine des Reinettes in 2001 and

145 2002 (Brun et al., 2008). Scab was present each year on Reine des Reinettes, whereas  
146 no scab was observed on Ariane. In 2004, scab was observed for the first time on two  
147 Ariane trees planted in the surrounding area, 14m away from the experimental orchard  
148 (Crété et al., 2013).

149

## 150 **2.2. Management strategy**

151 Since 2005, two modalities have been tested for each cultivar.

152 Three of the six plots (called ‘managed plots’ in the paper) for each cultivar were  
153 managed through sanitation by leaf removal and through chemical treatments in the case  
154 of moderate or severe risks for scab infection. The choice of this strategy was in  
155 agreement with both the recommendations given for a partially resistant cultivar for  
156 increasing the efficiency of scab control, and the recommendations given for an *Rvi6*  
157 cultivar for increasing the durability of scab control.

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

166

## 167 **2.3. Sanitation and chemical treatments**

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

171 Climatic risks for scab development were evaluated using Pulsowin 3.1 software  
172 (Pulsonic, Orsay, France) and based on hourly climatic data provided by an automatic  
173 weather station (Pulsiane, Pulsonic, Orsay, France) located 650 m from the orchard.  
174 Four levels of risk were considered (Olivier, 1986): very low (A, i.e. “Angers”), low  
175 (L), moderate (M) and severe (S). Ascospore release was also estimated by Pulsowin  
176 3.1 software. The experimental orchard was managed following the French Integrated  
177 Fruit Production rules, except for scab and powdery mildew chemical control. For  
178 powdery mildew (*Podosphaera leucotricha*) control, only the bupirimate (a specific  
179 fungicide for powdery mildew) was used. For scab control, the fungicide treatment was  
180 based on Olivier's (1986) model of treatment threshold validated and modified by  
181 Lefeuvre (1995). The strategy of Olivier is based on stop or curative treatments, which  
182 can induce the emergence of resistance to fungicides in the *V. inaequalis* populations.  
183 To reduce this risk, we chose a mixed strategy:

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

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

196

#### 197 ***2.4. Leaf litter density and potential ascospore dose assessments***

198 In October after the harvest, the number of leaves and number of scab lesions per leaf  
199 was counted on 25 to 42 shoots per plot. The leaf area of 280 leaves was measured by  
200 an Area Meter (Delta-T Devices, England) and estimated as 19.6 and 22.1 cm<sup>2</sup> for  
201 Reine des Reinettes and Ariane, respectively. The lesion density (LD) was evaluated as  
202 the number of lesions per cm<sup>2</sup> of leaf area. In March, before bud break, the orchard leaf  
203 litter density was evaluated around two trees per plot selected at random in the four  
204 inner rows. The presence/absence of leaves was evaluated every 30 cm along four  
205 transects of 5.5 m between each selected tree and four trees located in the adjacent rows,  
206 in order to assess the proportion of orchard floor covered by leaf litter, i.e. leaf litter  
207 density (LLD). There was no overlapping of the different transects. The potential  
208 ascospore dose (PAD) was then calculated each spring for each plot according to  
209 Gadoury and MacHardy (1986), and expressed as a potential number of ascospores per  
210 square meter of orchard floor.

$$211 \quad \text{PAD} = \text{LLD} \times \text{LD} \times \text{PD} \times \text{AD} \times n, \quad (1)$$

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

214 (1986) based on the value of 21.6 pseudothecia per fertile lesion, with two different  
215 equations depending on the % of scabbed leaves (I):

216 
$$I < 10\%: PD = (2.67 - 0.0496 \times I + 0.29 \times I^2) \times 21.6 \text{ (Gadoury and MacHardy,}$$
  
217 1986) (2)

218 
$$I > 10\%: PD = (9.6447 \times \ln I - 4.6545) \times 21.6, \text{ adapted from Gadoury and}$$
  
219 MacHardy (1986) (3)

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

222

### 223 ***2.5. Disease assessments***

224 Scab disease was assessed for 5 years from 2006 until 2010. The 78 trees of each plot  
225 were observed in June or July at the end of the primary infection phase, as the % of  
226 infected leaves on the whole tree. The scoring scale was an ordinal scale adapted from  
227 Lateur and Populer (1994), with nine levels of disease incidence, described as intervals  
228 around the following values of scabbed leaves percentages: 0% (score 1), 1% (score 2),  
229 3% (score 3), 12.5% (score 4), 25% (score 5), 37.5% (score 6), 50% (score 7), 75%  
230 (score 8) and 90% (score 9). Two annotators, one standing on each side of the tree,  
231 looked for the presence of sporulating scab lesions on the upper surface of all the leaves  
232 that could be observed during a period of 2 minutes and agreed on a common score for  
233 each tree. At harvest, fruits were sampled randomly on the 4 inner rows of each plot  
234 (approximately 26 kg of apples per row) and the % of scabbed fruits (at least one typical  
235 scabbed lesion observed) was evaluated.

236

### 237 ***2.6. Reference plot***

238 In the same experimental Unit, 800m from the experimental orchard, a plot of 2095 m<sup>2</sup>  
239 (4 rows of 202 trees, with 4 m between the rows and a tree-to-tree distance of 1.25 m)  
240 planted with the scab susceptible cultivar Mitchgla, a mutant of Gala, was managed  
241 following the French Integrated Fruit Production rules. The leaf litter was grinded each  
242 autumn. All scab risks were protected by preventive, stop or curative treatments from  
243 the green tip stage of Mitchgla until 3 days to 3 weeks after the last forecasted ascospore  
244 ejection if no or few lesions were detected in the orchard. However, in 2008 and 2009  
245 the last fungicide sprays against scab were done on the first of August and the 31<sup>th</sup> of  
246 July, respectively. At harvest, an estimation of the % of scabbed fruits was carried out  
247 by counting the number of scabbed fruits from a sample of 330 kg.

248

## 249 ***2.7. Statistical analyses***

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

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

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

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

265

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

268

269

## 270 **3 Results**

271

### 272 ***3.1. Predicted infection risks***

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

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

283

### 284 ***3.2. Number of fungicide treatments for scab control and active ingredients used***

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

295

### 296 ***3.3. Efficiency of leaf removal***

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

299

### 300 ***3.4. Disease***

301 In the control plots of Reine des Reinettes, the % of scabbed trees increased from 37%  
302 in 2006 to 99% in 2009 (mean of the three plots), the % of scabbed fruits from 2 to  
303 85%, and the PAD was multiplied by 40 (Fig. 1). These results can be explained by a  
304 high number of severe risks in 2009 (5 severe risks) following 2 years of high number  
305 of total risks (17-18 risks in 2007-2008). As the disease amount was above the defined  
306 threshold in 2009, we applied sanitation in autumn 2009. In 2010, which presented  
307 lower scab risks than in the previous years (including only one severe risk), the disease

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

310 In the managed plots of Reine des Reinettes, the disease remained low (between 3 and  
311 17% of scabbed trees, and between 0.2 and 1.5 % of scabbed fruits), even in 2007 when  
312 two moderate risks were unprotected, showing the stability of the disease control (Fig.  
313 1).

314

315 In the control plots of Ariane, apple scab was observed for the first time in 2006 (i.e. 2  
316 years after the first report of apple scab on Ariane in the surrounding areas). Then the  
317 disease increased quickly to reach 96% of scabbed trees, with 7% of scabbed leaves and  
318 35% of scabbed fruits in 2008 (Fig. 1). As the disease amount was above the defined  
319 threshold in 2008, we applied sanitation the following autumn and treated the control  
320 plots with fungicides to cover all scab risks in 2009 and 2010. As a consequence, the  
321 disease in the control plots was near to zero in 2009 and 2010, even if the PAD in 2010  
322 was still high.

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

330

331 In the reference plot of the cultivar Mitchgla, no scabbed fruit was observed except in  
332 2009 (2.3% of scabbed fruits).

333

#### 334 **4. Discussion**

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

341

##### 342 ***4.1. Sustainability of disease control***

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

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

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

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

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

389

390 ***4.2. Reduction of fungicides and implementation of a strategy based on an***  
391 ***association of control methods on a commercial scale***

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

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

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

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

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

426

427        **5. Conclusions**

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

439

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449

450

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 <sup>a</sup>		Reference plot <sup>b</sup>	
		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 + Pyrimethanyl	unisites	not used	not used	5	0
Hexaconazole	unisite	0	1	0	1
Tolyfluanid	unisite	0	1	1	1

453

454 <sup>a</sup> Application of fungicides to protect moderate and severe scab infection risks

455 <sup>b</sup> Application of fungicides to protect all scab infection risks

456

457

458 Table 2. Periods of primary scab infection, number of scab infection risks and description of the scab infection risks not protected in the  
 459 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 <sup>a</sup>	Number of scab risks in each level <sup>b</sup>					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 <sup>c</sup> )	1 (+1 <sup>c</sup> )	7 (+2 <sup>c</sup> )	18/05: 0 19/05: 1.2 20/05: 0.6	0	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	0	6
2008	14 March	17 March	26 May	3	7	6	2(+1 <sup>c</sup> )	18 (+1 <sup>c</sup> )	23/03: 0.4	13/04: 1.6 04/05: 8.4 22/05: 0.3	0	0	0	4
2009	24 March	24 March	19 May	0	4	2	5	11	0	27/04: 6.1 <sup>d</sup>	0	0	0	1 <sup>d</sup>
2010	26 March	31 March	8 June	2	1(+2 <sup>c</sup> )	2	1	6 (+2 <sup>c</sup> )	26/05: 0	27/03: 0.6 <sup>e</sup> 28/03: 0.6 <sup>e</sup>	0	0	0	1 (+2 <sup>e</sup> )

460

461 <sup>a</sup> End of ascospore ejections predicted by the software Pulsowin

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

464 <sup>c</sup> Into brackets additional risks concerning only Ariane due to the earlier green tip stage date

465 <sup>d</sup> Untreated risk concerning only Reine des Reinettes due to missing fungicide treatments

466 <sup>e</sup> 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 <sup>a</sup>			number of treatments in the reference plot <sup>b</sup>		
	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) <sup>c</sup>	8 (-2) <sup>c</sup>	14	7	21
2010	1	2	3	15	1	16

470

471 <sup>a</sup> Application of fungicides to protect moderate and severe scab infection risks

472 <sup>b</sup> Application of fungicides to protect all scab infection risks

473 <sup>c</sup> 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 <sup>a</sup>	<b>12</b>	* <sup>c</sup>	59	<b>6</b>	*
2007	45	<b>25</b>	*	45	<b>26</b>	*
2008	42	<b>16</b>	*	51	<b>8</b>	*
2009	<b>15<sup>b</sup></b>	<b>14</b>	NS	44	<b>15</b>	*
2010	<b>15</b>	<b>16</b>	NS	<b>8</b>	<b>11</b>	NS

476

477 <sup>a</sup> in italic, no removal of leaf litter

478 <sup>b</sup> in bold, removal of leaf litter

479 <sup>c</sup> \*significant difference between control and managed plots (p<0.05), NS non-significant difference

480 **Figure captions**

481

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

495

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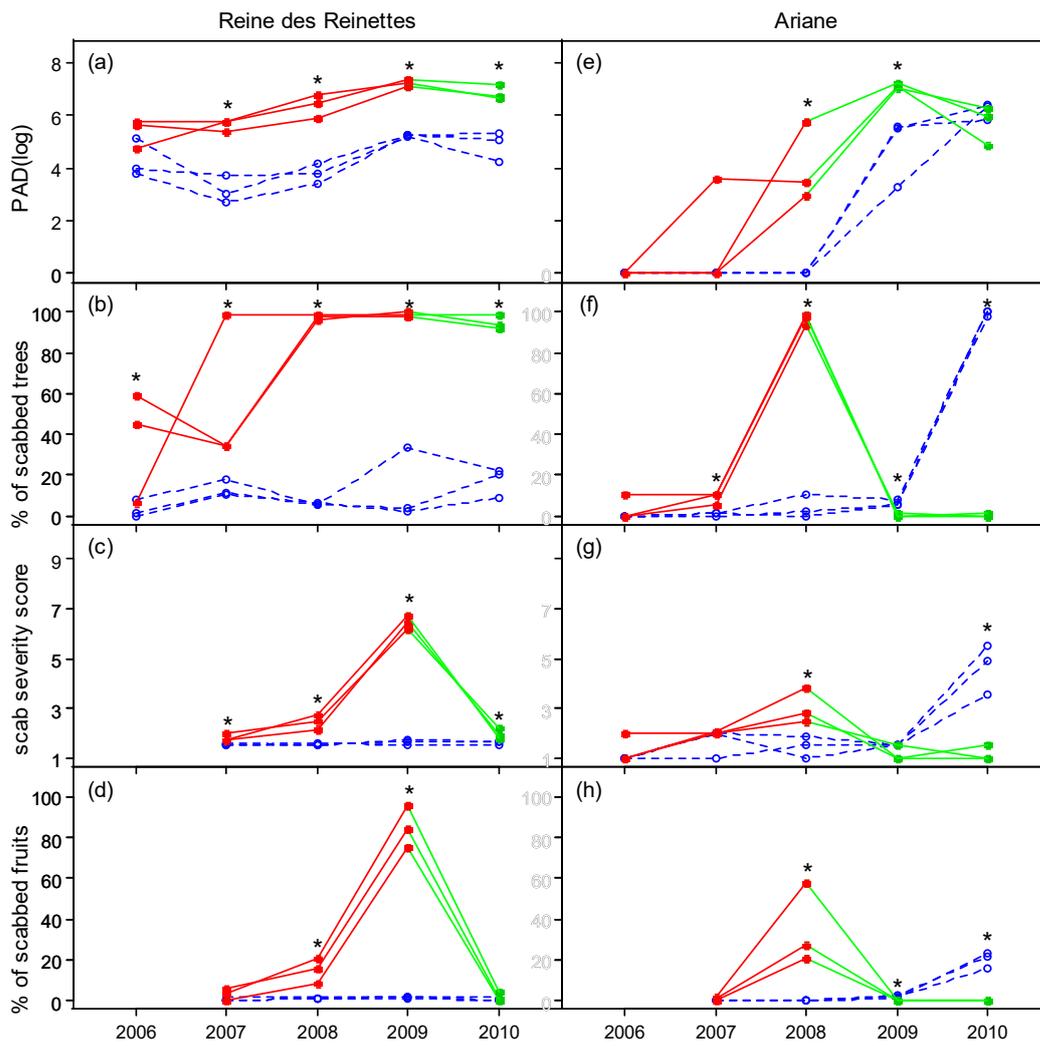
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642 Figure 1