

*The relative importance of conidia and ascospores as primary inoculum of Venturia inaequalis in a Southeast England orchard*

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1 **Title: The relative importance of conidia and ascospores as primary**  
2 **inoculum of *Venturia inaequalis* in a Southeast England orchard**

3

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9 Running head: Apple scab primary inoculum

10

11 Key Words: Apple scab, *Spilocaea pomi*, *Fusicladium dendriticum*, asexual over-wintering

## 12 **Abstract**

13 Apple scab, caused by *Venturia inaequalis*, can lead to large losses of marketable fruit if left  
14 uncontrolled. The disease appears in orchards during spring as lesions on leaves. These  
15 primary lesions are caused by spores released at bud burst from over-wintering sources; these  
16 spores can be sexually produced ascospores from the leaf litter or asexual conidia from  
17 mycelium in wood scab or within buds. We investigated the relative importance of conidia  
18 and ascospores as primary inoculum in an orchard in the United Kingdom. Potted trees not  
19 previously exposed to apple scab were placed next to (c. 1 m) orchard trees to trap air-  
20 dispersed ascospores. Number and position of scab lesions were assessed on shoots from both  
21 the potted trees (infection by airborne ascospores) and neighbouring orchard trees (infection  
22 by both ascospores and splash-dispersed conidia overwintered in buds). The distribution and  
23 population similarity of scab lesions were compared in the two tree types by molecular  
24 analysis and through modelling of scab incidence and count data. Molecular analysis was  
25 inconclusive. Statistical modelling of results suggested that conidia may have contributed  
26 approximately 20-50% of the total primary inoculum in this orchard: incidence was estimated  
27 to be reduced by 20% on potted trees, and lesion number by 50%. These results indicate that,  
28 although conidia are still a minority contributor to primary inoculum, their contribution in  
29 this orchard is sufficient to review current management. This might also be true of orchards  
30 with a similar climate.

31

## 32 **Introduction**

33 Annual epidemics of apple scab, caused by the ascomycete *Venturia inaequalis*, lead to large  
34 losses of marketable fruit worldwide if uncontrolled. The *V. inaequalis* life cycle sees  
35 overwintered spores released in the spring to infect newly emerged leaves. Lesions from  
36 these infections produce conidia which are dispersed by water splash, leading to secondary

37 infections which in turn continue the secondary inoculum cycle throughout the growing  
38 season (MacHardy, 1996). There are two possible sources of over-wintered inoculum, one  
39 sexual and the other asexual. Ascospores, released during spring rainfall from leaf litter and  
40 wind dispersed, have traditionally been believed to be the most important primary inoculum  
41 of *V. inaequalis*. As a result the majority of research into apple scab control has focused on  
42 reducing leaf litter in orchards (Sutton *et al.*, 2000; Vincent *et al.*, 2004; Gomez *et al.*, 2007)  
43 and inoculum forecasting based on ascospore development and release to aid the application  
44 of chemical control (Gadoury & MacHardy, 1986; Beresford & Manktelow, 1994; Berrie &  
45 Xu, 2003).

46 *V. inaequalis* can also overwinter as stromata on twigs or as viable inoculum (most likely  
47 conidia) between bud scales (Cook, 1974; Hill, 1975; Becker *et al.*, 1992).. It is likely asexual  
48 conidia are either washed on to leaves near to the source of overwintered scab, or they  
49 germinate and form a lesion on or around the bud forming conidia that are then released and  
50 dispersed by water. As with conidia from lesions in the main epidemic phase, these conidia  
51 from over-wintered sources will infect within an area close to the initial lesion site. In  
52 contrast, airborne ascospores will be turbulently dispersed or advected over longer distances .  
53 Thus, we would expect more leaves to be infected within the same flower truss or extension  
54 shoot, and more aggregation in lesions on individual leaves, if conidia are the primary  
55 inoculum. Studies (Holb *et al.*, 2004, 2005; Gao *et al.*, 2009) suggest that these conidial  
56 sources may be a significant part of the primary inoculum. This is important because  
57 reduction of overwintering inoculum and early season control measures differ for the two  
58 sources and because relatively lower levels of sexual reproduction (compared to all primary  
59 infections resulting from ascospores) in the population may affect the evolution of for  
60 pathogen virulence and fungicide resistance.

61 If conidia contribute to primary inoculum it means that a proportion of the lesions present in  
62 an orchard are not recombinant products of meiosis. As a result the population as a whole  
63 will evolve at a different rate from the population of an orchard where ascospores are the sole  
64 primary inoculum since a certain proportion of the primary inoculum has identical genotypes  
65 to the previous year. The size of this change will depend on the genetic architecture of the  
66 trait under study. Furthermore, if a race of scab with superior fitness caused by several  
67 weakly linked polymorphic loci develops in an orchard it is likely to become dominant in the  
68 orchard faster, as more of the primary inoculum in successive seasons will be the favoured  
69 genotype. Fitness might be increased because of virulence towards resistant cultivars or  
70 resistance to a fungicide -

71 We aimed to investigate the relative importance of conidia and ascospores as sources of  
72 primary inoculum in an orchard in Southeast England. As previously stated, conidia are  
73 dispersed by water splash, but it is difficult to trap conidia from buds reliably in splash water  
74 in the early season. Instead, we placed potted trees in an orchard with a history of scab. Scab  
75 on potted trees not previously exposed to scab should result from ascospores because they are  
76 air-borne and travel longer distances, whereas scab on orchard trees may result from both  
77 ascospores and overwintered conidia. We compared scab incidence and clustering on the two  
78 types of recipient tree. We inferred the relative importance of the two sources of initial  
79 infection under the assumptions that young leaves from both types of trees are equally  
80 susceptible, and that conidia and ascospores have an equal infection potential/efficiency. The  
81 latter assumption is realistic for temperatures in the spring in the Southeast England and the  
82 infection requirement for conidia and scab (MacHardy, 1996) . In addition, we compared the  
83 genetic structure of the *V. inaequalis* populations from potted and orchard trees using simple  
84 sequence repeat (SSR) markers.

85

## 86 **Materials and methods**

### 87 **Sampling and lesion assessments**

88 Orchard WM132 at East Malling Research (Kent, UK) has three consecutive rows of *Malus x*  
89 *domestica* ‘Cox’s Orange Pippin’ (Cox) next to 3 consecutive rows of *Malus x domestica*  
90 ‘Royal Gala’ (Gala) on M9 rootstocks (rows 4 m apart); each row has 12 trees planted 1.75 m  
91 apart. This orchard is not sprayed with fungicides, but is pruned, and is c. 15 years old. Six  
92 potted trees of each of Cox and Gala on M9 rootstocks (c. 10-12 years old) in 10 litre pots  
93 were placed within the orchard trees of the same cultivar, two positions randomly chosen in  
94 each row, at bud burst in 2012, 2013 and 2014 (these positions remained the same for all  
95 three years); therefore observations were carried out between paired samples, a potted tree  
96 with a partner orchard tree. Potted trees had been kept in a polytunnel, except for the  
97 experimental exposure period, to prevent surface wetness and so prevent *V. inaequalis*  
98 infection (hence remove the possibility of overwintering conidia from previous years). The  
99 distance between the potted tree and the nearest orchard tree was c. 1 m; potted trees were  
100 secured to the post of an orchard tree but the trees were arranged and pruned so that no  
101 branches of a potted tree touched or were directly above a branch of the corresponding  
102 orchard tree. Trees of both types were around 180-200 cm tall, with lowest shoots about  
103 80cm above ground level. Potted trees were watered (approx. 500ml) three times a week,  
104 directly onto the compost in the pot. The potted trees were returned to a polytunnel after  
105 sufficient infection events (3 to 5 weeks depending on weather), but before the first  
106 generation of conidia (i.e. visible lesions resulting from infection by primary inoculum) was  
107 produced, to ensure that infection on the potted trees all resulted from primary sources. The  
108 number of potential infection periods were 12, 3 and 3 for 2012, 2013 and 2014, respectively.  
109 Two weeks later, up to 15 shoots (flower trusses) were randomly sampled from across each  
110 potted tree and the nearest orchard tree (all available shoots were sampled when less than 15

111 were available). The number of scab lesions was counted on both sides of every leaf and the  
112 position of infected leaves on the shoot noted. On the few occasions when the scab was  
113 severe enough that discrete lesions could not be defined an estimate of the percentage of leaf  
114 covered in scab was made and this converted to an estimated number of lesions (assuming a  
115 single lesion corresponds to 1% scabbed area, based on empirical experiences). From each  
116 infected leaf the most clearly separated scab lesion was selected and cut out with a 5 mm  
117 cork-borer, placed in a 2 ml micro tube, left to air dry at room temperature and then  
118 transferred to a -20°C freezer until DNA extraction.

### 119 **DNA extraction and screening**

120 DNA was extracted from six lesions (where possible) per tree, no more than one lesion from  
121 any one shoot. As lesions were relatively sparse, few lesions will have resulted from infection  
122 by more than one spore; the rate at which this occurred was estimable from the genotype  
123 data. Therefore, DNA was extracted directly from the lesion on the leaf disc. Two 4 mm ball  
124 bearings were added to the leaf disc in the microtube and disrupted in an MM2 oscillating  
125 mill (Retsch). DNA was then extracted using a DNeasy Plant Mini Kit (Qiagen) following  
126 the manufacturer's instructions with all optional steps. DNA was quantified and quality-  
127 checked using a Nanodrop 1000 spectrophotometer (Thermo Scientific) and stored at -20°C.

128 The SSR primers used (Table 1), PCR and thermal cycle conditions, as well as the procedure  
129 for genotyping were all carried out as set out in Passey *et al.* (2016). PCR was repeated on  
130 any samples with no product for an SSR marker, alongside a positive control(s), so as to  
131 score a null allele, rather than a failed PCR, for that primer pair.

### 132 **Statistical analysis**

#### 133 *Molecular data*

134 Allele frequencies were estimated using Powermarker software (Liu & Muse, 2005).  
135 Analysis was run with and without rare alleles (frequency  $\leq 0.01$ ; i.e. an allele appearing only



136 once in the orchard in any given year) as very rare alleles have little effect on genetic  
137 diversity (Hale *et al.*, 2012). If two alleles were present at a locus it was assumed that the  
138 lesion had resulted from infection by more than one spore. If a sample had only one locus  
139 with two alleles one was randomly selected. If a sample had multiple loci with more than one  
140 allele then the sample was discarded.

141 We assessed differentiation between populations on the potted trees and the orchard trees by  
142 AMOVA (Analysis of Molecular Variance) in Arlequin version 3.5 (Excoffier & Lischer,  
143 2010). AMOVA significance tests, based on 1023 permutations, were carried out for ‘among  
144 tree type (Orchard vs. Potted)’ and ‘among cultivars (Cox vs. Gala)’.

145 Multi-locus Linkage Disequilibrium (LD) was estimated for scab populations on each tree  
146 type for each cultivar to determine whether associations between alleles were compatible  
147 with sexual reproduction. LD was calculated by a permutation test (1000 permutations) with  
148 Powermarker software. The null hypothesis of the test is that scab from a particular group is  
149 in linkage equilibrium, i.e. that the genotype frequency is equal to the product of the allele  
150 frequencies (Liu & Muse, 2005).

#### 151 *Lesions on leaves*

152 AGGREGATION OF LESIONS. The density of lesions is expected to be higher on leaves of  
153 orchard trees than on potted trees because of additional overwintered conidia in the orchard  
154 trees. For the same reason, lesions are expected to be more aggregated within an individual  
155 leaf on orchard trees than potted trees. We assessed aggregation by fitting the distribution of  
156 lesion counts on leaves to a Poisson or negative binomial distribution, separately for potted or  
157 orchard trees . We used generalised linear modelling (GLM) to make the fits. In the GLM  
158 analysis, cultivar and year were treated as factors; their interaction was not included. Errors  
159 were assumed to follow either a Poisson or a negative binomial distribution. Then we used

160 the average residual deviance per degree of freedom to compare the goodness of fit of the two  
161 models. The best fitting distribution was used in subsequent work,  
162 LESION DENSITY. We tested whether mean lesion counts per leaf were significantly  
163 greater for the orchard than for the potted trees using a hurdle model. A limitation of standard  
164 count models is that the zeros and the non-zeros (positives) are assumed to come from the  
165 same data-generating process; often this type of model cannot account for an excess of zero  
166 counts in the data. To overcome this shortcoming, two types of models have been proposed:  
167 hurdle models and zero-inflated models (Cameron & Trivedi, 1998, 2005). For hurdle models,  
168 a Bernoulli probability governs the binary outcome of whether a count variate has a zero or  
169 positive realisation, similar to the common logistic modelling in GLM. If the realisation is  
170 positive (i.e., the hurdle is crossed), positive count data are assumed to be governed by a  
171 truncated-at-zero count data model (e.g., Poisson or negative binomial model). On the other  
172 hand, zero-inflated models assume that the response variable is a mixture of a Bernoulli  
173 distribution and a discrete data-generating process (e.g. Poisson) distribution. Therefore, zero  
174 counts can result from a discrete data generating process as well as a Bernoulli process for the  
175 zero-inflated models but only from a Bernoulli process for hurdle models.

176 We chose to use the hurdle models because they enable easy interpretation of differences  
177 between potted and orchard trees in the incidence of scabbed leaves and in average lesion  
178 counts per scabbed leaf. The incidence of scabbed leaves was modelled as a binomial process  
179 and lesion density per scabbed leaf as or a negative binomial process. When fitting hurdle  
180 models, the origin of leaves (potted or orchard trees) was used as a factor in both parts of the  
181 hurdle model: incidence (logistic model) and density (truncated positive counts model). In  
182 addition to the comparison between the potted and orchard trees, year, cultivar and locations  
183 in the orchard were included the analysis – but all represented by a single factor of tree pairs:  
184 six locations (pairs of trees) within the Cox or Gala section within each year [giving 36 levels

185 for the factor ‘tree pairs’]. Therefore the effects of years and cultivars were already accounted  
186 for by the ‘tree pairs’ factor. We did not include cultivar or year explicitly in the analysis  
187 because the purpose of the present study was to study the overall difference in scab  
188 development between the potted and orchard trees. GLM was carried out using the MASS  
189 package (Venables & Ripley, 2002) and hurdle models using the pscl package (Zeileis *et al.*,  
190 2008) in R (version 3.2).

191 *Number of scabbed leaves per shoot*

192 The variance in the number of infected leaves on a shoot would be expected to be greater in  
193 orchard trees due to additional conidial infection localised on particular shoots. For each tree,  
194 we have 12-15 shoots. We cannot directly compare variances between trees for two reasons.  
195 First, shoots have an unequal number of leaves. Second, the variance of the distribution  
196 depends on the mean by the nature of binomial distribution. Therefore, a permutation test,  
197 conditioned on the total number of scabbed leaves in a tree, was used to compare the number  
198 of infected leaves in each shoot with that expected under the assumption of a random  
199 distribution of infected leaves. For each tree, we first conducted the following analysis: (1)  
200 find the total number of scabbed leaves; (2) for trees with more than one infected leaf,  
201 randomly assigning the same number of infected leaves to the shoots [taking into account the  
202 number of leaves on each shoot], (3) calculating the variance among shoots on each tree in  
203 the number of scabbed leaves on a shoot, (4) repeating steps 1-3 999 times, (5) calculating the  
204 variance of the observed data [we have 1000 variance values for each tree now: 999 variances  
205 for simulated data sets and one for the observed], (6) calculating the rank of the observed  
206 variance in the 1000 values [if there were ties, using the average rank; rank was calculated in  
207 descending order, i.e. the largest value has a rank of 1], and (7) calculating the ratio of the  
208 observed variance to the mean of the 999 permuted values. Thus, for each tree the analysis  
209 resulted in two values: the rank (frequency with which the observed variance would be seen if

210 the pattern were random), and the relative size of the observed variance to the mean of a  
211 random pattern. Then, ANOVA was applied to assess whether the rank (ln-transformed) or  
212 the ratio of variances differed significantly between potted and orchard trees. For the same  
213 reasons as outlined above, only tree pairs and the type of tree were included as factors in  
214 ANOVA of permuted data. Permutation and ANOVA were implemented in R (version 3.2).

215

## 216 **Results**

### 217 **Molecular data**

218 In total we screened 396 sampled leaf discs over the three years (2012-2014, Table 2): 202  
219 and 194 samples from potted and orchard trees, respectively. Populations with less than 36  
220 analysed samples were due to: a lack of scab (two potted Cox trees in 2013); samples failing  
221 to amplify; or, removal of samples from analysis because they had multiple alleles at more  
222 than one locus. A change of capillary in the ABI 3130xl, after the 2012 samples were  
223 analysed, led to a +2bp shift in markers Vica9/X, Vitc1/82 and Vitg9/129. This was  
224 ascertained by running a subset of the 2012 samples and crosschecking against their original  
225 allele sizes; an appropriate correction was made to the data. Tests were run with and without  
226 rare alleles (frequency  $\leq 0.01$ ) of the orchard population in a given year; however, there was  
227 no difference in results. Null alleles occur when a mutation in the flanking region of the  
228 sequence repeat stops the annealing of the primer and therefore stops amplification during  
229 PCR. Statistical tests were run twice, including the null as an extra allele for that marker or  
230 excluding the isolate. There were no differences that affected inferences.

231 AMOVA showed no evidence of difference between the orchard trees and the potted trees in  
232 any of the three years, nor any difference between the cultivars ( $P > 0.3$ ).

233 In 2012 and 2014 all of the multi-locus LD tests showed that the populations were in linkage  
234 equilibrium, indicating random mating (Table 3). In 2013 the *V. inaequalis* populations on

235 Gala potted trees were in linkage equilibrium but the scab populations on the Cox potted and  
236 orchard and the Gala orchard trees were in LD (Table 3).

237

### 238 **Analysis of scab lesion distributions**

239 GENERAL RESULTS. Scab was much more severe on Gala than on Cox (Table 4;  $P <$   
240  $0.001$ ): incidence of 5.7% ( $\pm 0.003$ ) (Cox) vs 21.6% ( $\pm 0.006$ ) (Gala) and average lesion  
241 counts per leaf of 0.16 ( $\pm 0.019$ ) (Cox) vs 1.11 ( $\pm 0.067$ ) (Gala). Scab development was more  
242 severe in 2012 and 2014 than in 2013 (Table 4). More scab was observed on orchard trees  
243 than on potted trees in 2012; however, slightly more scab was seen on potted trees in 2014  
244 than on orchard trees (Table 4). There were only slight differences in the overall scab  
245 incidence and density between potted and orchard trees in 2013 (Table 4). Average number  
246 of lesions on the scabbed leaves was 4.61 ( $\pm 0.224$ ). Overall, there was a larger proportion of  
247 leaves with high scab counts on orchard trees than on potted trees, except for Gala in 2014  
248 (Fig. 1).

249 LESION DISTRIBUTION. A Poisson distribution fitted the count data on potted trees fitted  
250 reasonably well (average residual deviance 1.58) but not the for the orchard trees (average  
251 residual deviance 3.23). The lack of fit of a Poisson distribution can be seen in Fig. 1,  
252 particularly for the susceptible cv. Gala. Both sets of lesion data were equally well described  
253 by a negative binomial distribution: average residual deviances were 0.327 and 0.363 for the  
254 potted and orchard trees, respectively.

255 The aggregation of lesions on leaves was further confirmed on fitting the hurdle distributions.  
256 A hurdle model based on the negative binomial distribution fitted the data much better than  
257 the corresponding model with a Poisson distribution. The hurdle model with a negative  
258 binomial error distribution was therefore adopted for further analysis.

259 LESION DENSITY. GLM analysis (using hurdle distributions) showed that the incidence of  
260 leaves with scab was significantly ( $P < 0.001$ ) greater on the orchard trees than on the potted  
261 trees. For the negative binomial part of the model, the parameter estimate for potted trees  
262 was 0.206 ( $\pm 0.063$ ) less than that of orchard trees; that is, the odds ratio of being scabbed for  
263 potted trees was c. 80% of corresponding orchard trees. Furthermore, average lesion counts  
264 on infected leaves were greater ( $P < 0.001$ ) on the orchard trees than on the potted trees.  
265 Potted trees had an intercept 0.701 ( $\pm 0.140$ ) less than that of orchard trees; that is, the average  
266 lesion number on potted trees was about 50% of that on the corresponding orchard trees.

267

### 268 **Number of infected leaves per shoot**

269 The variance in the number of infected leaves on a shoot (expressed as ratio of the observed  
270 to the mean of the permuted values) and the rank in a list of random permutations of the  
271 observations both differed greatly between potted and orchard trees (Fig. 2). For both  
272 variance ratio and log-transform rank variables, residual plots did not suggest any apparent  
273 violations of ANOVA assumptions. For potted trees, the ratio of the observed variance in the  
274 number of infected leaves on a shoot within each tree to the mean of the permuted values was  
275 0.98, close to the expected value of 1.0. For the orchard trees, this ratio was much greater at  
276 1.63 ( $F_{1,39} = 27.2$ ,  $P < 0.001$ ). The rank of the observed variance in a permuted dataset (Fig.  
277 2) was much greater in orchard trees (792) than in potted trees (467) ( $F_{1,39} = 25.1$ ,  $P < 0.001$ ;  
278 the average rank of variance of the permuted datasets was necessarily 500).

279

## 280 **Discussion**

281 Previous molecular comparisons of isolates from different cultivars within the same orchard  
282 indicated that conidia may overwinter in bud and/or wood scab and act, in addition to  
283 ascospores, as a source of primary inoculum (Xu *et al.*, 2013). Several other studies have also

284 suggested overwintered conidia are a source of primary inoculum (Becker *et al.*, 1992; Holb  
285 *et al.*, 2004, 2005; Gao *et al.*, 2009). In this study we showed that scab lesions on orchard  
286 trees were more aggregated on leaves and shoots than on adjacent potted trees not previously  
287 exposed to scab (i.e. not exposed to overwintered conidia). Both scab incidence and count  
288 data suggest that conidial primary inoculum may have contributed approximately 20-50% of  
289 the total inoculum: incidence was estimated to be reduced by 20% on potted trees, and lesion  
290 number by 50%, averaged over the three years of the study. This interpretation is under the  
291 assumptions that infection efficiency by both conidia and ascospores on orchard and potted  
292 trees is the same and that both potted and orchard trees are equally susceptible to infection.  
293 Infection efficiency in the spring temperatures that the orchard experienced was similar for  
294 ascospores and conidia (Reviewed by MacHardy, 1996). The initial infection process should  
295 have been completed when the potted trees were returned to the polytunnel; subsequent  
296 temperature should not have affected the number of lesions, since we allowed sufficient time  
297 for all infections to become visible, predicted on the basis of the relationship of incubation  
298 time to temperature (MacHardy, 1996). The likely causes of difference in susceptibility are  
299 “softer” tissue in potted plants and lack of resistance priming and induced resistance from  
300 phylloplane organisms in the potted plants. Both would produce effects in the opposite  
301 direction to those observed.

302 The scab populations on potted and orchard trees were in linkage equilibrium in both 2012  
303 and 2014. This fits the hypotheses of either predominantly ascospore primary inoculum or no  
304 deviation from linkage equilibrium within the conidial primary inoculum, due presumably to  
305 no selective changes (detectable with the set of SSRs used) in the population the previous  
306 year. In 2013 the population of *V. inaequalis* on potted trees of Gala was in linkage  
307 equilibrium but the orchard trees were in linkage disequilibrium. This would be expected if  
308 conidia were an important part of the primary inoculum, as the scab on the potted trees would

309 be from sexually produced ascospores and therefore from independent sampling, whereas the  
310 scab on the orchard trees would be from both (freely recombinant) ascospores and clonal  
311 conidia. However, the populations of *V. inaequalis* on both potted and orchard trees of Cox  
312 were in linkage disequilibrium in 2013; the potted trees more significantly than the orchard  
313 trees. This suggests that unexplained factors influenced our estimates of linkage  
314 disequilibrium, so no secure inferences can be drawn. .

315 Although wood scab in heavily infected orchards is commonly observed, it is believed that  
316 very few of these wood scab lesions produce viable conidia in spring, indicating that  
317 asexually overwintering scab is most likely to result from overwintering in buds (Becker *et*  
318 *al.*, 1992). Although the present study was conducted in an unsprayed orchard (WM132),  
319 scab was not very severe and there was no evidence of wood scab present. Furthermore,  
320 commercial pruning was applied to the orchard; heavily infected shoots will be likely to have  
321 been removed. Thus, conidia that overwintered in the buds are probably the main source of  
322 overwintered conidium inoculum in the spring.

323 We may conclude that ascospores are still the main source of primary inoculum (c. 80% in  
324 this specific orchard) in the spring for temperate growing regions such as Southeast England.  
325 Therefore, the current management practice of eliminating leaf debris in late autumn  
326 (MacHardy, 1996) needs to be retained. However conidia as primary inoculum cannot be  
327 ignored. The relative importance of conidia and ascospores as primary inoculum is likely to  
328 vary between orchards and years. In this study we have not compared the differences  
329 between years, cultivar or position within an orchard as the aim was to assess the overall  
330 importance of conidia primary inoculum. There are many other factors that could affect the  
331 relative proportion of conidia as primary inoculum, including pruning, leaf degradation, in-  
332 season control efficacy, cultivar, and epidemic severity. Most of the studies suggesting the  
333 importance of conidia as part of primary inoculum have been in areas with wet and mild



334 winters such as the United Kingdom (Present study; Cook, 1974; Hill, 1975), the Netherlands  
335 (Holb *et al.*, 2004, 2005) and west Norway (Stensvand *et al.*, 1996). Conditions in these  
336 regions are likely to be both more conducive to faster decomposition of leaf material,  
337 reducing ascospore levels, and more likely to allow survival of conidia or mycelia in buds  
338 than regions with colder winters. Warmer growing regions, where there is no winter chill  
339 necessary for pseudothecia development, only have clonal lineages of the apple scab  
340 pathogen (Boehm *et al.*, 2003).

341

342 Reducing the amount of inoculum in early season is paramount to good scab control. The  
343 main focus of forecast programmes designed to aid effective application of chemical control  
344 in spring is currently ascospore release. However, even with a perfect elimination of leaf  
345 debris, scab control in the early season is still essential as, based on this work, overwintered  
346 conidia are likely to be a source of primary inoculum. Consideration of release of conidia  
347 from bud scale should be incorporated into spray guidance programmes. Further, it might  
348 also be useful to spray when buds are forming, similar to a strategy being evaluated for  
349 reducing overwintering of powdery mildew in apple buds at East Malling.

350 In summary, we have shown that conidia play an important role as part of the primary  
351 inoculum of apple scab in the orchard studied; however, ascospores are still the predominant  
352 source. Due to the many factors that can affect the amount of overwintering conidia in  
353 orchards, the overall contribution of conidia as primary inoculum is expected to vary  
354 considerably with orchards and seasons. Sanitation practices are imperative, for example  
355 good winter pruning and removal of leaf litter are both important. Early season sprays are  
356 necessary for successful control of scab whether the primary inoculum is from ascospores or  
357 overwintered conidia; however traditional spray programmes may have to be revisited in  
358 light of these findings.

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Table 1 Sequences (5'-3') for SSR primer pairs used to genotype apple scab isolates

SSR	Fluorescent label-Forward primer	Reverse Primer	Allele range
EMVi029 <sup>a</sup>	HEX-ACGAGTCCCAGGTCTCACAG	TGTTGACGGTCACGGTGTAT	170-252
Vica9/X <sup>b</sup>	FAM-TCGCGCATCACTATCTACAC	AGACAGGAATGTGGTGGAAG	219-247
Vica10/154 <sup>b</sup>	HEX-CCTCCTTCCTATTACTCTCG	CTGAAGCGAACCTATGTCC	100-168
Vicacg8/42 <sup>b</sup>	FAM-TGTCAGCCACGCTAGAAG	CACCGGACGAATCATGC	200-240
Vict1/130 <sup>b</sup>	FAM-GATTGGTGACGCATGTGT	GCTGGAGATTGCGTAGAC	148-164
Vitc1/82 <sup>b</sup>	HEX-ACTGTCTCTAGGCGAAAG	ACTTGGAAAGCTCGCTAAG	227-243
Vitc2/16 <sup>b</sup>	FAM-ACATTGACGAAGACGAGC	TACAATTGAGGCGTGTCC	153-169
Vitg9/129 <sup>b</sup>	FAM-CTAATTCAACTCGCTGCGTC	TTTCAGCCAGCTAACCTAGG	277-291

<sup>a</sup>Xu *et al.*, 2009

<sup>b</sup>Guérin *et al.*, 2004

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**Table 2** Number of leaf discs with scab lesions screened for SSR markers to compare populations from potted trap trees and orchard trees

Type	2012		2013		2014	
	Cox	Gala	Cox	Gala	Cox	Gala
Potted	36	36	25	35	35	35
Orchard	31	29	34	31	36	33

**Table 3** Significance results in test for Linkage Disequilibrium of *V. inaequalis* populations of potted and orchard trees in different cultivars in an orchard in Southeast England

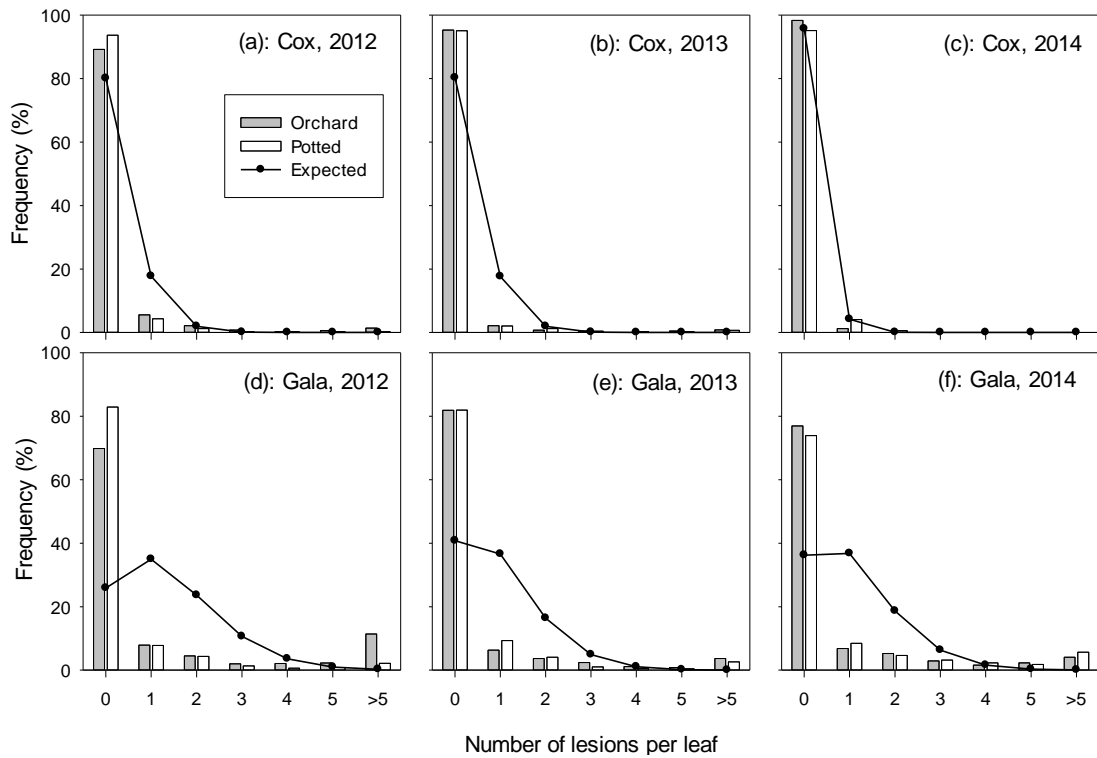
	Population(s)	2012	2013	2014
Cox	Orchard	1.00	0.01	1.00
	Potted	1.00	< 0.001	1.00
Gala	Orchard	1.00	0.01	1.00
	Potted	1.00	1.00	1.00

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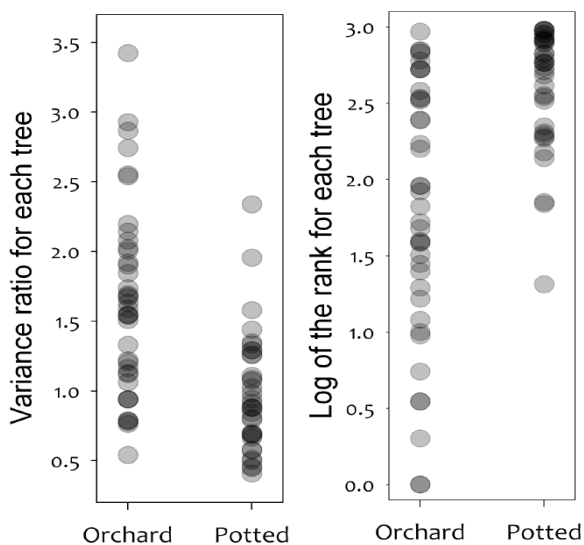
**Table 4** Incidence of leaves with scab and average number of lesions per leaf on orchard and potted trees of cvs. Cox and Gala in an orchard in Southeast England

Type	2012		2013		2014	
	Cox	Gala	Cox	Gala	Cox	Gala
Number of leaves assessed						
Potted	1201	1105	738	687	1051	602
Orchard	917	850	830	951	797	686
Incidence of leaves with scab						
Potted	0.063	0.171	0.049	0.180	0.049	0.261
Orchard	0.108	0.301	0.047	0.181	0.017	0.230
Average lesion counts						
Potted	0.118	0.536	0.172	0.646	0.059	1.228
Orchard	0.358	2.414	0.263	1.077	0.025	0.828



458  
 459 **Figure 1.** Distribution of apple scab lesions on individual leaves collected from potted trees  
 460 (non-shaded bar) and corresponding orchard trees (shaded bar) of two cultivars in three years  
 461 when both types of trees were exposed to the same conditions at the same locations. In  
 462 addition the expected frequency assuming a Poisson (random) distribution for number of  
 463 lesions on individual leaves is also shown (line). Observed data has a higher frequency than  
 464 expected for leaves with no lesions and more than four lesions per leaf indicating aggregation  
 465 of lesions within a single leaf.

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 469 **Figure 2.** Plot of the ratio between the observed variance in the number of scabbed apple  
 470 leaves in each shoot within each tree with the average variance of 999 permutations  
 471 assuming random distribution of infected leaves, and the log of the rank of the observed

472 variance among the 1000 variance values (999 permuted and one observed; in the  
473 descending order – i.e. the largest has the rank of one). Depth of grey indicates overlaying of  
474 observations. The rank of observed variance was significantly different ( $P < 0.001$ ) between  
475 orchard and potted trees in this Southeast England orchard.

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