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# Spider predation on rosy apple aphid in conventional, organic and insecticide-free orchards and its impact on aphid populations

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Abstract: Spiders dwelling in the canopy of apple trees may contribute to the control of the rosy apple aphid (*Dysaphis plantaginea*). They are expected to be very efficient early in season during aphid colony establishment. However, their predation on aphids may be impaired by insecticide treatments. We aimed to assess the effectiveness of spider predation on *D. plantaginea* in apple orchards. For this purpose, we monitored *D. plantaginea* abundance and sampled a total of 1281 canopy spiders in 8 orchards under IFP (Integrated Fruit Production), organic or insecticide-free management in early and mid-season. Using DNA analyses of the spider gut with specific *D. plantaginea* PCR primers we investigated which taxa had eaten this aphid pest. Lastly, we assessed effectiveness of aphid suppression by spiders in insecticide-free orchards. The most abundant spiders belonged to *Philodromidae*, *Salticidae* and *Anyphaenidae*. Spiders were more abundant in the insecticide-free orchards than in the other orchards in mid season but not in early season. Spiders detected positive for *D. plantaginea* DNA were observed in 8 out of 12 families and occurred in all orchards. The probability that a spider was positive did not depend on its size. Lastly, the increase in the number of aphid colonies early in season related negatively to the abundance of *Philodromus* spp. All these results showed that numerous spider taxa participate to the control of *D. plantaginea* in orchards and that *Philodromus* spp may be particularly efficient in early season in absence of insecticide treatments.



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Avignon, March 15<sup>th</sup> 2016.

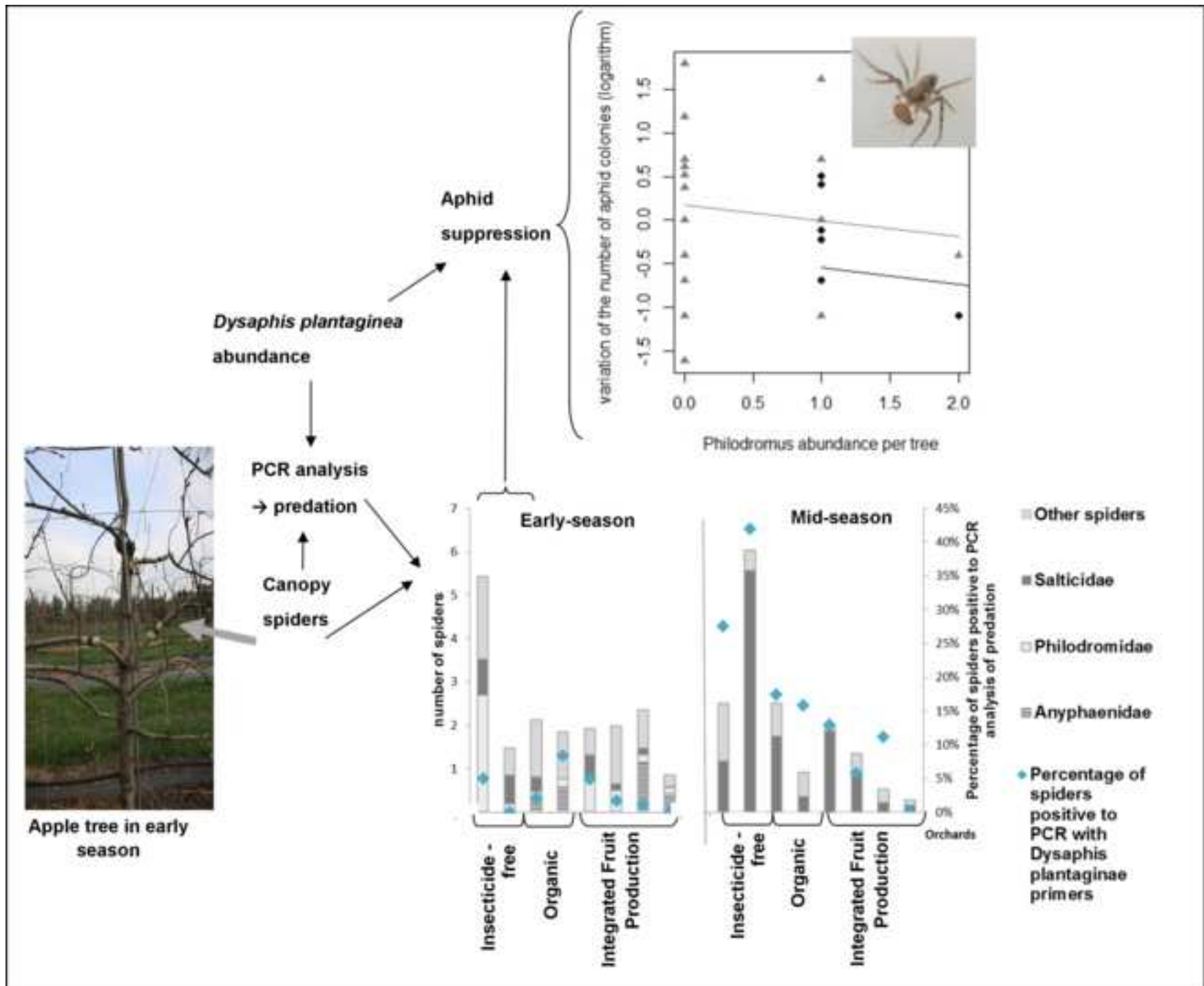
Dear editor

Please find in this submission a manuscript entitled "Spider predation on rosy apple aphid in orchards under insecticide-free, organic and integrated management, and its impact on aphid populations" that we would like to submit for publication in Biological Control. In this study we combined field monitoring of aphids and spiders with PCR analyses of spider gut content to investigate predation of the rosy apple aphid by canopy spiders. The study was carried out in orchards under three different management strategies and at four dates (February-June) during the growing season. We present evidence that spiders predate aphids in all types of orchards. Based on results in insecticide-free, we further highlight a role of Philodromidae in aphid suppression in early season.

We hope that you will find this manuscript suitable for your journal,

Sincerely

Claire Lavigne



## \*Highlights (for review)

- We monitored cursorial spiders and *Dysaphis plantaginea* abundance in apple orchards
- PCR gut content analyses showed that 8 of 12 spider families predated *D.plantaginea*
- Predation occurred in integrated production, organic and insecticide-free orchards
- Early-season variation in aphid colony numbers related to *Philodromus spp* abundance

1 Spider predation on rosy apple aphid in orchards under insecticide-free,  
2 organic and integrated management, and its impact on aphid populations.

3

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20

21 **Abstract**

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36 results showed that numerous spider taxa participate to the control of *D. plantaginea* in orchards  
37 and that *Philodromus spp* may be particularly efficient in early season in absence of insecticide  
38 treatments.

39

40 **Key words**

41 Agroecology; *Anyphaena accentuata*; conservation biological control; gut content analysis;  
42 *Philodromus spp.*; prosoma size; spider community.

43

#### 44 **Introduction**

45 Aphids are one of the main groups of apple tree pests and they are responsible for production loss,  
46 smaller fruits, and long term damages on apple trees. In apple orchards, the rosy apple aphid  
47 *Dysaphis plantaginea* (Passerini) is the main aphid pest with the wooly apple aphid *Eriosoma*  
48 *Lanigerum*. *D. plantaginea* can be most detrimental, causing yield reductions from 30% (Blommers et  
49 al., 2004; Deberardinis et al., 1994) to 80% (Qubbaj et al., 2005). The apple tree is its primary host. In  
50 the Mediterranean region, the eggs start to hatch in March and aphid colonies develop from asexual  
51 reproduction. The migration of *D. plantaginea* winged adults to their secondary host, the plantain  
52 herb, begins in May and lasts until the end of June (Bonnemaison, 1959). Alate individuals return to  
53 apple trees in autumn and females lay eggs close to buds.

54 Aphids are prey of a large number of arthropod predators. Among these, generalist predators  
55 may be of particular interest for aphid control. Their ability to feed on a range of prey allows them to  
56 persist in crops when pest numbers are low (Symondson et al., 2002) and thus prevent pest  
57 outbreaks early in the season before specialist natural enemies are abundant (Landis et al., 2000).  
58 Timing of predator arrival is a key point for aphid suppression because of their exponential  
59 population growth in season (Costamagna et al., 2015). Among generalist predators that feed on  
60 aphids, canopy dwelling spiders are of particular interest: some canopy spider species are active  
61 despite low temperatures in winter or early spring (Korenko et al., 2010). Thus, they could predate  
62 prey very early during aphid colony establishment (Boreau de Roince et al., 2013). Such mechanism is  
63 at the basis of the efficient suppression of psyllids in pear orchards (Pekár et al., 2015). Aphid  
64 predation can be characterized by several technics: field observations, laboratory tests of food  
65 preference, ELISA analyses of gut content, and more recently gut content analyses by PCR (Harwood  
66 and Obrycki, 2005). Further than predation, aphid suppression (sensu (Schellhorn et al., 2014)) is the  
67 key of natural biological control. Promising results showed that the abundance of spiders cumulated  
68 from March to May limited the presence of aphid colonies on apple trees (Boreau de Roince et al.,  
69 2013).



70 Aphid predation by spiders may be affected both by the species composition of spider  
71 communities, by the distribution of their ecological traits and by the availability of alternative preys  
72 (Madsen et al., 2004). First, spider species may differ intrinsically in their appetite for aphids: the  
73 canopy spiders of genus *Philodromus* have a high appetite for aphids compared to other insects  
74 (Michalko and Pekar, 2015a) while some ground spiders prefer fruit flies to cereal aphids (Toft,  
75 1995); second, prey consumption by wandering and active hunters depends on local prey abundance  
76 (Michalko and Pekar, 2015a) and may exhibit an aggregative response (Riechert and Lockley, 1984);  
77 third, aphids are considered as low food value for spiders, which may need alternative preys to  
78 complement their diet (Madsen et al., 2004); fourth, selection is likely to enhance a specialization of  
79 generalist arthropod predators on the most abundant prey present in their environment (Rana et al.,  
80 2002). Further than taxonomy, traits such as individual size also appear relevant to explain prey  
81 preferences. Both inter and intra-specific size differences may be relevant, because active hunters  
82 and wandering spiders are likely to eat preys within a certain range of predator-prey body size ratio  
83 (Michalko and Pekar, 2015b). Besides, the anti-predator behavior of the prey can be related to the  
84 predator-prey body size ratio (Binz et al., 2014).

85 To prevent multiplication of the fundatrices in apple orchards, treatments with mineral oils or  
86 insecticides are applied early in season. Such insecticide treatments may impair the spontaneous  
87 aphid biocontrol by natural enemies (Cross et al., 2015). Insecticides may affect spider communities  
88 directly by reducing prey availability, reducing their fitness, and modifying their behavior (Marc et al.,  
89 1999) resulting in lower abundance of canopy spiders in treated orchards as compared to untreated  
90 ones (from spring to autumn (Mansour, 1987; Marko et al., 2009; Pekar, 1998), and in winter (Pekár,  
91 1999)) but in no difference between organic and conventional orchards (spring to autumn (Pekár and  
92 Kocourek, 2004)). Among treated orchards, species diversity of canopy spiders, however, was higher  
93 in organic orchards than in orchards treated with broad-spectrum insecticides or following IPM  
94 (integrated pest management) guidelines (Pekár and Kocourek, 2004). The environment surrounding  
95 the orchard may also influence strongly the abundance and community composition of canopy

96 spiders within orchard, in particular in autumn because of the recolonization of the orchard after  
97 insecticides spraying stops (Lefebvre et al., 2016; Marko et al., 2009; Wisniewska and Prokopy, 1997).  
98 Winter/early spring communities that may be responsible for early aphid control may thus depend  
99 less on crop protection practices than later communities. Further than a taxonomical  
100 characterization, orchard management can change the distribution of spiders' ecological traits. The  
101 mean body length of canopy spiders was larger in untreated orchards than in IPM orchards  
102 (Wisniewska and Prokopy, 1997). Similarly, conventional and IPM orchards hosted communities of  
103 ground spiders with a higher proportion of small individuals than organic or abandoned orchards in  
104 spring and summer (Mazzia et al., 2015). As a consequence, although spiders were shown to eat  
105 aphids in organic apple orchards from March (when aphids are at the fundatrix stage) to May  
106 (Boreau de Roince et al., 2013), this does not entail that they do so to a similar extent in orchards  
107 treated with chemical insecticides.

108 In the present study, our objective was to assess which canopy spiders ate the pest aphid *D.*  
109 *plantaginea* according to individual size and species, in IFP (Integrated Fruit Production), organic and  
110 insecticide-free orchards. We thus first assessed the impact of orchard management on spider  
111 abundances, sizes and species composition and then characterized their predation on *D. plantaginea*  
112 using PCR gut content analyses. We further assessed effectiveness of aphid suppression by spiders in  
113 insecticide-free orchards at two periods independently: early and mid-spring.

114

## 115 **Material and methods**

### 116 **2.1 Sampled orchards**

117 Sampling and monitoring were carried out in 2014 on 8 apple orchards from 4 experimental farms in  
118 France. Five orchards were located in three farms in south-eastern France, under a Mediterranean  
119 climate; and the last 3 orchards were located in one farm in the north-western part of France, under  
120 a temperate oceanic climate. Two south-eastern farms only comprised one insecticide free orchard  
121 (*Ifree1* and *Ifree2*); the other two farms comprised two orchards under Integrated Fruit Production

122 management (*S\_IFP1*, *S\_IFP2*, for the south-eastern region and *N\_IFP1*, *N\_IFP2* for the northern  
123 region) and one organic orchard (respectively *S\_orga* and *N\_orga*) (figure 1, supplementary material  
124 table S1).

125 The organic orchards were distant of approximately 500m from the IFP orchards that were distant  
126 of approximately 50m from each other in both farms. All orchards were grown with vertical axis  
127 training of the cultivar, "Ariane". The IFP orchards received 3 or 4 chemical insecticides from March  
128 to May, while organic orchards received paraffinic and vaseline oils and/or natural pyrethrins. The  
129 Ifree orchards did not receive any insecticide and only synthetic fungicides and thinning products  
130 during this period (Details on orchard descriptions in supplementary material: table S1 and table S2).

## 131 **2.2 Sampling of spiders**

132 Spiders were sampled on four occasions. Sampling dates were chosen in each farm to match *D.*  
133 *plantaginea* stages: eggs in February; fundatrices in March; young colonies in April; mature colonies  
134 in May. 18 to 45 apple trees were sampled per orchard, on a regular canvas. Sampled trees were  
135 distant of approximately 6 trees and distributed on 3 up to 7 rows according to the orchard size  
136 (supplementary material: table S1).

137 Spiders were collected using cardboard trap bands (10 x 30cm) wrapped around apple tree  
138 branches. These bands were replaced by new ones on each sampling occasion. Two trap bands per  
139 tree were first placed in January. In February, spiders were collected from the all trap bands; in  
140 March, April and May spiders were collected alternately from one of the two bands per tree. Despite  
141 being non exhaustive in terms of spider species that were caught, this method was chosen because it  
142 did not disturb aphids in branches, contrarily to the beating method.

143 To ensure DNA conservation for later PCR analyses, spiders were immediately placed individually  
144 in Eppendorf tubes with Ethanol 90°, and then stored in the freezer (-20°C) in the laboratory  
145 approximately 1hour later. All the spiders were identified by first author to species if possible and to  
146 genus in other cases. Their prosoma widths (Michalko and Pekar, 2014) were measured using a  
147 binocular loupe set with 125x magnification (the precision considered is 0.1mm).

### 148 **2.3 Aphids monitoring**

149 *D. plantaginea* was monitored on trees used for the sampling of spiders and at the same dates. To  
150 assess aphid infestation at tree level, we counted all the growing shoots from the basis of each tree  
151 to 1m70 height and recorded the presence or absence of aphid fundatrices or aphid colonies on each  
152 of them. From these we obtained the variable “*number of aphid colonies*” per tree. The size of each  
153 colony was characterized visually by an index ranging from 1 to 4 and was later summarized by the  
154 median number of aphids of the index classes (1=1-5, median 3; 2=5-50 median 28; 3=50-250 median  
155 150; 4≥250 median 300). The total number of aphids per tree was approximated by the sum of  
156 colony sizes and was called the “*infestation index*” of the tree in further analyses.

157 Aphid growth was estimated between March and April (growth rate called *r1*) and/or between  
158 April and May (called *r2*). The growth rates were calculated as the ratio  $(\text{Aphid}(t+1) + 1) / (\text{Aphid}(t)$   
159  $+ 1)$ , where *Aphid*(*t*) is the *number of aphid colonies* at time *t*.

### 160 **2.4 PCR-based analysis of aphid predation**

161 The assessment of *D. plantaginea* predation by the spiders was carried on 1017 individuals out of the  
162 1281 spiders collected. The 1017 selected spiders belonged to the most abundant genus or species  
163 taxa (at least 20 individuals per taxa). Total DNA of each spider individual was extracted using the  
164 DNeasy® 96 blood & tissue kit (Qiagen, Germany) following the manufacturer’s protocol. The entire  
165 spider body was previously ground using an agitator-ball mill excluding the legs of the biggest  
166 individuals. To assess aphid predation, PCR were performed on each spider individual with specific *D.*  
167 *plantaginea* DNA primers (Boreau de Roince, 2012) (see supplementary material S3 for details on  
168 primer design and specificity). PCR amplifications were carried out in a 10 µl reaction volumes  
169 containing 2 µl of DNA template, 1 unit of GoTaq® Flexi DNA Polymerase (Promega) 2.5 mM MgCl<sub>2</sub>,  
170 0.1 mg/ml Bovine Serum Albumin, 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 200 mM of each dNTPs, 1X Promega PCR  
171 buffer, and 0.4 mM of each *D. plantaginea* specific primer (*Dp*-F: GTTATCAAATAATATTGCACAC  
172 labeled on 5' with Rhodamine Atto-565 dye and *Dp*-R: CAAATAAAATTAATTGCTCCTAG). The PCR were  
173 performed on a Mastercycler thermocycler with the following conditions: initial denaturation at 95°C

174 for 3 minutes followed by 35 cycles at 95°C for 30s, 56°C for 30s, and 72°C for 30s and a final  
175 elongation step at 72°C for 20 min. Each PCR product was diluted (0.4% dilution with 0.02%  
176 GeneScan™- 600 LIZ® Size standard in HiDi formamide, Applied Biosystems) and 2µl of this dilution  
177 was injected on an ABI 3730xl DNA Analyzer using the POP-7 polymer. Specific amplifications of *D.*  
178 *plantaginea* (105pb DNA fragments) were scored using Genemapper® V4.1 Analysis Software  
179 (Applied Biosystems). Amplification in the spider samples with intensity upper than control  
180 amplifications with 1pg of *D. plantaginea* DNA were considered as positive.

## 181 **2.6 Statistical analyses**

182 All analyses were performed using packages implemented in the R software (R 3.0.1; R Development  
183 Core Team 2012). Linear and generalised linear mixed effect models were performed by the  
184 functions lmer and glmer of R package lme4. Significances of the fixed effect were tested by model  
185 comparison by dropping successively each independent variable of the full model (drop1 function).  
186 When a qualitative fixed effect (factor) with more than two levels was significant according to model  
187 comparison, pairwise multiple comparisons by Tukey test (function glht, R package multcomp) were  
188 performed to quantify its effect.

189

### 190 2.6.1 Response to management of spider abundances and size of individuals

191 The effect of the orchard management (IFP, organic or insecticide-free) was tested on three  
192 characteristics of the spider communities: (1) the total abundance of spiders captured per trap band  
193 (generalised mixed model with a Poisson distribution and log link function); (2) the size of the spiders  
194 measured by their prosoma width (linear mixed model); (3) the cumulated size of the spiders per  
195 trap band (linear mixed model). For all analyses, two mixed models examined independently the  
196 early-season data (February +March) and the mid-season data (April +May). A random effect  
197 described the orchard where sampling occurred.

198

### 199 2.6.2 Traits of the spiders and orchard management affecting the predation

200 We performed generalized linear mixed models to assess if the probability of detecting *D.*  
201 *plantaginea* DNA in the spider guts depended on orchard management and spider traits, in mid-  
202 season only. In early-season, two few spiders were detected positive to PCR analyses (see below) for  
203 statistical analyses. The dependent variable took value 1 if a spider was positive in the PCR  
204 assessment and 0 otherwise. A random effect described the orchard where sampling occurred. We  
205 also tested for overdispersion in the data by adding an individual random effect but this was never  
206 significant and it was removed from further analyses. Because the rate of positive spiders may  
207 primarily depend on aphid availability, we included the log (*infestation index* + 1) as a covariate in all  
208 models. The fixed independent variables of interest in the models were either (1) the orchard  
209 management (insecticide-free, organic or IFP) or (2) the prosoma width of the spider individuals.

210

### 211 2.6.3 Effectiveness of aphid suppression

212 The impact of spiders on growth of aphid colonies was analyzed in insecticide-free orchards only.  
213 Indeed, in organic and IFP orchards, we considered that the effect of insecticides, in particular on  
214 aphid fundatrices, was too strong to observe an effect of spiders on aphid increase. To assess the  
215 effect of spiders on the variation of aphid abundances, linear mixed model were performed to  
216 explain the log of the aphid growth rates. Early season aphid variations ( $\log(r1)$ ) were analyzed  
217 considering all the spiders collected in February and March ; mid-season aphid variations ( $\log(r2)$ )  
218 were analyzed considering spiders collected in April. A random effect described the orchard where  
219 sampling occurred. Fixed effects of interest were tested in independent models. They were the  
220 abundances per trap at time *t* of either all spiders or of some spider taxa that were most numerous in  
221 insecticide-free orchards. In the models which analyzed early season growth rates, spider taxa were  
222 either 'All spiders', Philodromidae (*Philodromus spp*), Salticidae or 'Other spiders'; in models that  
223 analyzed the mid-season growth rates, taxa were 'All spiders', Salticidae or 'Other spiders'.

224 To question the effect of cumulated spider sizes on the variation of aphid abundances, similar  
225 models were performed with the per tree cumulated prosoma widths as independent variable.

226

## 227 **Results**

### 228 **3.1 Aphid infestation of the orchards**

229 All orchards harbored similar numbers of fundatrices in March. N\_IFP1 and N\_IFP2 did not have any  
230 aphid colonies later, and S\_IFP1 and S\_IFP2 harbored very few in April and May (Table1). In organic  
231 and Ifree orchards, from 30 to 73% of the apple trees in April, and from 50 to 94% in May, harbored  
232 an aphid colony. One of the insecticide free orchards was as much infested by *D. plantaginea* in May  
233 as the organic orchards, but the second insecticide-free orchard was more infested (Table1).

### 234 **3.2 Phenology and diversity of the spiders captured according to the management.**

235 Spiders differed according to sampling date. In February, Philodromidae (with the unique species  
236 *Philodromus cespitum* and *Philodromus aureolus*) and Anyphaenidae (with the unique species  
237 *Anyphaena accentuata*) were abundant in traps and all the orchards harboured at least one of those  
238 two taxa independently of region or management (Figure 2). Further than those taxa, abundant taxa  
239 differed among orchards with no clear pattern. March communities were intermediate between  
240 those of February and of mid-season (April and May), particularly because of the capture of  
241 Salticidae, Thomisidae and Gnaphosidae (data not shown). In mid-season (April and May), most of  
242 the spiders captured were Salticidae that mated in silken retreats and laid eggs in the trap bands. The  
243 Salticidae species varied according to the orchard with no clear pattern (supplementary material  
244 table S4). Overall, 'Other spiders' represented 4 to 40% of the spiders in mid-season according to the  
245 orchard, but no family of this category comprised more than 10 individuals per orchard at that period  
246 (supplementary material table S4).

### 247 **3.3 Abundance per trap and size of the spiders**

248 On average, 0.8, 1.3, 1.9 and 1.7 spiders were captured per trap band, in February, March, April and  
249 May. Considering all the taxa, the abundance of spiders and the cumulated prosoma width per tree  
250 did not depend on orchard management in early-season ( $\Delta AIC=3.1$ ,  $p=0.62$ ) but did in mid-season  
251 (abundance and cumulated prosoma width respectively:  $\Delta AIC=-1.1$   $p=0.07$  ;  $-3$   $p=0.02$ ): at that

252 period spiders were more abundant in insecticide-free orchards than in IFP orchards which resulted  
253 in a higher cumulated prosoma width (pairwise comparison lfree-IFP for abundance: estimate=0.84  
254 p=0.04 ; for cumulated prosoma width : estimate= -2.5 p=0.016); their abundance and cumulated  
255 prosoma width in organic orchards was intermediate (Figure 2).

256 The prosoma width of the spiders was (mean±SE) 1.47±0.028 mm in early season and 1.53±0.026  
257 mm in mid-season. It did not depend on the orchard management in either season (early and mid-  
258 season respectively: deltaAIC=4, p=0.81; deltaAIC=3, p=0.71). Besides, *Philodromus spp*, the single  
259 taxon that occurred in a sufficient number of orchards for statistical analyses, had similar prosoma  
260 widths in lfree and IFP south-eastern orchards (number of *Philodromus*, mean prosoma width in mm  
261 and sd: S\_lfree1: n=51, 1.05±0.026; S\_lfree2: n=10, 1.1±0.06; S\_IFP1: n=36, 1.06±0.005; S\_IFP2: n=22,  
262 1.06±0.05).

### 263 **3.4 Predation detected by PCR**

264 The probability of detecting *D. plantaginea* DNA in the spider gut depended on aphid infestation, i.e  
265  $\log(\text{infestation index}+1)$ , in the three analyzed models ( $\Delta\text{AIC}$  in [-18;-15] and pvalue in [ $1 \cdot 10^{-5}$ ;  $4 \cdot 10^{-5}$ ],  
266 estimate in [0.17; 0.18], sd=0.04). In addition to the effect of the infestation index, the probability of  
267 detecting *D. plantaginea* DNA depended on orchard management ( $\Delta\text{AIC}=-6$ , pvalue= $6 \cdot 10^{-3}$ ): it was  
268 significantly higher in insecticide-free orchards compared to the organic and the IFP orchards and did  
269 not differ between the organic and IFP orchards (pairwise comparison estimate and pvalue: lfree-  
270 Organic= 1.0  $p < 1 \cdot 10^{-3}$ , lfree-IFP= 1.0 and  $p = 10^{-2}$ , IFP-Organic=-0.1  $p = 0.96$ ). Besides, the probability of  
271 detecting *D. plantaginea* DNA in the spider gut did not depend on their prosoma width ( $\Delta\text{AIC}=1$ ,  
272 pvalue=0.33).

273 Considering all spider taxa, the percentages of spiders that were positive to PCR analysis in early-  
274 season varied from 0 to 13% according to the orchard. They were high in March in IFP orchard  
275 (S\_IFP1 and S\_IFP2) and remained stable later (Table 1). In insecticide-free orchards, the percentage  
276 of positive spiders increased strongly between March, April and May, reaching 50 and 60% of the  
277 tested spiders. Organic orchards showed intermediate pattern (Table 1). Positive spiders were



278 observed for 8 out of the 12 families for which more than 10 individuals were analyzed (table2, table  
279 S4). In early season (February and March), 9 individuals of Anyphaenidae, 3 individuals of  
280 Philodromidae, 3 individuals of the Salticidae and 3 of 'other spiders' were positive to *D. plantaginea*  
281 primer (Table 2). The number of positive individuals was higher in mid-season for Salticidae (26%  
282 positive i.e 102 individuals) and 'other spiders' (19% i.e 19 individuals). It could not be estimated for  
283 Anyphaenidae and Philodromidae because these taxa were too few in traps. At genus level, all taxa  
284 from which more than 10 individuals were tested, had at least one individual that was positive to PCR  
285 except *Nursia albomaculata*, *Olios argelasius*, *Lathys humilis* and *Synaema globosum* (Table 2).

### 286 **3.5 Effectiveness of aphid suppression in insecticide-free orchards**

287 The variations between March and April of the *number of aphid colonies*  $r_1$  related negatively to the  
288 total number of spiders per tree, among which Philodromidae (*Philodromus spp*) were dominant. The  
289 abundance of Philodromidae alone explained better than 'All spiders' the decrease of  $r_1$  (better  
290 pvalue, deltaAIC and stronger estimate)(Table 3, Figure 3). Contrarily,  $r_1$  did not depend significantly  
291 on the early season abundance per tree of Salticidae, of 'Other spiders', or on the 'cumulated  
292 prosoma width'. In mid-season,  $r_2$  did not depend significantly on any spiders' abundance or on the  
293 'cumulated prosoma width' per tree (Table 3).

294

### 295 **Discussion**

296 Because of their predatory behavior, the diversity of their hunting strategies and their abundance in  
297 orchards, spiders have long raised an interest as generalist predators of pests (Nyffeler and Benz,  
298 1987). Our results confirm their potential effectiveness for controlling *D. plantaginea* thanks to PCR  
299 analyses of gut content (Harwood and Obrycki, 2005) and monitoring of aphid growth and spider  
300 abundances in insecticide free orchards. Besides, we highlighted that predation of aphids occurred in  
301 insecticide-free, organic and IFP orchards when aphids were present.

302 The species we captured were canopy spiders frequently found in orchards of southern Europe  
303 (Bogya et al., 1999; Isaia et al., 2010, 2006; Pekár, 1999). They were mostly represented by

304 wandering spiders in early season (February, March) and jumping spiders later in spring. Early-season  
305 species were similar between north-western and south-eastern orchards, but the species captured in  
306 mid-season were different, confirming the effect of geography on agrobiont spiders (Samu et al.,  
307 2011). The same species were present in IFP and organic orchards in north-western orchards;  
308 contrarily, the species present in the IFP, the organic and the insecticide-free south-eastern orchards  
309 differed to a large extent (table1, figure2). This absence of pattern is consistent with the observation  
310 that local and landscape scale environment may influence spider species composition further than  
311 the management of the orchard (Herrmann et al., 2010; Lefebvre et al., 2016).

312 The pattern was clearer concerning spider abundance. In early season, spider abundance did not  
313 differ according to the orchard management, and in mid-season, it tended to be higher in insecticide-  
314 free orchards (Tables 3 and S6). This pattern is consistent with the reported higher abundances in  
315 untreated orchards compared to treated ones in spring and summer (Mansour, 1987; Marko et al.,  
316 2009; Pekar, 1998) and the similar abundances in organic, IFP and conventional orchards (Pekár and  
317 Kocourek, 2004) at the same period. The absence of differences in early season could result from  
318 immigration and/or reproduction of individuals in late-summer, autumn and winter that would allow  
319 populations from treated orchards to recover from the decrease in abundance caused by spring and  
320 summer insecticides spraying (Pekár, 1999). Note, however, that the observed pattern of abundance  
321 may be partly oversized by the trapping method because the mid-season pattern was mainly  
322 observed on Salticidae while the early season pattern was mainly observed on the winter-active  
323 spiders *Philodromus spp* (Philodromidae) and *A. accentuata* (Anyphaenidae) (Korenko et al., 2010).  
324 *Philodromus spp* are little sensitive to synthetic and organic insecticides (Rezac et al., 2010), they may  
325 be abundant during summer in IPM orchards (Wisniewska and Prokopy, 1997) but they were not  
326 caught by our trap bands at that period. Beating is a more appropriate technique for their capture in  
327 late-spring and summer; however, beating was not performed on the sampled trees to avoid  
328 disturbing aphid colonies. Less information is available about the ecology *A. accentuata* and its  
329 sensitivity to pesticides or orchard management. This species is hardly caught by any technique in

330 late spring in orchards of the study region. We could thus not assess if *Philodromus spp*  
331 (*Philodromidae*) and *A. accentuata* taxa were as abundant in IFP as in untreated orchards in mid-  
332 season, as was observed in early-season.

333 Gut content molecular analysis is becoming a popular way of quantifying predation under  
334 naturally occurring conditions, including for spider species (Gurr et al., 2012; Harwood and Obrycki,  
335 2005). Among the 25 genera of spiders tested by PCR in the present study, 19 were detected positive  
336 for predation on *D. plantaginea* (Table 2). Besides, in all the orchards but one in early season and one  
337 in mid-season, the most abundant taxon predated on *D. plantaginea* (table 2). This confirms that a  
338 diversity of cursorial spiders may contribute to aphid control. We assessed predation by wandering  
339 spiders, ambush spiders and active hunters forage on aphid colonies, which must be complemented  
340 by the action of web spiders that capture passively alate aphids (Birkhofer et al., 2008; Boreau de  
341 Roince et al., 2013; Dib et al., 2010; Marc and Canard, 1997). Further, PCR positive individuals were  
342 observed in orchards whatever their management strategy (IFP, insecticide-free, organic) indicating  
343 that spiders may act as biocontrol agent in different types of orchards.

344 Although results appear straightforward, interpreting similarities or differences in rates of positive  
345 individuals among management strategies in terms of potential pest control is difficult. Indeed many  
346 factors may affect these rates. First, differences in the rate of positive spiders may result from  
347 differences of prey availability (Kerzicnik et al., 2012) and indeed rates increased with aphid  
348 infestation in the present study. Nevertheless, in mid-season, the probability of detecting aphid DNA  
349 was still highest in insecticide-free orchards after accounting for aphid infestation. It is possible, that  
350 spiders of conventional and organic orchards are less frequently positive as they switch to other  
351 preys than aphids because of aphid rarity as compared to alternative prey (Koss and Snyder, 2005;  
352 Pearce and Zalucki, 2006). It is also possible that IFP orchards were inhabited by spiders' species, or  
353 spiders with functional traits less likely to predate on aphids than spiders inhabiting insecticide free  
354 orchards. We only measured spider size as functional trait and it is unlikely to explain the observed  
355 differences because spiders size did not differ between management, and it did not relate to the

356 probability of aphid predation (as detected by PCR). Constraints on prey/predator size for hunting  
357 spiders exists (Michalko and Pekar, 2014; Sanders et al., 2015) but aphids are a small prey that is  
358 likely to be eaten by both small and bigger canopy spiders (Michalko and Pekar, 2015b).

359 The impact of spiders on aphid suppression, defined as an effect on aphid population growth  
360 (Desneux et al., 2006), was only assessed here in insecticide free orchards to avoid confounding  
361 effects of insecticides. This impact was expected to be stronger when aphids were not abundant  
362 because later in season many other natural enemies may affect aphid growth, including more  
363 efficient species such as syrphids and coccinellids (Minarro et al., 2005). Consistently with  
364 expectations, we found a positive effect of the abundance of the dominant spider taxon, *Philodromus*  
365 spp, on the decrease of the number of aphid colonies in early season, despite only few *Philodromus*  
366 individuals (3/129) were positive to *D. plantaginea*. That few individuals were positive early in  
367 season, although *Philodromus* appeared to affect aphid population, is consistent with low aphid  
368 abundance. Further, pest suppression can also be due to non-consumptive or trait-mediated non-  
369 lethal effects (Beleznai et al., 2015; Bucher et al., 2015) such as individuals dropping or losing energy  
370 due to disturbance. Few studies report aphid suppression by natural enemies in the field by relating  
371 the temporal variation of aphids with that of predators or parasitoids (e.g. Boreau de Roince et al.,  
372 2013; Chaplin-Kramer et al., 2013; Dib et al., 2010). To our knowledge only Boreau et al. (2013)  
373 specifically addressed the role of canopy spiders on aphids' colonies in spring and the authors did not  
374 report this possibly important role of *Philodromus* spp. This role is consistent with the high  
375 appetite of *Philodromus* for aphids (Michalko and Pekar, 2015b). Such appetite for aphids is not  
376 observed for all spider species. Aphids were, for example, shown to be of low food value to some  
377 ground species (Toft, 1995) and euryphagous species (i.e. with a large diet breadth) need alternative  
378 preys to complement their diet (Madsen et al., 2004). But other studies suggest that the diet of  
379 generalist predators, during aphids' infestation pic, is mainly based on aphids in cultivated fields  
380 (Staudacher et al., 2015). Further than the abundance of total predators or of specific taxa, the  
381 functional composition of predator assemblages has been suggested be a more pertinent indicator of

382 their potential impact on preys (Long and Finke, 2014). Here we found no effect of the cumulated  
383 sizes of spiders on aphid suppression (table 3), possibly because aphid are small preys (see above),  
384 but also possibly because larger predators are those that exert more intra-guild predation (Rusch et  
385 al., 2015).

386 Our result, using a combination of field observations and molecular analyses thus demonstrate  
387 the potential of spiders for reducing aphid populations in insecticide-free orchards, particularly very  
388 early in season. Further, our observations in treated orchards indicate that spiders in these orchards  
389 are also abundant early in season and that they predate on aphids, suggesting that their potential  
390 effect could be taken into account in decision rules governing early season insecticide treatments.

391

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398

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541

542 **Figure legend**

543 **Figure 1:** Location of the sampled orchards within France. IFP: orchards under integrated fruit  
544 production; organic: orchards under organic management; Ifree: insecticide-free orchards. N: north-  
545 western orchards; S: south-eastern orchards.

546 **Figure 2:** Mean abundance per trap band of different spider taxa. Values are cumulated for the two  
547 months of each season (early and mid-season), for the different orchards. Groups of orchards with  
548 different letters are significantly different based on glmer tests comparing the abundance per trap  
549 band according to the management of the orchard and pairwise multiple comparisons by Tukey  
550 tests.

551 **Figure 3:** Relation between  $\text{Log}(r1)$ , the ratio of the number of aphid colonies between March and  
552 April, and the number of *Philodromus* spp individuals captured per tree in February and March. In  
553 black, data from Ifree1; in grey, data from Ifree2. The lines represent predictions from the linear  
554 models considering the random orchard effect.

555

556 **Tables**

557 **Table 1:** *D. plantaginea* abundance in orchards and results of PCR tests: percentage of spiders that were found positive for *D. plantaginea* consumption  
 558 (Pos), total number of spiders tested by PCR (PCR); means and standard deviations (sd) of aphid infestation calculated as log(infestation index +1) (Inf) and  
 559 of the number of colonies (c).

	Feb		March				April						May					
	Pos	PCR	Pos	PCR	c	sd	Pos	PCR	Inf	sd	c	sd	Pos	PCR	Inf	sd	c	sd
lfree1	4.3%	23	5.1%	59	0.7	(0.9)	17.9%	28	1.8	(2.1)	2.0	(1.8)	50.0%	12	1.2	(1.7)	1.4	(1.7)
lfree2	0.0%	13	0.0%	32	1.3	(1.9)	24.7%	89	1.1	(1.2)	2.7	(4.1)	60.0%	85	3.1	(2.9)	13	(10)
S_organ	2.4%	41	1.8%	57	0.8	(0.7)	10.5%	57	0.5	(0.9)	1.0	(2.7)	23.4%	64	1.2	(1.4)	3.2	(5.9)
S_IFP1	0.0%	28	8.8%	34	0.9	(0.8)	8.6%	35	0.0	0	0.0	(0)	18.5%	27	0.1	(0.3)	0.1	(0.2)
S_IFP2	0.0%	30	3.4%	29	2.6	(3.6)	5.9%	17	0.1	(0.4)	0.1	(0.4)	5.6%	18	0.0	0	0	(0)
N_organ	13.2%	53	0.0%	31	0.3	(0.7)	14.3%	14	0.3	(0.6)	2.5	(2.8)	20.0%	5	1.1	(1.0)	5.3	(4.4)
N_IFP1	2.4%	41	0.0%	48	0.8	(1.0)	0.0%	8	0.0	(0)	0.3	(0.6)	100.0%	1	0.0	(0.2)	0.7	(2.6)
N_IFP2	0.0%	27	0.0%	8	0.4	(0.7)	0.0%	3	0.0	0	0.0	0	0.0%	1	0.0	0	0	(0)

560

561

562 **Table 2:** Taxa captured and PCR results in early and mid-season. Number of individuals captured (N);  
 563 number of individuals tested (PCR) and number of individuals positive to PCR analysis (+).

		early-season			mid-season		
		N	PCR	+	N	PCR	+
Amaurobidae	<i>Nursia albomaculata</i>	42	29	0			
Anyphaenidae	<i>Anyphaena accentuata</i>	157	148	9	1	1	0
Araneidae	spp	74	21	1	25	11	2
Clubionidae	<i>Clubiona sp</i>	34	24	0	17	15	2
Dictynidae	spp	48	24	0	8	4	0
Eutichuridae	<i>Cheiracanthium mildei</i>	9	9	0	14	11	4
Gnaphosidae	spp	11	6	0	23	20	6
Linyphiidae	spp	2	0		3	0	
Liocranidae	spp	3	0		1	0	
Philodromidae	<i>Philodromus spp</i>	162	130	3	4	3	1
Salticidae	spp	122	89	5	402	387	102
Segestridae	<i>Segestria senoculata</i>	0	0		5	0	
Sparassidae	<i>Olios argelasius</i>	23	20	0	2	2	0
Theridiidae	spp	36	19	0	15	6	2
Thomisidae	spp	29	17	0	9	3	0

565 **Table 3:** Test of variables (taxa or traits) affecting aphid suppression measured as the variation of the *number of colonies* in early and mid-season (r1 and r2).  
 566 Estimates of effects and their standard deviations are provided together with the variation of AIC when removing each factor from the full model, and the  
 567 corresponding p-value. Independent variables were tested in different models (see material and methods).

	r1			r2		
	Estimate (sd)	ΔAIC	p-value	Estimate (sd)	ΔAIC	p-value
Philodromidae	<b>-0.18 (0.06)</b>	<b>-6</b>	<b>4. 10<sup>-3</sup></b>			
Salticidae	-0.05 (0.15)	2	0.7	-0.08 (0.05)	0	0.14
Other spiders	-0.1 (0.2)	1	0.4	-0.17 (0.2)	2	0.4
All spiders	<b>-0.08 (0.05)</b>	<b>-3</b>	<b>0.03</b>	-0.09 (0.05)	0	0.1
Cumulated prosoma width	-0.04 (0.04)	0.6	0.2	-0.03 (0.02)	0.8	0.09





Figure1

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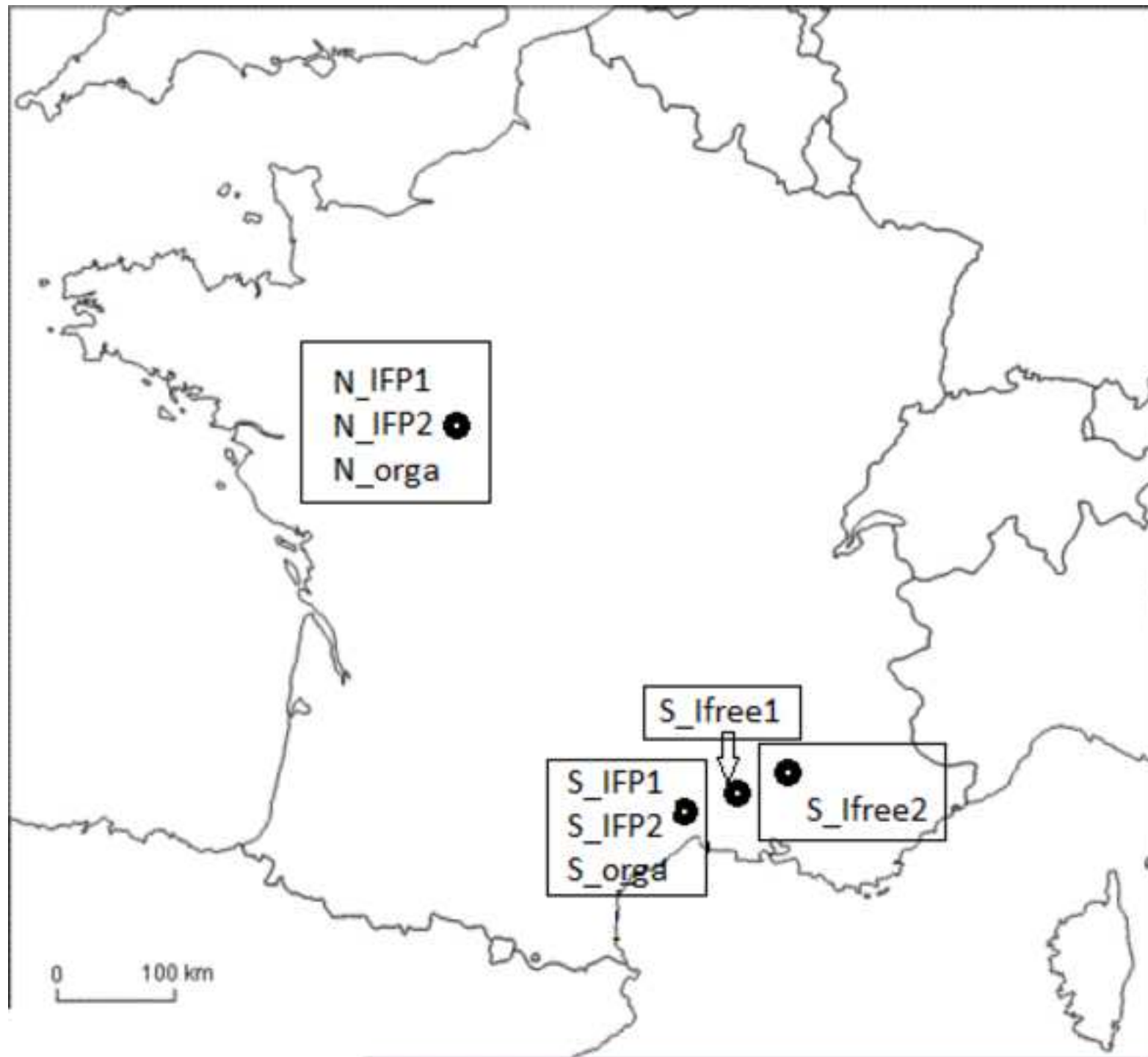


Figure2

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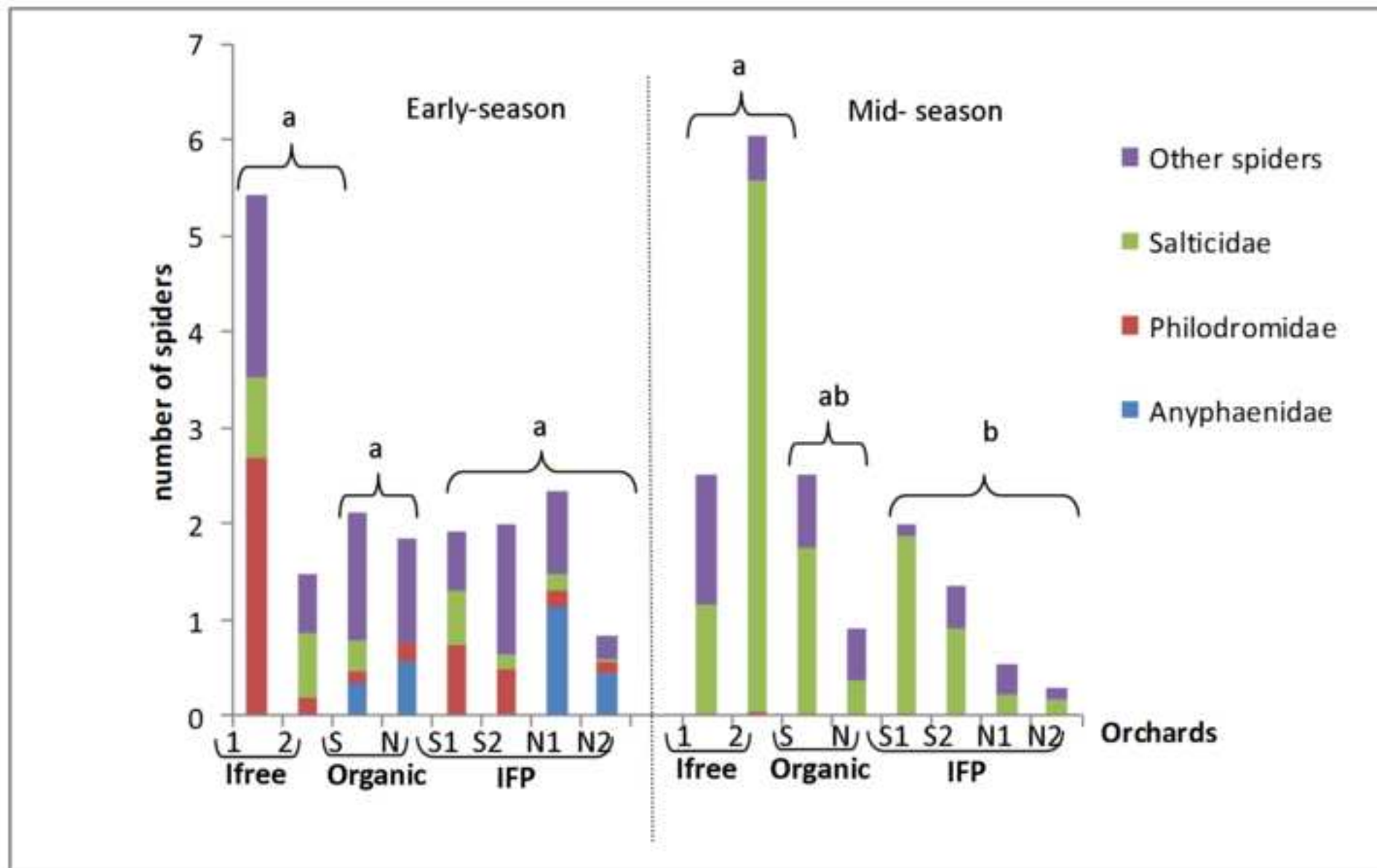
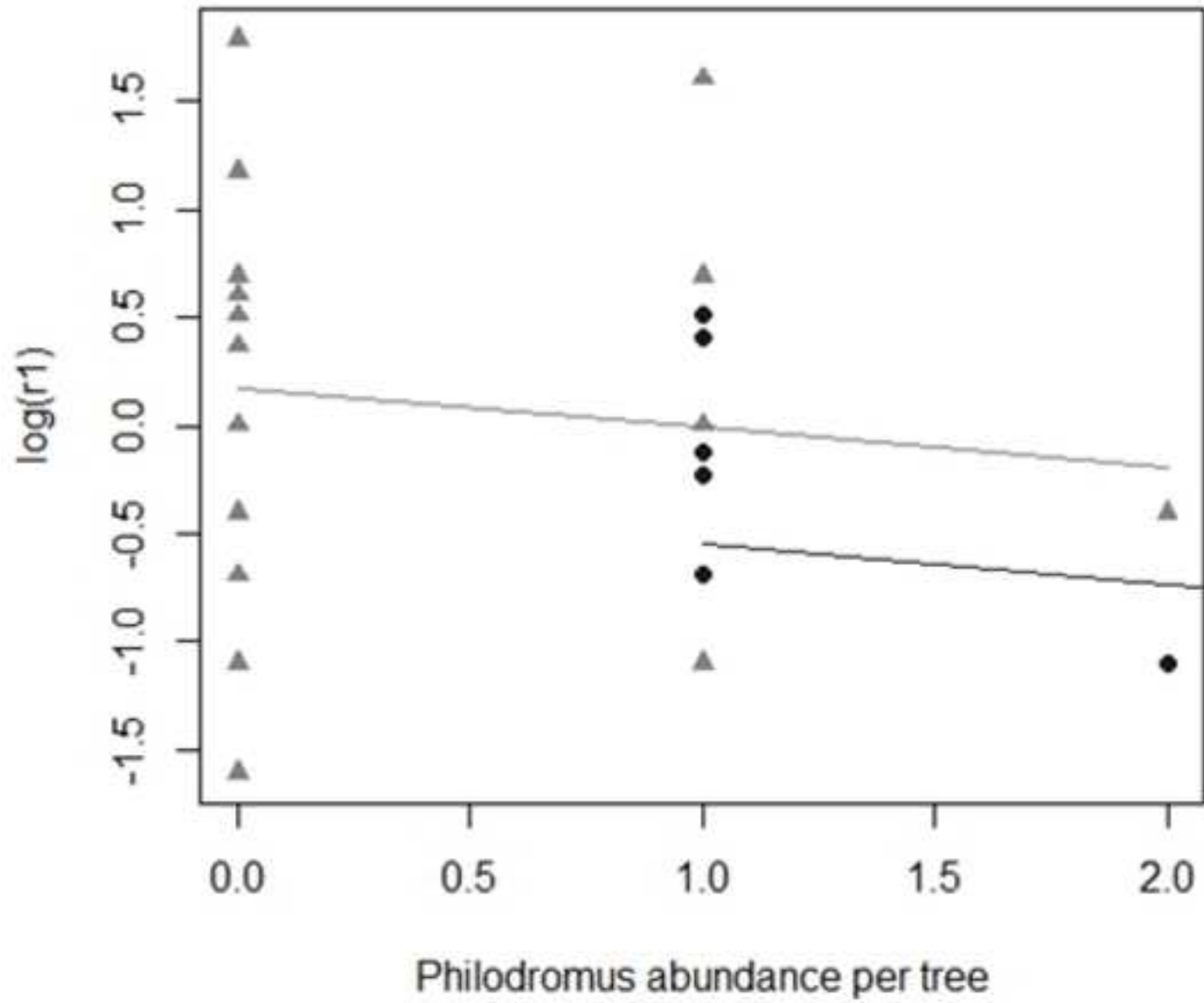


Figure3

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**Supplementary Material**

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