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## How the use of nitrogen fertiliser may switch plant suitability for aphids: the case of Miscanthus, a promising biomass crop, and the aphid pest *Rhopalosiphum maidis*

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1 **How the use of nitrogen fertiliser may switch plant suitability for aphids: the case of**  
2 **Miscanthus, a promising biomass crop and the aphid pest *Rhopalosiphum maidis*.**

3 Running title: Nitrogen fertilizer and Miscanthus-aphid complex

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9

10 **Abstract**

11 BACKGROUND: The use of nitrogen fertiliser in agrosystems can alter plant nitrogen and  
12 consequently improve nutrient availability for herbivores, potentially leading to better  
13 performance for herbivores and higher pest pressure in the field.

14 RESULTS: We compared, in laboratory conditions, the effects of nitrogen fertilisation on a  
15 promising biomass crop, *Miscanthus x giganteus*, and its parents *Miscanthus sinensis* and  
16 *Miscanthus sacchariflorus*. The plant-mediated effects were compared on the second trophic  
17 level, the green corn leaf aphid *Rhopalosiphum maidis*.

18 Results showed that the biomass and leaf C:N ratio of *M. sinensis* plants treated with nitrogen  
19 fertiliser were significantly greater than those of non-treated plants. Concerning  
20 *M. x giganteus* and *M. sacchariflorus*, the only reported change was a significantly smaller  
21 leaf C:N ratio for treated *M. sacchariflorus* compared to non-treated plants.

22 Surprisingly, nitrogen fertilisation had opposite consequences on plant-herbivores  
23 interactions. Following N treatments, *M. sinensis* was less suitable in terms of intrinsic rate of  
24 increase for *R. maidis*, whose feeding behavior was negatively affected, while  
25 *M. sacchariflorus* and *M. x giganteus* exhibited greater suitability in terms of aphid weight.

26 CONCLUSION: Nitrogen fertilisation had contrasting effects on the three species of  
27 *Miscanthus* plants. These effects cascaded up to the second trophic level, *R. maidis* aphid  
28 pests, either through a modification of their weight or demographic parameters. The  
29 implications of these results were discussed in the context of agricultural sustainability and  
30 intensive production practices.

31 **Keywords:** Aphididae, Aphid performance, Electropenetrography, Leaf C:N ratio,  
32 *Miscanthus* species, Nitrogen input, Pest management

## 33 1. Introduction

34

35 Nitrogen (N) is a key element for plants. Its availability in agrosystems can be improved with  
36 the use of fertilisers. Plants are capable of plastic responses following fertilising, as evidenced  
37 by the profound reprogramming of their N and carbon (C) metabolism.<sup>1</sup> Such metabolic  
38 changes can impact plant quality and ultimately influence trophic-level interactions, thus  
39 affecting the performance of herbivores.<sup>2</sup> Plant response following the use of fertiliser may  
40 vary between different species but also between cultivars as shown by various studies on  
41 maize or oilseed rape.<sup>3,4</sup> Nitrogen inputs have usually been linked with decreased C:N ratio in  
42 plants, correlated to an improvement of plant quality for herbivores.<sup>5</sup> A higher plant N content  
43 has been shown to positively impact their feeding behaviour<sup>6</sup> or life history traits<sup>5,7,8</sup>, and thus  
44 pest pressure in the field. However, excessive nutrient intake by herbivores can negatively  
45 affect their fitness and population dynamics.<sup>9</sup> Altogether the N content may influence plant  
46 resistance to higher trophic levels, mostly through the alteration of primary and secondary  
47 metabolites production in plants.<sup>1,10</sup> According to the Carbon-Nutrient-Balance hypothesis,<sup>11</sup>  
48 a plant subjected to an abundant amount of nitrogen should allocate relatively more to  
49 nitrogen-containing defence metabolites and reduce secondary carbon-based substances.

50 Nitrogen requirement is a particularly significant issue, since both the manufacturing process  
51 of nitrogen fertilisers and losses following application can have local and global  
52 environmental impacts as well as significant implications to greenhouse-gas balances.<sup>12</sup> Smith  
53 *et al.*<sup>13</sup> reported that biomass crops have great potential to mitigate carbon emissions and are  
54 likely to be major contributors to the renewable energy mix in the future.  
55 *Miscanthus x giganteus*, a vigorous sterile hybrid between *M. sinensis* and *M. sacchariflorus*,  
56 is a promising crop dedicated to biomass and biofuel production. The potential of *Miscanthus*

57 is attributed to high productivity and long term perenniality, together with nutrient  
58 requirements that are generally considered low, although the exact needs of this crop are not  
59 yet defined.<sup>14</sup>

60 The two cultivated species of *Miscanthus* exhibit different productivities, the crops of the  
61 sterile hybrid *M. x giganteus* leading to higher yields than those of the parental species  
62 *M. sinensis*. However, as European crops of *M. x giganteus* consist of a single clone, *M.*  
63 *sinensis* is still regarded as a potential alternative for *Miscanthus* production as certain *M.*  
64 *sinensis* clones display a high biomass potential.<sup>15,16</sup> Although the parental species  
65 *M. sacchariflorus* is not a dedicated biomass crop, it is also interesting as a progenitor for  
66 breeding programs, due to its low ash content making it suitable for the different bioenergy  
67 conversion processes.<sup>17</sup> Interestingly, the three species of *Miscanthus* differ in terms of  
68 susceptibility to pests, in particular to aphids.<sup>18</sup> The corn leaf aphid *Rhopalosiphum maidis* is  
69 considered as the main *Miscanthus* pest, as *R. maidis* colonies can develop on *Miscanthus*  
70 host-plants<sup>19</sup>, to which they can also transmit the *Barley yellow dwarf virus*<sup>20</sup>. Indeed, some  
71 studies using vitro-plants have pointed out that *M. sacchariflorus* shows a greater resistance to  
72 the corn leaf aphid *R. maidis* than *M. sinensis* and *M. x giganteus*.<sup>21,22</sup> Another study using  
73 potted plants has shown that *M. sinensis* was more resistant than *M. x giganteus*, raising the  
74 possibility that *M. sinensis* could represent a better alternative to *M. x giganteus* under heavy  
75 aphid pressure.<sup>20</sup> These studies suggest that *Miscanthus* breeding programs should also take  
76 into account traits that are related to resistance to insect pests.<sup>21</sup>

77

78 To understand the possible consequences of fertilisers on pest pressure in bioenergy crops, we  
79 compared the effects of nitrogen input on the interactions between three species of  
80 *Miscanthus* (*M. x giganteus*, *M. sinensis* and *M. sacchariflorus*) and the green corn leaf aphid  
81 *Rhopalosiphum maidis*. To date, regarding the different nitrogen supply programs studied,

82 none has converged towards a consensual understanding of *Miscanthus* response to  
83 fertilisation. This might be attributed to the fact that, so far, the majority of these studies were  
84 solely performed in field setups and therefore submitted to variations and potential biases  
85 inherent to natural conditions.<sup>12</sup> The aim of the present study was to compare, in controlled  
86 and standardised conditions, the effects of nitrogen input on *M. x giganteus* and its two  
87 parents, as well as the plant-mediated effects on the second trophic level, the aphid *R. maidis*.  
88 We predicted that nitrogen inputs should positively impact (i) the plants biomass and N  
89 contents; (ii) the feeding behaviour of aphids; (iii) the performance of aphids reared on these  
90 host-plants, including weight and demographic parameters.

91

## 92 2. Materials and Methods

93

### 94 2.1 Insects and Plants

#### 95 2.1.1 Insect cultures

96 A laboratory colony of the aphid *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) was  
97 initiated from a parthenogenetic aphid population. Aphids were reared on plants of winter  
98 barley (*Hordeum vulgare* cv. “Cervoise”). Pots (90 × 90 × 70 mm) containing each 15–20  
99 barley plants were placed in ventilated Plastic ® cages (240 × 110 × 360 mm) and maintained  
100 in a growth chamber under 20 ± 1°C, 60 ± 5 % RH, and a 16:8 L:D light cycle.

#### 101 2.1.2 Plants material

102 Plantlets of the three Miscanthus species, *i.e.*, *M. x giganteus* (cv. “GigB”, 2n = 3x = 57),  
103 *M. sacchariflorus* (cv. “Sac”, 2n = 2x = 38) and *M. sinensis* (cv. “Goliath”, 2n = 4x = 76)<sup>23</sup>  
104 were obtained by *in vitro* multiplication as described in previous study.<sup>24</sup> Single rooted shoots  
105 coming from clusters, rooted in perlite for eight weeks, were transplanted into plastic pots  
106 (firstly 9 × 9 × 10 cm, then 13 × 16 cm and 15 × 20 cm (height x diameter)) containing  
107 potting soil (NPK 18-10-20, 0.5 kg/m<sup>3</sup>, FLORAGARD) and kept in a growth chamber under  
108 20 ± 1°C, 60 ± 5 % Relative Humidity (RH), and a 16:8 (L:D) photoperiod for ten weeks.

109

#### 110 2.2 Nitrogen treatments

111 After development for ten weeks in the growth chamber, the potted plants were randomly  
112 assigned to one of the two following treatments: (i) a low nitrogen input, consisting of the  
113 potting soil only (non-treated, referred to as “-N”) (ii) potting soil supplemented with 0.76

114 gN.plants<sup>-1</sup> of ammonium nitrate (treated, referred to as "+N"). This dose of nitrogen (*i.e.*  
115 120 kgN.ha<sup>-1</sup>) was chosen in accordance with previous works.<sup>25</sup> Application of the  
116 nitrogenous solution was carried out using two half-doses. The first half-dose  
117 (0.38 gNH<sub>4</sub>NO<sub>3</sub>) was diluted in 100 mL of water and was applied over the entire soil surface  
118 of each pot three weeks before use for the experiments. The second half-dose was applied one  
119 week after the first one. All *Miscanthus* plants were placed randomly (*i.e.* regardless of the  
120 species or nitrogen status) in a greenhouse under controlled conditions (20 ± 1 °C, 60 ± 5 %  
121 RH, photophase 16h at 4 klux) for three weeks after the first N application, until they were  
122 used for experiments (plant biomass measurement and introduction of aphids onto the plants),  
123 *i.e.* thirteen weeks following potting. Watering was performed every 48 hours using volumes  
124 (100 ml) of water that had been calibrated during preliminary tests to prevent any leachage.  
125 No experimental blocking was performed.

126

## 127 **2.3 Effects of nitrogen treatments on plants aerial biomass and quality**

### 128 **2.3.1 Plants biomass measurements**

129 For each treatment and species, plant sprouts were individualised and weighed using an  
130 electronic balance (Mettler Toledo ML204, Max: 220 g, d = 0.1 mg), then placed in the  
131 freezer at -80 °C for a later use in carbon and nitrogen content measurements as described in  
132 previous works.<sup>26</sup> For *M. sacchariflorus*, twelve plants were weighed for the "-N" treatment  
133 and seven for the "+N" one. Eleven plants of *M. x giganteus* were weighed for the "-N"  
134 treatment and ten plants for the "+N" one. For *M. sinensis*, ten plants were weighed for each  
135 treatment ("-N" and "+N").

136

### 137           **2.3.2 Plants quality**

138   The quality of plants was assessed through the calculation of the Carbon:Nitrogen (C:N) ratio  
139   of their aerial organs. To perform the measurement of the C and N contents, the samples from  
140   the previous experiments (stored at -80 °C) were lyophilised at 4 °C for 48 hours using a  
141   lyophiliser (Alpha I-5). Samples of *Miscanthus* plantlets were processed individually. Each  
142   sample was ground for one minute with a ball mill (Retsch MM 400), then placed in a tin  
143   basket and weighed using a precision balance (Sartorius Genius ME415S). The carbon and  
144   nitrogen concentrations were determined using an elemental analyser (Flash EA 1112 series  
145   Thermo Electron, Bremen Germany). For *M. sacchariflorus*, twelve plants were analysed for  
146   the "-N" treatment and ten for the "+N" one. Eleven plants of *M. x giganteus* were analysed  
147   for each treatment ("-N" and "+N"). For *M. sinensis*, ten plants were analysed for each  
148   treatment ("-N" and "+N").

149

### 150           **2.4 Plant-mediated nitrogen effects on *R. maidis* feeding behaviour**

151   The electrical penetration graph DC-system was used.<sup>27</sup> To insert one aphid and one plant into  
152   an electrical circuit, a thin gold wire (20 mm diameter and 2 cm long) was tethered on the  
153   insect's dorsum using conductive silver glue (EPG systems, Wageningen, The Netherlands).  
154   Eight aphids were connected to the Giga-8 DC-EPG amplifier and each one was placed on the  
155   leaf of an individual plant. A second electrode was inserted into the soil of each of the potted  
156   plants to complete the electrical circuits. The recordings were performed continuously for six  
157   hours during the photophase. Each aphid-plant system was placed as a whole inside a Faraday  
158   cage at  $20 \pm 1$  °C. Acquisition and analysis of the EPG waveforms were carried out with  
159   PROBE 3.5 software (EPG Systems, [www.epgsystems.eu](http://www.epgsystems.eu)). Parameters from the recorded  
160   EPG waveforms were calculated with EPG-Calc 6.1 software.<sup>28</sup> These parameters were based

161 on different EPG waveforms corresponding to:<sup>29</sup> (C) stylet pathways in plant tissues except  
162 phloem and xylem; (pd) potential drops (intracellular stylet punctures); (E1) salivation in  
163 phloem elements; (E2) passive phloem sap ingestion; (E1 + E2) activity within phloem  
164 vessels, (G) active xylem sap ingestion; and (F) derailed stylet mechanics. In this study, the  
165 feeding behaviour of *R. maidis* on *M. sinensis*, *M. sacchariflorus* or *M. x giganteus*, treated or  
166 not, was investigated using 24–39 individuals.

167

## 168 **2.5 Plant-mediated nitrogen effects on *R. maidis* aphid performance**

169 Pools of synchronised first instar nymphs (less than 24-hours old) were obtained from  
170 parthenogenetic adult females placed on leaves of their host-plant set in 1.5 % agar in Petri  
171 dishes (90 mm diameter). To obtain synchronised young adults, first instar nymphs were kept  
172 in the same device for a further eight days. For the nymph survival study, groups of five first  
173 instar nymphs were gently transferred, using a small paintbrush, onto the plantlets to be  
174 tested. These groups of aphid nymphs were enclosed in plastic clip-cages (15 mm diameter,  
175 10 mm height) ventilated by a grid and held by a metal clip on the lower face of a leaf at the  
176 mid-height of each plantlet, and their survival was recorded every day. To study the adult  
177 performance, young adults were individually transferred onto the plantlets to be tested.  
178 Survival and fecundity of adult individuals were recorded every 24 hours. Each adult  
179 individual was placed individually in a clip-cage in order to count the number of nymphs  
180 produced. The newly larviposited individuals were counted and removed with a brush every  
181 24 hours to estimate the daily fecundity of each individual parent. A total of 120 adult aphids  
182 were followed, with 20 aphids for each host-plant species and each treatment. Their daily  
183 fecundity was assessed for a duration equivalent to twice the pre-reproductive period  
184 duration. The intrinsic rate of natural increase ( $r_m$ ) was calculated using the DEMP 1.5.2

185 Software,<sup>30</sup> as  $\sum e^{-r_m x} l_x m_x = 1$ , where  $x$  was the age,  $l_x$  the age-specific survival, and  $m_x$  the  
186 mean number of female offspring produced in a unit of time by a female aged  $x$ .<sup>31</sup> This  
187 parameter was selected to compare the ability of *R. maidis* to establish a population on each  
188 of the three *Miscanthus* species treated or not with  $\text{NH}_4\text{NO}_3$  addition.

189 To measure aphid weight, synchronisations of aphids were carried out in plastic boxes (125 x  
190 115 x 55 mm) on each of the three species of *Miscanthus*, for the two treatments (“-N” and  
191 “+N”). A 30 cm<sup>2</sup> opening covered with nylon mesh was made on each box for ventilation. A  
192 single *Miscanthus* leaf was slid inside each box, on which 100 newly larviposited *R. maidis*  
193 nymphs were deposited. For each species of *Miscanthus*, eight plants were used for the  
194 experiments, with four plants per treatment (“-N” and “+N”). For each plant, four plastic  
195 boxes containing a leaf with 100 aphids were used. At eight days post-larviposition, 30 aphids  
196 were randomly selected from each box and individually weighed using an electronic precision  
197 balance (Mettler M3, class 1, Max: 3g Low: 1 µg, T = -3G [dd] = 1 µg).

198

## 199 **2.6 Statistical analyses**

200 As a Shapiro-Wilk test showed that data were not normally distributed, non-parametric tests  
201 were used for all analyses. The impacts of nitrogen fertilisation within the three species of  
202 *Miscanthus* on the aboveground biomass, leaf nitrogen content and leaf C:N ratio were  
203 analysed using Mann–Whitney *U* tests. Plant-mediated nitrogen effects on the feeding  
204 behaviour of *R. maidis* were analysed using Mann–Whitney *U* tests. The combined effects of  
205 the *Miscanthus* host species and N treatment on *R. maidis* performance parameters and weight  
206 were analysed using GLM with quasipoisson distribution (link: log) followed by pairwise  
207 comparisons using least-squares means (package R: “lsmeans”). All statistical analyses were  
208 performed in the R software.<sup>32</sup>

## 209 3. Results

210

### 211 3.1 Effects of nitrogen addition on aboveground biomass and quality of the three 212 species of *Miscanthus*

213 Following addition of  $\text{NH}_4\text{NO}_3$  (+N) on *M. sinensis*, the biomass of plants was significantly  
214 greater than that of non-treated (-N) *M. sinensis* plants ( $U = 19, P = 0.007$ ) (Table 1a). For  
215 *M. x giganteus* and *M. sacchariflorus*, the addition of  $\text{NH}_4\text{NO}_3$  had no significant effect on the  
216 aboveground biomass of plants (*M. x giganteus*:  $U = 45, P = 0.332$ ; *M. sacchariflorus*:  $U =$   
217  $66, P = 0.722$ ) (Table 1b and 1c). The C:N leaf ratio of *M. sinensis* exposed to  $\text{NH}_4\text{NO}_3$  (+N)  
218 was significantly greater than that of non-treated (-N) *M. sinensis* plants ( $U = 19, P = 0.007$ )  
219 (Table 1a). For *M. x giganteus*, the leaf C:N ratio was not significantly affected by the  
220 treatment ( $U = 65, P = 0.797$ ) (Table 1b). The leaf C:N ratio of treated (+N) *M. sacchariflorus*  
221 was significantly smaller than the leaf C:N ratio of non-treated (-N) *M. sacchariflorus* plants  
222 ( $U = 93, P = 0.035$ ) (Table 1c).  $\text{NH}_4\text{NO}_3$  treatment did not have any significant effect on leaf  
223 nitrogen content for *M. x giganteus* ( $U = 59, P = 0.949$ ) (Table 1b and 1c). Treated (+N)  
224 *M. sinensis* and *M. sacchariflorus* respectively showed significantly lower and higher leaf  
225 nitrogen content compared to non-treated (-N) plants (*M. sinensis*:  $U = 93, P = 0.021$ ;  
226 *M. sacchariflorus*:  $U = 24, P = 0.017$ ) (Table 1a).

227

### 228 3.2 Plant-mediated nitrogen effects on aphid feeding behaviour

229 Whatever the modality (plant species or nitrogen treatment), individuals exhibited sustained  
230 phloem sap ingestion (E2). During the six hours of recording, the total duration of stylet  
231 activity in the plant lasted on average about five hours. When submitted to treated (+N)  
232 *M. sinensis* plants, *R. maidis* aphids exhibited a significantly longer duration of the pathway

233 phase (C) ( $U = 370, P = 0.003$ ) and a significantly shorter hydration phase (G) ( $U = 839, P =$   
234  $0.0004$ ) than aphids on non-treated (-N) *M. sinensis* plants. On treated (+N) *M. sacchariflorus*  
235 plants, aphids derailment phase of stylets (F) was significantly shorter than on non-treated (-  
236 N) *M. sacchariflorus* plants ( $U = 399, P = 0.024$ ). On treated (+N) *M. x giganteus* plants,  
237 *R. maidis* salivation period (E1) was significantly shorter than on non-treated (-N)  
238 *M. x giganteus* plants ( $U = 261, P = 0.037$ ) (Table 2).

239

### 240 **3.3.Plant-mediated nitrogen effects on aphid performance**

241 The average rate of natural increase  $r_m$  of *R. maidis* aphids was significantly affected by the  
242 plant species ( $\chi^2 = 0.676, df = 127, P = 1.238 \times 10^{-14}$ ) and by the N treatment ( $\chi^2 = 0.044, df =$   
243  $126, P = 0.041$ ). The  $r_m$  was significantly lower when aphids were reared on *M. x giganteus*  
244 compared to *M. sinensis* or *M. sacchariflorus*. When aphids developed on treated (+N)  
245 *M. sinensis*, the  $r_m$  was significantly smaller compared to that of aphids on non-treated (-N)  
246 *M. sinensis* plants. There was no interaction between the two factors ( $\chi^2 = 0.026, df = 124, P =$   
247  $0.286$ ) (Fig. 1a.).

248 The weight of *R. maidis* aphids was significantly affected by the plant species ( $\chi^2 = 1231.87,$   
249  $df = 177, P = 2.848 \times 10^{-14}$ ) and by the N treatment ( $\chi^2 = 2131.62, df = 176, P < 2.2 \times 10^{-16}$ )  
250 (Fig. 1b.). There was a significant interaction between the plant species and the N treatment  
251 ( $\chi^2 = 932.49, df = 174, P = 5.579 \times 10^{-11}$ ) (Fig. 1b.). The weights of aphids developing on  
252 non-treated (-N) *M. x giganteus* were significantly smaller than those on non-treated (-N) *M.*  
253 *sinensis*. The weights of aphids developing on treated (+N) plants were significantly greater  
254 on *M. x giganteus* (2.5 times) and *M. sacchariflorus* (4 times) compared to those developing  
255 on the respective non-treated (-N) host-plants.

256

#### 257 4. Discussion

258

259 To our knowledge, this is the first study not only reporting the effects of nitrogen input on the  
260 three main species of the *Miscanthus* genus, but also investigating the consequences on aphid  
261 herbivores. Nitrogen fertilisation had contrasting effects on the three species of *Miscanthus*  
262 plants. These effects cascaded up to the second trophic level, *R. maidis* aphids, either through  
263 a modification of their weight or demographic parameters.

264 We showed that nitrogen input had no significant impact on the aboveground biomass of the  
265 hybrid *M. x giganteus* and of the parental species *M. sacchariflorus*. To our knowledge, this is  
266 the first study of nitrogen input consequences on *M. sacchariflorus*. Our results are in line  
267 with those obtained in field conditions,<sup>33,12</sup> according to which nitrogen fertilisation had no  
268 impact on the aboveground biomass of *M. x giganteus* crops during the first years after  
269 rhizome transplanting. On the contrary, the aboveground biomass of the second parental  
270 species, *M. sinensis*, was significantly greater for treated (+N) plants.

271 In our study, nitrogen input differently impacted the C:N ratio of each *Miscanthus* species:  
272 *M. x giganteus* hybrid leaf C:N ratio was not affected by nitrogen input contrary to those of  
273 parental species. The leaf C:N ratio of treated (+N) plants was significantly smaller for  
274 *M. sacchariflorus* but significantly greater for *M. sinensis*. The results obtained on *M. sinensis*  
275 could be explained by the nitrogen dilution when the biomass increased.<sup>34</sup> Most studies show  
276 that decreased plant C:N ratios are generally linked to an increased biomass following  
277 nitrogen fertilisation.<sup>25</sup> The differences observed between the C:N ratio of the three species of  
278 *Miscanthus* could be due to their different use of nitrogen in our experimental conditions.

279 Previously published papers highlight the relationships between a high nitrogen content in the  
280 host-plant and a high performance in terms of population growth rate,<sup>5</sup> weight increase,<sup>7</sup> or

281 both for the phytophagous.<sup>8</sup> Regarding the nitrogen leaf content of treated (+N) Miscanthus,  
282 we could have expected the performance (in terms of  $r_m$  and/or weight) of the *R. maidis* aphid  
283 to be (i) worse on *M. sinensis*, (ii) better on *M. sacchariflorus* and (iii) not different on  
284 *M. x giganteus* in comparison with the non-treated (-N) respective host-plants.

285

286 Consistent with our assumption about *M. sinensis*, aphids  $r_m$  was significantly smaller on  
287 treated (+N) plants (although their weight was not different), whose leaf C:N ratio was  
288 significantly higher. This seemed consistent with the study,<sup>35</sup> where a decrease of aphid  
289 performance was imputed to a high C:N in plant tissues. The significantly smaller *R. maidis*  
290  $r_m$  measured on treated (+N) plants can be explained by the negatively altered feeding  
291 behaviour revealed by a significantly longer duration of stylet pathways (C) and shorter  
292 duration of xylem (G) phases. Ameline *et al.*<sup>36</sup> showed that an increase of this hydration phase  
293 induced better aphid demographic performance. Therefore, the alteration of *R. maidis*  
294 performance could be due to a decrease of xylem sap ingestion. However, the phloem sap  
295 intake duration was unchanged although phloem quality had possibly been lower, as indicated  
296 by the greater leaf C:N ratio. This could explain why *R. maidis* weight was not impacted and  
297 also, why its  $r_m$  was negatively affected.

298 Consistent with our assumption about *M. sacchariflorus*, *R. maidis* aphids weight was  
299 significantly greater in treated (+N) plants and their feeding behaviour seemed to be  
300 positively affected. Indeed, we recorded a significantly shorter duration of stylet derailment  
301 (F), a phase generally considered as an indicator of plant resistance.<sup>26</sup> The phloem sap intake  
302 duration was similar for both treatments although phloem quality was probably better in  
303 treated plants, as indicated by their smaller leaf C:N ratio. This could explain why *R. maidis*  
304 weight was so remarkably greater. However, despite this smaller leaf C:N ratio, aphid  $r_m$  was  
305 similar to that on non-treated plants.

306 On the treated (+N) hybrid *M. x giganteus*, aphids  $r_m$  was also similar to that on non-treated  
307 plants but their weight was greater. Nitrogen treatment did not affect the *M. x giganteus* leaf  
308 C:N ratio, contrary to both treated (+N) parents in which opposite effects were recorded  
309 (smaller C:N for *M. sacchariflorus* and greater C:N for *M. sinensis*). This could be the  
310 consequence of the hybrid status of *M. x giganteus*. Accordingly, the consequences of  
311 fertiliser use on *M. x giganteus* were less important on aphids than those on its parents. The  
312 changes of aphid feeding behaviour following N fertilising were minor.

313

314 The present study shows that aphids  $r_m$  and weight can be differently affected by the quality  
315 of their host-plants, as described in the theoretical model.<sup>37</sup> Indeed, according to this model,  
316 when the nutritional quality of the host is low, the herbivore will allocate or invest a greater  
317 amount of energy to its body weight than to the production of offspring, thus suggesting the  
318 existence of trade-offs. Indeed, in our study, the possible trade-offs between the parameters  
319 taken into account for  $r_m$  calculation and aphid growth depended on the host-plant: (1) on  
320 treated *M. x giganteus* and *M. sacchariflorus*, the host-plant quality was better, aphids  
321 weights were greater but their  $r_m$  unchanged; (2) on treated *M. sinensis*, the host-plant quality  
322 was lower, aphid  $r_m$  was smaller but the weight was unchanged.

323 In our study, we showed that nitrogen treatment could influence *Miscanthus* suitability for *R.*  
324 *maidis* aphids. When host-plants were not treated (-N), *R. maidis* aphids reared on the hybrid  
325 *M. x giganteus* showed smaller larval weights compared to those developing on *M. sinensis*,  
326 and smaller  $r_m$  compared to aphids on both parental species. These results appeared similar to  
327 the previous study,<sup>18</sup> demonstrating that *M. x giganteus* exhibited a stronger resistance to *R.*  
328 *padi* than *M. sinensis*, and that *M. sacchariflorus* was the most sensitive host-plant. Hence,  
329 both parents exhibited the best suitability for *R. maidis* regarding aphid weight and  $r_m$ .  
330 Following nitrogen input, *M. sacchariflorus* remained the most suitable host-plant. The other

331 parent, *M. sinensis* was less suitable for *R. maidis* compared to (i) *M. sacchariflorus* when  
332 considering aphids  $r_m$ , and (ii) both *M. sacchariflorus* and the cultivated hybrid  
333 *M. x giganteus* regarding aphid weight. This switch in suitability could be attributed to the  
334 difference in C:N ratio between *M. x giganteus* and *M. sinensis*, and thus in their nutritional  
335 quality for aphids in terms of primary and/or secondary metabolites.<sup>1,10</sup> Other explanatory  
336 factors, such as physical features, can be ruled out considering the absence of significant  
337 interspecific differences between non-treated (-N) and treated (N) host-plants in the following  
338 EPG parameters: time to first probe, time to first phloem phase, duration of pathways phase  
339 (data not shown).

340

341 Most studies have shown that nitrogen fertilisation improves crop yields and also leads to  
342 enhanced pest pressure<sup>2,8</sup>. However, the results of our study showed this was not the case for  
343 the cultivated species of *Miscanthus*. Indeed, in the case of *M. x giganteus* and in line with the  
344 sustainable agriculture practices, we confirmed the previous fields results<sup>12</sup> as nitrogen  
345 fertilisation did not impact plant productivity in terms of aboveground biomass. Our work  
346 also suggested that the suitability of *M. x giganteus* for *R. maidis* pests was not affected by  
347 nitrogen fertiliser. In the case of *M. sinensis* and in line with the intensive production  
348 practices, nitrogen fertilisation did improve plant productivity, but *M. sinensis* suitability for  
349 pests was unexpectedly lower.

350 Finally, as *Miscanthus* crops can act as host reservoirs for the *Barley yellow dwarf virus*,<sup>20,38</sup>  
351 our results underline the importance to consider both the selected host-plant species and the  
352 agricultural practices in terms of fertility programs as they can modulate the population  
353 dynamics of aphids vectors.

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355

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361

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507 **Tables**

508 **Table 1** Influence of nitrogen fertiliser treatment on biomass and quality of *M. sinensis* (a),  
 509 *M. sacchariflorus* (b) and *M. x giganteus* (c) host-plants.

a)			
<i>M. sinensis</i>			
Parameter	- N <sup>°</sup>	+ N <sup>†</sup>	Statistics‡
Aboveground biomass (g)	26.66 ± 3.23	48.85 ± 8.41	**
Leaf C:N ratio	14.71 ± 0.65	17.35 ± 0.44	**
Leaf nitrogen content (%)	3.23 ± 0.15	2.78 ± 0.06	*

b)			
<i>M. sacchariflorus</i>			
Parameter	- N <sup>°</sup>	+ N <sup>†</sup>	Statistics‡
Aboveground biomass (g)	2.32 ± 0.44	2.16 ± 0.52	NS
Leaf C:N ratio	18.72 ± 1.55	15.43 ± 0.91	*
Leaf nitrogen content (%)	2.46 ± 0.17	2.95 ± 0.12	*

c)			
<i>M. x giganteus</i>			
Parameter	- N <sup>°</sup>	+ N <sup>†</sup>	Statistics‡
Aboveground biomass (g)	23.95 ± 5.77	30.55 ± 6.65	NS
Leaf C:N ratio	15.60 ± 0.59	15.25 ± 0.36	NS
Leaf nitrogen content (%)	3.14 ± 0.12	3.19 ± 0.05	NS

512 Asterisks indicate statistically significant differences (Mann–Whitney *U* tests, \*  $P < 0.05$ ; \*\*  
 513  $P < 0.01$ , \*\*\*:  $P < 0.001$ )

514 ° Mean values ± SEM for plants without N solution (-N)

515 † Mean values ± SEM for plants with N solution (120 kgN/ha) (+N)

516

**Table 2:** Feeding behaviour parameters (mean  $\pm$  standard error of the mean) of *R. maidis*, developing on *M. sinensis*, *M. x giganteus* or *M. sacchariflorus* host-plants on depleted soil (-N) or treated with a nitrogenous fertiliser 120 kg.N.ha<sup>-1</sup> (+N).

		<i>M. sinensis</i> (-N)	<i>M. sinensis</i> (+N)		<i>M. x giganteus</i> (-N)	<i>M. x giganteus</i> (+N)		<i>M. sacchariflorus</i> (-N)	<i>M. sacchariflorus</i> (+N)	
Parameter		n=32	n=39		n=38	n=24		n=37	n=38	
Pr	s_Pr	305.44 $\pm$ 9.86	292.69 $\pm$ 10.16	NS	308.04 $\pm$ 6.83	308.21 $\pm$ 12.1	NS	302.53 $\pm$ 8.64	315.26 $\pm$ 7.12	NS
(min)	n	32	39		38	24		37	38	
C	s_C	122.72 $\pm$ 11.07	168.74 $\pm$ 10.57	**	140.83 $\pm$ 30.09	170.31 $\pm$ 15.94	NS	169.64 $\pm$ 10.31	177.46 $\pm$ 13.36	NS
(min)	n	32	39		38	24		37	38	
F	s_F	45.31 $\pm$ 9.86	63.89 $\pm$ 11.56	NS	85.72 $\pm$ 11.51	47.87 $\pm$ 10.84	NS	58.52 $\pm$ 12.24	35.73 $\pm$ 9.05	*
(min)	n	23	30		28	17		25	22	
G	s_G	137.79 $\pm$ 15.65	69.11 $\pm$ 8.37	***	97.63 $\pm$ 12.17	84.13 $\pm$ 17.07	NS	90.31 $\pm$ 14.9	89.15 $\pm$ 12.46	NS
(min)	n	31	36		36	20		30	35	
E1	s_E1	19.36 $\pm$ 4.5	14.69 $\pm$ 2.05	NS	13.32 $\pm$ 2.61	6.84 $\pm$ 1.41	*	5.61 $\pm$ 1.36	7.33 $\pm$ 1.39	NS
(min)	n	16	26		22	17		18	24	
E2	s_E2	64.27 $\pm$ 50.43	22.70 $\pm$ 13.71	NS	57.65 $\pm$ 41.35	87.14 $\pm$ 35.25	NS	107.33 $\pm$ 46.49	88.79 $\pm$ 28.69	NS
(min)	n	4	2		3	8		6	13	

517

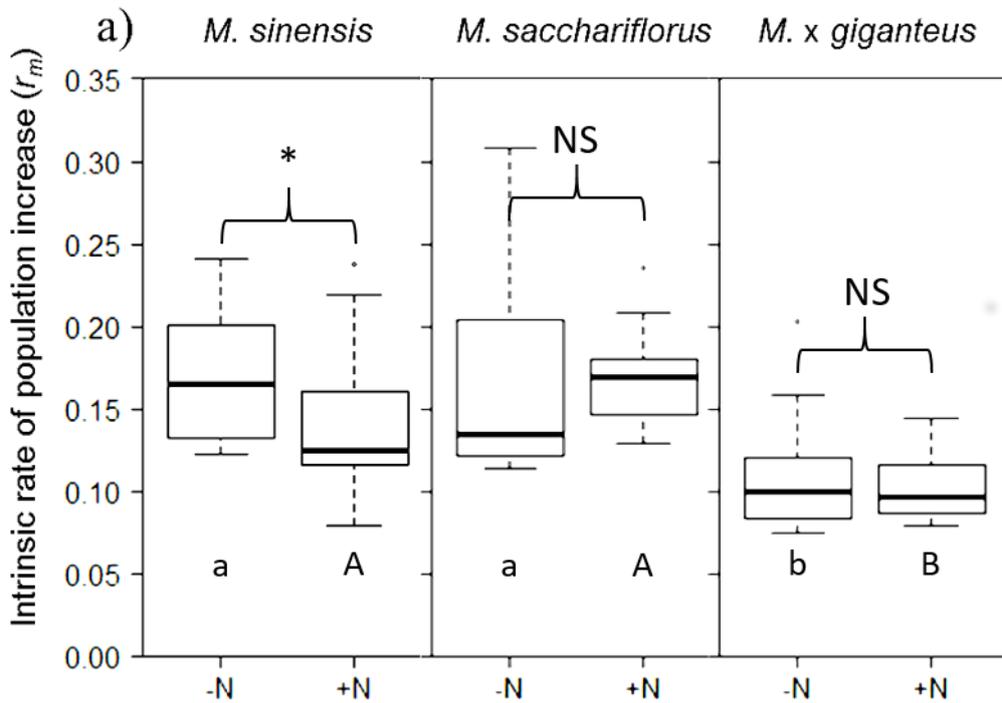
518 The asterisk \* indicates a significant difference between the two treatments (-N and +N) (Mann–Whitney *U* tests, \*  $P < 0.05$ ; \*\*  $P < 0.01$ , \*\*\*:  
519  $P < 0.001$ ). Pr: probing phase, C: stylet pathways in plant tissues except phloem and xylem, F: derailed stylet mechanics, G: active xylem sap  
520 ingestion, E1: salivation in phloem elements, E2: passive phloem sap ingestion.

521

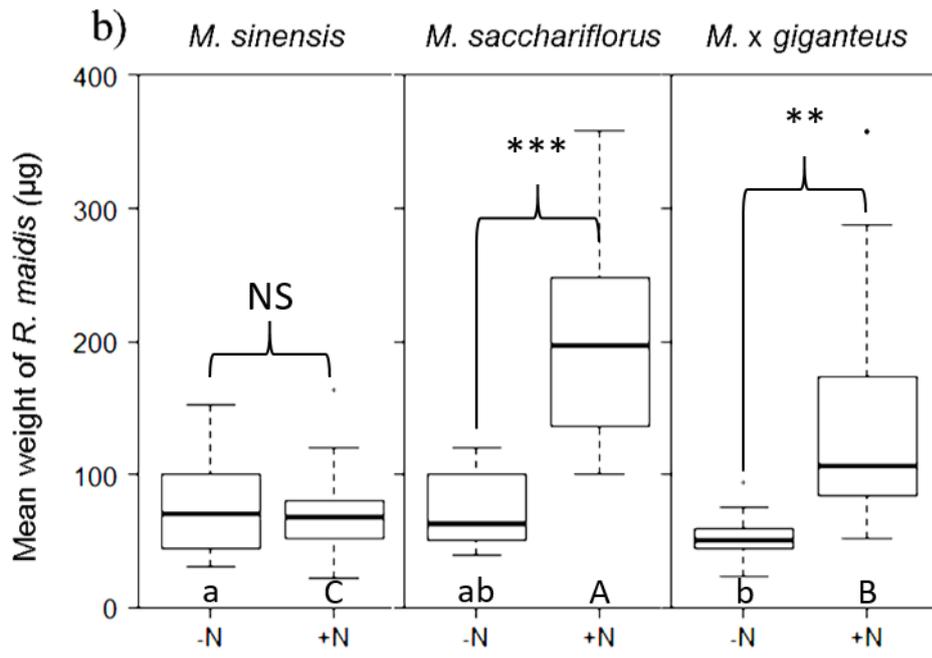
522 **Figures caption**

523

524 **Fig. 1** Performance parameters of *R. maidis* developing on three species of Miscanthus  
 525 without (-N) or with (+N) nitrogen input. Box-plots show median (*line*), 25-75 % percentiles  
 526 (*box*), 10-90 % percentiles (*whisker*) and outliers (*dots*). a) Intrinsic rate of population  
 527 increase ( $r_m$ ) of *R. maidis*. b) Weight of eight day-old *R. maidis* aphids. The asterisk \* indicates  
 528 a significant difference between the two treatments (-N and +N) for a plant host species and  
 529 letters indicate significant differences between plant species associated with 1smeans (lowercase  
 530 letters for non-treated plants, capital letters for treated plants) (\*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*:  
 531  $P<0.001$ ).  
 532



533



534