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Abstract

Miscanthus spp. (Poaceae) are large perennial C4-grasses that are receiving considerable attention as bioenergy crops. Therefore, the introduction of miscanthus crops in Europe needs continuous monitoring and risk assessment because they may serve as a refuge or a reservoir for aphid pests and/or pathogens of conventional crops. Here we first report the results of two field surveys conducted in northern France on the species composition of alate aphids flying above *Miscanthus* x *giganteus* crops. Then, in a first laboratory experiment, we investigated the colonization process on *M.* x *giganteus* of the four major aphid pests (Hemiptera: *Aphididae*) trapped in the field study. Results showed that the performances of these species in terms of feeding, survival and reproduction, on *M.* x *giganteus,* depended on their degree of specialization towards Poaceae. The suitability of this plant was moderate for the Poaceae specialist aphid *Rhopalosiphum padi* (L.), low for the polyphagous aphid species, *Aphis fabae* (Scop) and *Myzus persicae* (Sulzer) and very low for the Brassicaceae specialist aphid *Brevicoryne brassicae* (L.). Nevertheless, *M.* x *giganteus* cannot be considered as a reservoir crop for these common aphid pests as their progenies did not reach the adult stage. In a second laboratory experiment, the ability of the Poaceae specialist aphid *R. padi* to colonize *M.* x *giganteus* and its putative parents, *M. sinensis* and *M. sacchariflorus* was assessed. Results showed that *R. padi* was able to achieve its life cycle on *M. sacchariflorus* but not on *M. sinensis*. The consequences of the introduction of miscanthus in the north of France are discussed in terms of phytoviruses spreading and in terms of potential reservoir for aphid pests from conventional neighboring crops.

Keywords: *Miscanthus* x *giganteus*, Field survey, Host plant suitability, Aphididae, EPG, Demographic parameters, Phytoviruses, Plant resistance

1. Introduction

The use of perennial herbaceous energy crops dedicated to the production of biofuels in order to substitute fossil fuels is one way to reduce CO2 emissions (Smith et al., 2000). In this context, the countries of the European Union are committed to producing an increasing proportion of their energy needs from renewable resources (Ericsson et al., 2009; Ferreira et al., 2009; Lewandowski et al., 2006; Perry and Rosillo-Calle, 2008). Among all potential plants, *Miscanthus* x *giganteus*, the sterile hybrid between *Miscanthus sinensis* and *Miscanthus sacchariflorus*, has been extensively trialed as a biofuel in Europe since the early 1980s. Indeed, this promising candidate as a bioenergy crop is characterized by high biomass yields, even in cool northern European conditions (Beale and Long, 1995), a C4 photosynthetic pathway, a high tolerance to abiotic stresses, a perennial growth and a sustainable production (Heaton et al., 2004). Therefore, as planting miscanthus for energy production develops in Europe, an increased pathogen and pest pressure is likely to occur, and the risk of severe damage must be carefully examined by continuous monitoring and risk assessments. Miscanthus fields may indeed serve as a refuge or a reservoir for pests and/or diseases of conventional crops (Jørgensen, 2011). For example, it has been shown that *M.* x *giganteus* could be a suitable host for major maize pests such as the western corn rootworm *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) and the fall armyworm

Spodoptera frugiperda (Lepidoptera: Noctuidae) (Gloyna et al., 2011; Prasifka et al., 2009; Spencer and Raghu, 2009).

Among the agricultural pests, aphids are considered as the most serious ones, mainly because of the indirect damage they cause through the spread of phytoviruses. Most of these phytovirus vector species belong to the Aphidinae subfamily (Hemiptera: Aphididae), which includes the genus *Aphis*, *Myzus* and *Macrosiphum* (Eastop, 1983). Phytoviruses are divided into two main categories depending on their transmission mode. Non-persistent viruses are spread by transient or non-colonizing alate aphids that make brief intracellular probes ("potential drops") when moving from plant to plant, whereas persistent viruses are transmitted by colonizing aphids species and imply sustained feeding in the phloem (Hooks and Fereres, 2006).

Some studies have shown that miscanthus may act as a perennial reservoir of phytoviruses such as the barley yellow dwarf virus (BYDV) which can be transmitted in a persistent manner by *Rhopalosiphum maidis* (Christian et al., 1994; Huggett et al., 1999), the switchgrass mosaic virus (Agindotan et al., 2013) and the sorghum mosaic virus (Grisham et al., 2012).

During a field sampling study in *M.* x *giganteus* crops in the UK, Semere and Slater (2007) showed that Homoptera samples were dominated by Aphididae. However, in this broad scale biodiversity study, identification was not carried out up to the species level. In an extensive field survey set up in four different USA states, Bradshaw et al. (2010) recorded large populations of the yellow sugarcane aphid, *Sipha flava* which is only present in America (Blackman and Eastop, 2000) and the corn leaf aphid, *Rhopalosiphum maidis* which is virtually cosmopolitan but absent in regions with severe winter conditions such as northern Europe (Blackman and Eastop, 2000). The potential for the large-scale production of miscanthus has also led to its evaluation as a host through laboratory experiments for *S. flava*

(Pallipparambil et al., 2014) and for *R. maidis* (Huggett et al., 1999). For instance, Coulette et al. (2013) demonstrated that vitroplants of *Miscanthus sacchariflorus* were not suitable hosts for the black bean aphid *Aphis fabae*, the green peach aphid *Myzus persicae*, and the bird cherry aphid *Rhopalosiphum padi*. More recently, Pointeau et al. (2014) showed that *Miscanthus sacchariflorus* and, to a lesser extent, *M. sinensis* were less suitable and acceptable host plants for *R. maidis* than *M.* x *giganteus*.

In France, the first miscanthus crops were planted in 2006 and the surface area was essentially localized in the northern part of the country. The introduction of such crops in an agricultural landscape dominated by plants belonging to the Poaceae taxa (barley, wheat, maize) may create new refuges or reservoirs not only for monocot specialist aphids but also for polyphagous aphids associated with other main crops such as rapeseed, potato, legumes (typically beans and peas), sugar beet, turnip, carrots and lettuce.

In the present study conducted in northern France, we first carried out a field survey of alate aphids flying above *M.* x *giganteus* crops. We then made the following assumptions: (1) the ability of the most abundant aphid pests trapped in the field to successfully feed and reproduce on *M.* x *giganteus* would depend on their degree of specialization towards Poaceae and (2) within the *Miscanthus* genus, there are different levels of resistance which can modulate the performances of Poaceae aphid specialists. We tested these hypotheses through laboratory bioassays. In a first one, we investigated the colonization process on *M.* x *giganteus* of four major aphid pests (Hemiptera: *Aphididae*) trapped in the field study: the two polyphagous species *Aphis fabae* (black bean aphid) and *Myzus persicae* (green peach aphid), the Brassicaceae specialist *Brevicoryne brassicae* (cabbage aphid) and the Poaceae specialist *Rhopalosiphum padi* (bird cherry aphid). In a second laboratory bioassay, we investigated the colonization process of *R. padi* (i.e. the aphid species that performed the best

in the first laboratory experiment) on the three *Miscanthus* species studied in Europe for biomass production, i.e., *M.* x *giganteus, M. sinensis* and *M. sacchariflorus*.

2. Materials and methods

2.1. Field studies

The experiments were conducted in *M.* x *giganteus* fields located on three different sites in Picardy, northern France: two fields of 25 ha in Bougainville (49°51'18"N, 2°01'29"E and 49°51'21"N, 2°01'44"E) and one of 8 ha in Dreuil-les-Molliens (49°54'03"N, 2°02'23"E). Fields in Bougainville were planted with *M.* x *giganteus* in 2008 and the one in Dreuil-les-Molliens was planted in 2007. The aphid survey was conducted a first time in 2011 from May 2nd to July 29th and a second time in 2014 from May 7th to July 2nd. The field study started immediately after crop harvesting and when it was stopped ca. two months later, the plants had grown ca. 150 cm high. In each site, a yellow water trap used to catch different species of alate aphids was placed on a pole just above the plant canopy and 50 meters away from the border (Marame et al., 2010). Traps were checked every week and the insects caught were kept in small plastic containers with 70 % ethanol until identification under a stereomicroscope (Leica M165C).

All aphids trapped were identified at species level and only those considered as the main pests in Picardy (FREDON PICARDIE Pest Monitoring Network) were taken into account for this study. Eleven species were thus numbered: the green peach aphid *Myzus persicae* (Sulzer), the black bean aphid *Aphis fabae* Scopoli, the cabbage aphid *Brevicoryne brassicae* (L.), the willow-carrot aphid *Cavariella aegopodii* Scopoli, the pea aphid *Acyrthosiphon pisum* (Harris), the birdcherry-oat aphid *Rhopalosiphum padi* (L.), the blackcurrant-sowthistle aphid *Hyperomyzus lactucae* (L.), the grain aphid *Sitobion avenae* (F.), the potato aphid *Macrosiphum euphorbiae* (Thomas), the lettuce aphid *Nasonovia ribisnigri* (Mosley) and the willow-carrot aphid *Cavariella theobaldi* (Gillette & Bragg).

2.2. Insects and Plants for laboratory experiments

The *M. persicae* colony was established from one female collected in 1999 in a potato field near Loos-en-Gohelle (France) and was reared on turnip plants (*Brassica rapa* cv. *"*purple top white globe*"*). Both the colonies of *R. padi* and *B. brassicae* were provided in 2008 by INRA-Le Rheu (Rennes, France) and they were reared on barley (*Hordeum vulgare* cv. *"*Cervoise*"*) and rapeseed (*Brassica napus* cv. *"*Stego*"*) respectively. The colony of *A. fabae*, provided in 2012 by Gembloux Agro-Bio-Tech (Belgium) was reared on broad beans (*Vicia faba* cv. *"*Maya*"*).

For each aphid species, colonies were initiated from a single apterous parthenogenetic female and maintained on their respective host plant in a ventilated Plexiglas® cage in different growth chambers under 20 ± 1°C, 60 ± 5 % R.H., and 16:8 (L:D) photoperiod to induce parthenogenesis.

Plantlets of the three *Miscanthus* species, i.e., *M.* x *giganteus* (cv. *"*GigB*"*, 2n = 3x = 57), *M. sacchariflorus* (cv. *"*Sac*"*, 2n = 2x = 38) and *M. sinensis* (cv. *"*Goliath*"*, 2n = 4x = 76) (Zub et al., 2012) were obtained by *in vitro* multiplication as described by Rambaud et al. (2013). Single rooted shoots coming from clusters, rooting in perlite, were potted in plastic pots (firstly 9 x 9 x 10 cm, then 16 x 13 cm and 20 x 15 cm) containing potting soil in a growth chamber under 20 ± 2°C, 60 ± 5 % R.H, and a 16:8 (L:D) photoperiod. Plants used in the experiment were 8 to 12 weeks old (after potting) and 60 to 80 cm high.

2.3. Feeding behavior studies

The Electrical Penetration Graph DC-system described by Tjallingii (1978, 1988) was used to investigate the feeding behaviour of alate aphids on *Miscanthus* spp. In a first bioassay, the feeding behaviour of *M. persicae*, *R. padi*, *B. brassicae* and *A. fabae* was investigated on

166 *M.* x *giganteus* and in a second bioassay, the feeding behaviour of *R. padi* was investigated on
167 the three *Miscanthus* species.

168 To insert one aphid and one plant into an electrical circuit, a thin gold wire (20 µm diameter
169 and 2 cm long) was stuck on the insect's dorsum by conductive silver glue (EPG systems,
170 Wageningen, The Netherlands). Eight aphids were then connected to the Giga-8 DC-EPG
171 amplifier and each one was placed on a plantlet leaf of a different plant. A second electrode
172 was inserted into the soil of each of the potted plants to complete the electrical circuits. The
173 recordings were performed continuously for 8 hours during the day. Alate aphids in their
174 dispersal phase were collected on the inner wall of the rearing cages. Owing to their variable
175 propensity to fly or probe they were standardised in a Plexiglas® chamber (305 mm high,
176 152 mm diameter) as described by Brunissen et al. (2009). The whole aphid-plant system was
177 placed inside a Faraday cage at 20 ± 1°C. Acquisition and analysis of the EPG waveforms
178 were carried out with PROBE 3.5 software (EPG Systems, www.epgsystems.eu). Parameters
179 from the recorded EPG waveforms were calculated with EPG-Calc 6.1 software
180 (Giordanengo, 2014). These parameters were based on different EPG waveforms described by
181 Tjallingii and Hogen Esch (1993) corresponding to: (C) stylet pathways in plant tissues
182 except phloem and xylem; (pd) potential drops (intracellular stylet punctures); (E1) salivation
183 in phloem elements; (E2) passive phloem sap ingestion; (E1+E2) activity within phloem
184 vessels, (G) active xylem sap ingestion; and (F) derailed stylet mechanics. For the study
185 related to the feeding behavior of the four aphid species on *M.* x *giganteus*, 20 to 24
186 individuals were tested and for the study relating to the feeding behavior of *R. padi* on the
187 three *Miscanthus* species, 19 to 23 individuals were tested.

188

189 **2.4. Survival and reproductive traits bioassays**

In a first bioassay, the performances of *M. persicae*, *R. padi*, *B. brassicae* and *A. fabae* were investigated on *M.* x *giganteus* and in a second bioassay the performances of *R. padi* were investigated on the three *Miscanthus* species.

Pools of synchronized first instar nymphs (less than 24-hour old) of each aphid species were obtained from parthenogenetic adult females placed on leaves of their host plant set in 1.5 % agar in Petri dishes (90 mm diameter). To obtain synchronized young adults, first instar nymphs were further kept in the same device for six to eight days, depending on the aphid species.

For the nymph survival study, groups of five first instar nymphs were transferred onto the plantlets to be tested. These groups of aphid nymphs were enclosed in clip cages on leaves at mid-height of each plantlet and their survival was recorded every two days. For each of the two bioassays, six to ten replicates were performed.

For the adult performance study, young adults were individually transferred onto the plantlets to be tested. Survival and fecundity were assessed every day until the female died. For each of the two bioassays, 25 to 40 replicates were performed.

2.5. Statistical analysis

Because the homoscedasticity of all distributions was not confirmed, non-parametric tests were used. EPG parameters and demographic parameters were compared between aphid species for the first bioassay and between plants for the second bioassay by using a Kruskal-Wallis one-way analysis of variance (*H* value). Post-hoc multiple comparisons were carried out with the non-parametric pairwise Mann-Whitney *U* test. The false discovery rate (FDR)

approach (Benjamini and Hochberg, 2009) was used to control the family-wise error rate. All statistics were performed using R (R Development Core Team 2014).

3. Results

3.1. Field study

A total of 2436 alate aphids belonging to 50 different species were trapped in 2011 and 2014. The 11 focal species represented 65 % of the identified individuals in the 2011 campaign and 80 % of the identified individuals in the 2014 campaign (Table 1). The two most abundant species were the two polyphagous species *M. persicae* and *A. fabae* (54 % of the captures). Conversely, the polyphagous species *M. euphorbiae* was hardly ever captured. *B. brassicae* which feeds on a wide range of Brassicaceae plants was abundantly captured in 2011. The Asteraceae specialists (*Hyperomyzus lactucae* and *Nasonovia ribisnigri*) represented less than 3 % of the captures. Finally, the Fabaceae specialist *Acyrtosiphum pisum*, the Apiaceae specialist (*Cavariella* sp.), the Poaceae specialists (*Rhopalosiphum padi* and *Sitobion avenae),* represented respectively less than 2 % of the captures.

These field results led us to evaluate through laboratory bioassay, the ability of miscanthus colonization by the three main trapped aphid species (*Myzus persicae, Aphis fabae*, *Brevicoryne brassicae*). Even if *R. padi* was not frequently trapped during these two field surveys, it is probably the most important cereal pest and can be hosted by a large number species belonging to more than 30 genera of Poaceae including maize, sorghum, barley (Blackman and Eastop, 2000). Therefore, it was also chosen for subsequent laboratory bioassays.

3.2. Bioassay 1: feeding behavior and performance of the four aphid species on *M.* x *giganteus*

3.2.1 Electrical penetration graph studies

There was a significant effect of the aphid species for the following parameters (Table 2): number of probes ($H = 16.06$; $P < 0.01$), total duration of probing ($H = 30.4$; $P < 0.001$), number and total duration of pathway phases ($H = 15.92$; $P < 0.01$ & $H = 19.11$; $P < 0.001$), number of potential drops ($H = 21.26$; $P < 0.001$), time of phloem phase ($H = 13.23$; $P < 0.01$), total duration of phloem phase ($H = 20.41$; $P < 0.001$), total duration of xylem ingestion ($H = 15.52$; $P < 0.05$).

The number of probes was significantly lower for *R. padi* compared to *A. fabae* and *M. persicae* (Mann-Whitney *U* test, $P < 0.05$) but not compared to *B. brassicae*. The stylet activities within plant tissues (over the 8-hour recording) ranged from 42 % for *B. brassicae* to 74 % for *R. padi*. The Poaceae specialist *R. padi* exhibited the longest duration of probing and *B. brassicae*, the shortest one, whereas the two polyphagous species exhibited intermediate durations of total probing.

The number of pathway phases was significantly higher for *M. persicae* (Mann-Whitney *U* test, $P < 0.05$). The total duration of this phase was significantly longer for the cereal aphid *R. padi* than for *B. brassicae* and *M. persicae* (Mann-Whitney *U* test, $P < 0.05$). *R. padi*, performed twice as many potential drops as *B. brassicae* (Mann-Whitney *U* test, $P < 0.05$) and the other two aphid species presented intermediate values (Mann-Whitney *U* test, $P < 0.05$).

As for the phloem phase parameters, *R. padi and M. persicae* took significantly less time to access phloem vessels than *A. fabae* (Mann-Whitney *U* test, $P < 0.05$). The total duration of the activity within phloem vessels (E1+E2) was weak for all aphids (less than 6 % of the 8-hour recording). Nevertheless, this phase was significantly longer for *M. persicae* and *R. padi* than for *B. brassicae* and *A. fabae* (Mann-Whitney *U* test, $P < 0.05$). All the species exhibited phloem sap ingestion but the total duration of this phase, which was not significantly different between species ($H = 3.71$; $P > 0.05$), was trivial and represented less than 4 % of the 8-hour recording for all species.

Finally, the total duration of xylem sap ingestion (G) performed by *B. brassicae* was significantly shorter than when it was performed by *A. fabae* and *R. padi* (Mann-Whitney *U* test, $P < 0.05$), but not significantly so when it was performed by *M. Persicae*. The total duration of stylet derailment phase (F) was not significantly different between aphid species ($H = 7.40$; $P > 0.05$).

3.2.2. Aphid performance on *M.* x *giganteus*

Kruskal-Wallis statistical analysis showed an aphid species effect on all parameters presented in Table 3: adult survival ($H = 25.73$; $P < 0.05$), fecundity ($H = 71.75$; $P < 0.05$), nymph survival ($H = 26.52$; $P < 0.05$). Inter-specific pairwise comparisons showed that the adult survival was significantly shorter for *B. brassicae* (ca. 50 %) compared to all other species of aphid (Mann-Whitney *U* test, $P < 0.05$). Fecundity was significantly higher in *R. padi* (Mann-Whitney *U* test, $P < 0.05$) than in the other species. Concerning the nymph performance study, none of the individuals reached the adult stage. In addition, the nymph survival was longer for *R. padi* in comparison to *B. brassicae* and *M. persicae* (Mann-Whitney *U* test, $P < 0.05$).

3.3. Bioassay 2: feeding behavior and performance of *R. padi* on the three *Miscanthus* species

3.3.1 Electrical penetration graph studies

There was a significant effect of the plant species on the total duration of probing ($H = 13.97$; $P < 0.001$) and the total duration of phloem sap ingestion ($H = 8.58$; $P < 0.05$) (Fig 1). The total duration of probing was significantly shorter on *M. sinensis* in comparison to the two other plant species (Mann-Whitney *U* test, $P < 0.05$). Indeed, *R. padi* spent 60 % of the recorded time in plant tissue (versus at least 69 % for the two other plant species). The total duration of phloem sap ingestion was more than four times higher on *M. sacchariflorus* (16.5 % of the time over the 8 h duration of recording) than on *M.* x *giganteus* (3.7 % of the time over the 8 h recording) (Mann-Whitney *U* test, $P < 0.05$).

3.3.2 *R. padi* performances on the three *Miscanthus* species

Concerning the aphid performance study presented in Table 4, Kruskal-Wallis statistical analysis showed a plant species effect on *R. padi* adult survival ($H = 41.20$; $P < 0.001$), fecundity ($H = 20.11$; $P < 0.05$) and nymph survival ($H = 12.55$; $P < 0.01$). When aphids were reared on *M. sacchariflorus*, nymphs survived twice longer than when they were reared on the two other plant species (Mann-Whitney *U* test, $P < 0.05$), but only 3 nymphs out of the 31 individuals tested reached the adult stage. Similarly, adult survival was significantly longer on *M. sacchariflorus*. The fecundity was negatively affected for aphids reared on *M. sinensis* (Mann-Whitney *U* test, $P < 0.05$).

4. Discussion

Our study demonstrated that none of the four aphid species considered as the main crop pests in Northern France (FREDON Picardie Pest Monitoring Network), which were also abundantly trapped M. x *giganteus fields*, were able to achieve their life cycle on this plant because their progeny did not reach the adult stage. Even if *M.* x *giganteus* did not represent a reservoir for these common aphid pests of northern France, some differences in the suitability of this plant appeared regarding to the aphid degree of specialization towards Poaceae. The Poaceae specialist aphid *R. padi*, which performed better on *M.* x *giganteus* than the other aphid species, was able to achieve its life cycle on *M. sacchariflorus*.

4.1. *M.* x *giganteus* colonization ability by the four main aphid pest species

Host plant selection by alate aphids is achieved through a sequence of several steps defined by Niemeyer (1990) and Powell et al. (2006) : (1) pre-alighting behaviour, (2) landing, (3) probing the epidermis, (4) stylet pathways activity in the mesophyll, (5) sieve element puncture and salivation, (6) phloem acceptance and sustained sap ingestion and finally (7) survival and reproduction. Our field study showed that the trapped aphid species were the ones that are most frequently found in the main crops of the northern France and this, regardless of their potential relationship with Poaceae. Indeed, aphids have little control over the direction of their flight and the pre-alighting step appears to have negligible effect on the host-plant selection (Dixon, 1998). Aphids do not exhibit clear discrimination between host and non-host plants before they have landed and inserted their stylets (Kennedy and Booth, 1961; Kennedy et al., 1959a, 1959b). The two main trapped species were two polyphagous species, *Myzus persicae* and *Aphis fabae*. These species are considered to be the main pests of northern France crops as their host plant spectrum comprises a wide range of plant species belonging to a large number of plant families including Poaceae (Blackman and Eastop,

2000). Surprisingly, the Brassicaceae specialist *Brevicoryne brassicae* was extensively trapped in 2011 whereas the two cereal specialists *Rhopalosiphum padi* and *Sitobion avenae* were much less frequently trapped.

The analysis of EPG parameters such as frequency, duration, and sequence of different waveforms, are considered as valuable indicators for defining plant suitability or probing interference by chemical and/or physical factors in plant tissues (Mayoral et al., 1996). In the present EPG study, the total duration of pathway phases, the total duration of activity within phloem vessels and more generally the total activity of the recorded time in plant tissues were the highest for the Poaceae specialist *R. padi*, the lowest for the Brassicaceae specialist *B. brassicae* and intermediate for the two polyphagous species. Aphids usually have a low number of probes when feeding on suitable hosts (Cole, 1997). This was the case for *R. padi* on *M.* x *giganteus* in our study. Moreover, the time to reach phloem vessels was the shortest for *R. padi* and the longest for *B. brassicae*. Therefore, as expected, the suitability of *M.* x *giganteus* varied according to the degree of specialization towards Poaceae of each aphid species. And indeed, the Brassicaceae specialist cabbage aphid, *Brevicoryne brassicae*, usually feeds on plants that accumulate glucosinolates, which stimulate its feeding and oviposition (Ahuja et al., 2010; Wittstock et al., 2004). The lack of such secondary metabolites in miscanthus plants could also explain its weak performances, i.e. feeding behavior, survival and reproduction, of *B. brassicae* compared to the other aphid species. Conversely, *R. padi* was the aphid species that performed the best on *M.* x *giganteus*, although it performed less well than it did on barley, its conventional host plant (Chesnais et al., 2014; Schliephake et al., 2013). The relatively weak performances of *R. padi* on *M.* x *giganteus* could result from the different photosynthetic pathways occurring in its common C3 host plant and in the C4 miscanthus. Indeed, Weibull (1990) demonstrated a preference of *R. padi* for grasses with a C3-metabolic pathway. He hypothesized that *R. padi*,

353 having evolved in the Palaearctic region, has not had yet the adequate opportunity to adapt to
354 C4-grasses that grow mainly in warmer regions. The polyphagous species *Aphis fabae* and
355 *Myzus persicae* exhibited intermediate performances in comparison to the two specialist aphid
356 species. This is in accordance with Tosh et al. (2003) who found that, during the host plant
357 selection phases 4, 5 and 6 (see above, Powell et al. (2006)), aphid specialists reject more
358 easily and efficiently non-host plants than generalists do.

359 Chesnais et al. (2014) evaluated the intrinsic rate of increase of the same four aphid species as
360 the ones tested in this study and it clearly appeared that *M.* x *giganteus* was less suitable for
361 each aphid species than their respective rearing host plants. Accordingly, their feeding
362 behaviour was also drastically affected on *M.* x *giganteus* in comparison to what is reported in
363 the literature when they feed on their host plant (Boquel et al., 2012; Gabrys et al., 1997;
364 Powell and Hardie, 2001; Slesak et al., 2001). The unsuitability of *M.* x *giganteus* is also
365 supported by the presence of stress indicators such as a high xylem sap consumption and the
366 occurrence of stylet derailments (Prado and Tjallingii, 1997; Sauge et al., 2002).

367

368 **4.2. Suitability of the three *Miscanthus* species to *R. padi***

369 Two main basic modalities of plant resistance to insects have been defined by Panda and
370 Khush (1995) and Painter (1951): Antixenosis affects insect behavior by deterring or reducing
371 the colonization process whereas antibiosis affects insect life history traits (survival,
372 development, fecundity). Our study revealed that *M.* x *giganteus* and *M. sinensis* exhibited a
373 higher resistance level to *R. padi* than *M. sacchariflorus* thanks to a combination of both
374 resistance mechanisms.

375 Indeed, on a susceptible host plant such as *Hordeum vulgare*, the high population rate of
376 increase of *R. padi* was associated with a high duration of phloem feeding (58 % of the

12 hour recording) (Schliephake et al., 2013). Compared to such data obtained on susceptible plants, our study suggested the occurrence of an antixenosis resistance mechanism through a drastic reduction of phloem sap ingestion of *R. padi* on *M. sinensis* and *M.* x *giganteus*, and to a lesser extent on *M. sacchariflorus*, (9.3, 3.7 and 16.5 % of the 8-hour recording respectively). This data was consistent with the demographic performance results which showed a high antibiosis resistance mechanism when *R. padi* was reared on *M.* x *giganteus* and *M. sinensis.* Accordingly, Huggett et al. (1999) demonstrated that *R. padi* was unable to reproduce and to exhibit prolonged feeding on *M. sinensis.* In our study, *M. sacchariflorus* was a more susceptible host for *R. padi* than *M.* x *giganteus* and *M. sinensis.* However, the suitability of *M. sacchariflorus* to *R. padi* remained moderate as only three *R. padi* nymphs out of the 31 individuals tested reached the adult stage. In contrast, Pointeau et al. (2014) demonstrated that *Rhopalosiphum maidis* was able to develop and reproduce and exhibited long phases of phloem sap ingestion on the three Miscanthus species (23 % to 40 % of the time over the duration of probing depending on the *Miscanthus* species). This could be explained by the fact that, contrary to *R. padi*, the corn leaf aphid *R. maidis* is also adapted to feed on C4-plants (Blackman and Eastop, 2000).

4.3. Epidemiologic and agronomic implications

The inability of *A. fabae, M. persicae, B. brassicae* and even *R. padi* to produce nymphs that could reach the adult stage on *M.* x *giganteus* does not allow conferring to this plant the status of reservoir as defined by Spencer and Raghu (2009). *M.* x *giganteus* has therefore to be considered as a "transitional plant" that would allow the aphids to survive through the consumption of xylem sap and low quantities of phloem sap. The existence of such refuge

401 perennial plants is crucial for aphid pests that use annual plant crops that are harvested in
402 summer. Moreover, as the four aphid species studied cannot complete their life cycle on
403 *M.* x *giganteus,* they can be considered as non-colonizing or transient species (Fereres and
404 Moreno, 2009; Irwin et al., 2007). In the context of plant virus spreading, transient aphid
405 species alighting on non-host plants are known to transmit non-persistent viruses before
406 taking off in search of a suitable host plant (Boquel et al., 2012; Gray et al., 2010; Radcliffe
407 and Ragsdale, 2002). Our EPG study clearly showed that the four aphid species performed the
408 brief intracellular punctures (potential drop waveforms) which are directly involved in the
409 transmission of non-persistent viruses (Martín et al., 1997). To our knowledge, only the work
410 by Grisham et al. (2012) reported that *M. sinensis* could be infected by the non persistent
411 Sorghum mosaic virus (SrMV) which is not present in Europe.

412 The observation of sustained phloem ingestion phase, particularly for *R. padi*, also makes the
413 transmission of persistent viruses theoretically possible (Martín et al., 1997). Christian et al.
414 (1994) and Huggett et al. (1999) demonstrated that *M. sinensis* could be susceptible to some
415 persistent viruses such as the barley yellow dwarf virus (BYDV), and could therefore be a
416 perennial reservoir of phytoviruses. However, another study by Drechsler et al. (2014) shows
417 that different *M. x giganteus* cultivars were resistant to the persistent Maize streak
418 virus (MSV).
419

420 Thus, the introduction of miscanthus in the north of France could not only have an effect on
421 the spread of phytoviruses related to Poaceae but also on those related to the other
422 conventional crops. Indeed, *Miscanthus* spp. could constitute a tall barrier which may reduce
423 potential virus dissemination by aphid vectors when aphid vectors migrate between crops.
424 Hooks and Fereres (2006) propose that barrier plants may act as a sink for non-persistent
425 viruses. After landing on the barrier crop, the viruliferous aphid loses its virus “charge” by

making a few brief probes on the plant. Consequently, a virus-free aphid entering an area with susceptible primary crops will no longer be able to transmit a viral disease. For example, Fereres (2000) studied the use of sorghum and maize as barrier crops to protect pepper plants against the potato virus Y (PVY) and the cucumber mosaic virus (CMV). He concluded that these tall barrier plants did not reduce the number of vectors entering in pepper habitats but protected the pepper plant by acting as a natural sink for non-persistent viruses. In Picardy, the agricultural landscape being mainly dominated not only by cereal crops but also by crops such as rapeseed, potato, legumes (typically beans and peas), sugar beet, turnips, carrots and lettuce, attention should be paid to where miscanthus is being planted. Miscanthus could be used as a virus sink to prevent the dissemination of non persistent viruses of Brassicaceae (e.g., *Cauliflower mosaic virus*, CaMV, the Turnip yellow mosaic virus, TuMV), Solanaceae (*Potato virus Y* PVY) and Cucurbitaceae (*Cucumber mosaic virus*, CMV). However, it should not be planted as a barrier crop nearby arable crops such as wheat or maize, as in this case it could possibly act as a reservoir for the BYDV.

5. Conclusion

Despite the probable modification of the equilibrium of local agrosystems, the introduction of miscanthus in northern France may not contribute to creating a new reservoir for aphid pests issued from susceptible crops. Moreover, its possible role as a barrier crop could also limit aphid movements between crops, reducing the risk of virus spreading. Our work also demonstrates that, the most interesting species for biomass production, *M.* x *giganteus*, is also the species which exhibited the highest level of resistance towards *R. padi* in comparison to its parents *M. sinensis* and *M. sacchariflorus*.

In the future, field surveys should continue to be used to predict aphid pest problems before they develop in the miscanthus crop. Indeed, our study did not take into account the genetic

variability of *R. padi*. Within the same geographic location, Lushai et al. (2002) revealed two genetic profiles of the grain aphid, *Sitobion avenae* (Fabricius) that exhibited different levels of specialization towards different grasses and cereals. Therefore, some other natural populations of *R. padi* may be adapted to feed and reproduce on *Miscanthus* spp. Otherwise, even if *R. maidis* populations are negligible in the northern France, their abundance is likely to increase in cooler regions in response to climate warming (Harrington, 2007), which could in turn enhance its pest status of Poaceae such as *Miscanthus* spp.

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605 **Table 1** - Species and total number of alate aphids from yellow water traps placed in three
606 *Miscanthus* x *giganteus* fields of northern France in 2011 (from May 2nd to July 29th) and in
607 2014 (from May 7th to July 2nd). Among the 50 species identified, the eleven most abundant
608 pest species were numbered.

Species	Common name	Total collected in 2011	Total collected in 2014	Total collected in 2011 and 2014	Percentage collected in 2011 and 2014
Myzus persicae	Green peach aphid	175	845	1020	41.87
Aphis fabae	Black bean aphid	239	72	311	12.77
Brevicoryne brassicae	Cabbage aphid	231	3	234	9.61
Cavariella aegopodii	Willow-carrot aphid	53	15	68	2.79
Acyrthosiphon pisum	Pea aphid	20	25	45	1.85
Rhopalosiphum padi	Birdcherry-oat aphids	25	1	26	1.07
Hyperomyzus lactucae	Blackcurrant-sowthistle aphid	24	19	43	1.77
Sitobion avenae	Grain aphid	16	1	17	0.70
Macrosiphum euphorbiae	Potato aphid	4	7	11	0.45
Nasonovia ribisnigri	Lettuce aphid	0	2	2	0.08
Cavariella theobaldi	Willow - carrot aphid	4	1	5	0.21
Others		414	240	654	26.85
Total		1205	1231	2436	100.00

609

610 **Table 2 -** Electrical penetration graph parameters (means ± SEM) calculated for four aphid species during an 8-h monitoring session on

611 *Miscanthus* x *giganteus* plants.

	Kruskal-Wallis test	*A. fabae*		*B. brassicae*		*M. persicae*		*R. padi*	
EPG classes	H(*P*)	n = 22		n = 20		n = 24		n = 23	
General probing behaviour									
1. Number of probes	16.06 (**)	22.70 ± 2.20	a	21.50 ± 3.10	ab	25.80 ± 2.00	a	14.70 ± 1.20	b
2. Total duration of probing (min)	30.4 (***)	295.10 ± 14.58	b	202.33 ± 17.90	c	314.85 ± 16.82	ab	357.28 ± 12.34	a
Pathway phase									
3. Number of pathway phases	15.92 (**)	28.20 ± 2.10	b	28.00 ± 3.60	b	44.40 ± 4.00	a	29.72 ± 1.50	b
4. Total duration of pathway phases	19.11 (***)	185.01 ± 12.78	ab	137.79 ± 13.49	c	161.49 ± 9.75	bc	219.12 ± 11.52	a
5. Mean number of potential drops (pd)	21.26 (***)	110.27 ± 9.2	b	70.16 ± 9.97	c	119.25 ± 10.68	b	146.52 ± 8.55	a
Phloem phase									
6. Time of first phloem phases (min)	13.23 (***)	279.71 ± 33.44	a	186.58 ± 43.71	ab	151.76 ± 34.88	b	101.64 ± 9.41	b
7. Total duration of phloem phases (salivation E1+ ingestion E2) (min)	20.41 (***)	3.81 ± 0.99	c	8.11 ± 2.96	bc	29.21 ± 9.54	ab	21.27 ± 7.55	a
8. Total duration phloem sap ingestion (E2) (min)	3.71 (NS)	1.60 ± 0.44		3.96 ± 1.80		9.52 ± 3.35		17.83 ± 7.45	
Other parameters									
9. Total duration of xylem ingestion (G) (min)	15.52 (*)	100.41 ± 11.93	a	58.13 ± 11.98	b	78.62 ± 9.28	ab	90.79 ± 6.52	a
10. Total duration of stylet derailment (F) (min)	7.40 (NS)	9.13 ± 4.63		14.07 ± 8.21		54.64 ± 12.53		40.88 ± 16.72	

612 Asterisks indicate a significant difference*:* * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ associated with H (Kruskal-Wallis test); within a row,

613 different letters indicate significant differences (pairwise comparisons using Mann-Whitney U test).

614

615

Table 3 - Mean (± SEM) population parameter values of four aphid species reared on *Miscanthus* x *giganteus*.

		Kruskal-Wallis test	*A. fabae*		*B. brassicae*		*M. persicae*		*R. padi*	
Adults		H(*P*)	n = 34		n = 38		n = 40		n = 31	
	Survival (days)	25.73 (***)	4.49 ± 0.25	a	2.58 ± 0.23	b	4.44 ± 0.36	a	4.18 ± 0.26	a
	Fecundity	71.75 (***)	1.39 ± 0.30	b	0.04 ± 0.04	c	0.56 ± 0.24	bc	6.75 ± 0.68	a
Nymphs			n = 38		n = 58		n = 24		n = 32	
	Survival (days)	26.52 (***)	2.16 ± 0.09	ab	2	b	2	b	2.69 ± 0.17	a

Asterisks indicate a significant difference*:* * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ associated with H (Kruskal-Wallis test); within a row, different letters indicate significant differences (pairwise comparisons using Mann-Whitney U test).

628

629 **Table 4 -** Mean (± SEM) performance parameter values of *Rhopalosiphum padi* on the three miscanthus species (*Miscanthus* x *giganteus*,

630 *Miscanthus sacchariflorus* and *Miscanthus sinensis*).

	Kruskal-Wallis test	*Miscanthus* x *giganteus*		*Miscanthus sacchariflorus*		*Miscanthus sinensis*	
Adults	H(*P*)	n = 28		n = 25		n = 27	
Survival (days)	41.20 (***)	4.18 ± 0.26	b	9.28 ± 0.76	a	4.04 ± 0.27	b
Fecundity	20.11 (***)	6.75 ± 0.68	a	8.88 ± 0.85	a	3.67 ± 0.65	b
Nymphs		n = 32		n = 31		n = 25	
Survival (days)	12.55 (**)	2.69 ± 0.17	b	4.71 ± 0.7	a	2.4 ± 0.16	b

631 Asterisks indicate a significant difference*:* * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ associated with H (Kruskal-Wallis test); within a row,

632 different letters indicate significant differences (pairwise comparisons using Mann-Whitney U test).

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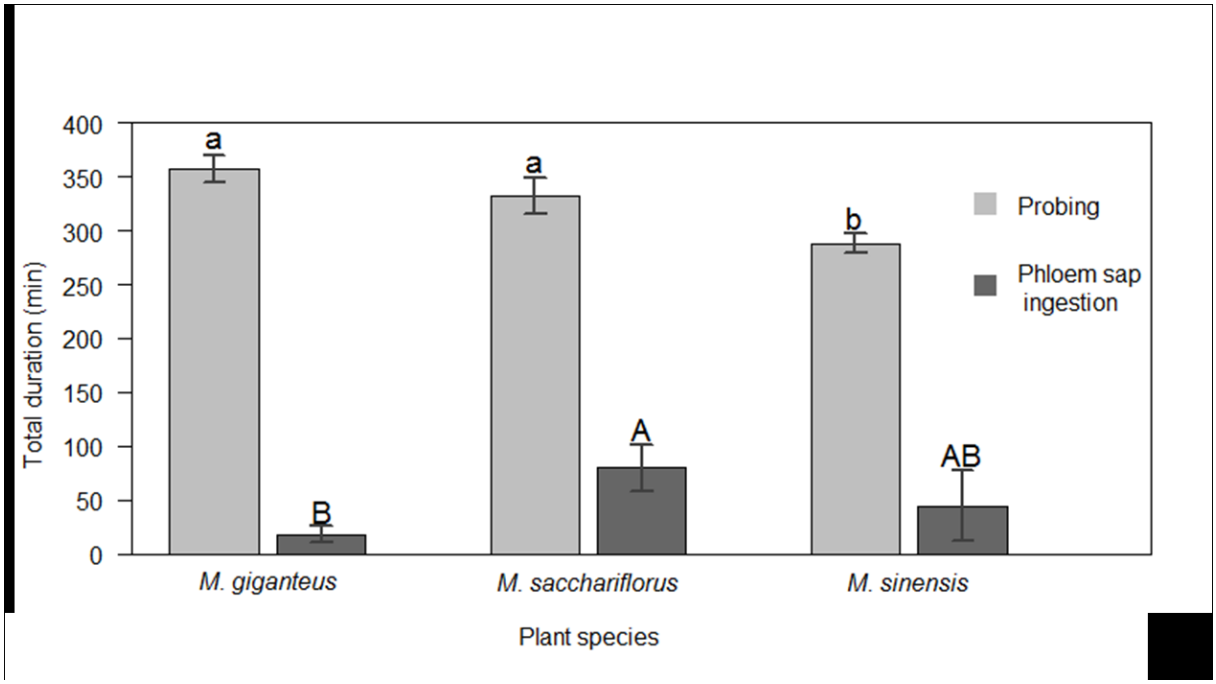
634

635 **Fig. 1 -** Two electrical penetration graph parameters (total duration of probing ± SEM in light
636 grey bars and total duration of phloem sap ingestion ± SEM in dark grey bars) calculated for
637 *Rhopalosiphum padi* during an 8 h monitoring session on the three miscanthus species
638 (*Miscanthus* x *giganteus*, *Miscanthus sacchariflorus* and *Miscanthus sinensis*).

639



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