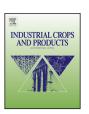
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Status of the bioenergy crop miscanthus as a potential reservoir for aphid pests



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ARTICLE INFO

Article history: Received 8 January 2015 Received in revised form 23 April 2015 Accepted 26 April 2015 Available online 21 May 2015

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

ABSTRACT

Miscanthus spp. (Poaceae) is 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 × giganteus crops. Then, in a first laboratory experiment, we investigated the colonization process on M. × 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 $M \times giganteus$, depended on their degree of specialization toward 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. × 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. × giganteus and its putative parents, Miscanthus sinensis and Miscanthus 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.

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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* × *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 are 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).

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For example, it has been shown that $M. \times giganteus$ 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 $M. \times giganteus$ 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, R. 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 M. 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 M. sacchariflorus and, to a lesser extent, M. sinensis were less suitable and acceptable host plants for R. maidis than $M. \times giganteus$.

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 land-scape dominated by plants belonging to the Poaceae taxa (barley, wheat, and 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 $M. \times giganteus$ 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 $M. \times giganteus$ would depend on their degree of specialization toward 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 $M. \times giganteus$ of four major aphid pests (Hemiptera: Aphididae) trapped in the field study: the two polyphagous species A. fabae (black bean aphid) and M. persicae (green peach aphid), the Brassicaceae specialist Bervicoryne Bervic

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. \times giganteus, M. sinensis, and M. sacchariflorus.

2. Materials and methods

2.1. Field studies

The experiments were conducted in M. \times 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, $2^{\circ}02'23''E$). Fields in Bougainville were planted with M. \times 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 m 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 *M.persicae* (Sulzer), the black bean aphid *A. fabae* Scopoli, the cabbage aphid *B. brassicae* (L.), the willow-carrot aphid *Cavariella aegopodii* Scopoli, the pea aphid *Acyrthosiphon pisum* (Harris), the birdcherry-oat aphid *R. 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, $60\pm5\%$ R.H., and 16:8 (L:D) photoperiod to induce parthenogenesis.

Plantlets of the three *Miscanthus* species, i.e., $M. \times giganteus$ (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 $9 \times 9 \times 10$ cm, then 16×13 cm and 20×15 cm) containing potting soil in a growth chamber under 20 ± 2 °C, $60 \pm 5\%$ R.H, and a 16:8 (L:D) photoperiod. Plants used in the experiment were 8-12 weeks old (after potting) and 60-80 cm high.

2.3. Feeding behavior studies

The electrical penetration graph DC-system described by Tjallingii (1978, 1988) was used to investigate the feeding behavior of alate aphids on *Miscanthus* spp. In a first bioassay, the feeding behavior of *M. persicae*, *R. padi*, *B. brassicae*, and *A. fabae* was investigated on *M. × giganteus* and in a second bioassay, the feeding behavior of *R. padi* was investigated on the three *Miscanthus* species.

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

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. \times 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-h 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–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-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, 25–40 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

Species and total number of alate aphids from yellow water traps placed in three *Miscanthus* × *giganteus* fields of northern France in 2011 (from May 2nd to July 29th) and in 2014 (from May 7th to July 2nd). Among the 50 species identified, the eleven most abundant 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

Table 2 Electrical penetration graph parameters (means \pm SEM) calculated for four aphid species during an 8-h monitoring session on *Miscanthus* \times *giganteus* plants.

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

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).

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 (*H. lactucae* and *N. ribisnigri*) represented less than 3% of the captures. Finally, the Fabaceae specialist *Acyrtosiphum pisum*, the Apiaceae specialist (*Cavariella* sp.), the Poaceae specialists (*R. padi* and *S. 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 (*M. persicae*, *A. fabae*, and *B. 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, and 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. \times 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-h recording) ranged from 42% for B. brassicae to 74% for B. padi. The Poaceae specialist B. 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-h 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-h 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 B. padi (Mann–Whitney B test, B0.05), but not significantly so when it was performed by B0. Persicae. The total duration of stylet derailment phase (B1) was not significantly different between aphid species (B1.40; B2.005).

3.2.2. Aphid performance on $M. \times giganteus$

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. P

Table 3 Mean (\pm SEM) population parameter values of four aphid species reared on *Miscanthus* \times giganteus.

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

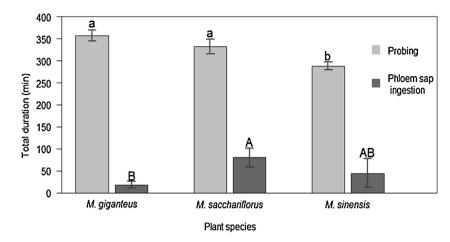


Fig. 1. Two electrical penetration graph parameters (total duration of probing ±SEM in light grey bars and total duration of phloem sap ingestion ±SEM in dark grey bars) calculated for Rhopalosiphum padi during an 8 h monitoring session on the three Miscanthus species (Miscanthus × giganteus, Miscanthus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

(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. × 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 $M. \times giganteus$ fields, were able to achieve their life cycle on this plant because their progeny did not reach the adult stage. Even if $M. \times giganteus$ 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 toward Poaceae. The Poaceae specialist aphid $R. \ padi$, which performed better on $M. \times giganteus$ than the other aphid species, was able to achieve its life cycle on $M. \ sacchariflorus$.

4.1. $M. \times giganteus \ colonization \ ability \ by \ the four \ main \ aphid pest \ species$

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 behavior, (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, and (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,b). The two main trapped species were two polyphagous species, M. persicae and A. fabae. These species are considered to be the main pests of northern France crops as their host

Mean (\pm SEM) performance parameter values of *Rhopalosiphum padi* on the three miscanthus species (*Miscanthus* \times giganteus, *Miscanthus sacchariflorus*, and *Miscanthus sinensis*).

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

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).

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 Brassicaeea specialist *B. brassicae* was extensively trapped in 2011 whereas the two cereal specialists *R. padi* and *S. avenae* were much less frequently trapped.

The analysis of EPG parameters such as frequency, duration, and sequence of different waveforms, is 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. × 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 $M. \times giganteus$ varied according to the degree of specialization toward Poaceae of each aphid species. And indeed, the Brassicaceae specialist cabbage aphid, B. 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 $M. \times giganteus$, although it performed less well than it did on barley, its conventional host plant (Chesnais et al., 2015; Schliephake et al., 2013). The relatively weak performances of R. padi on M. \times 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 C3metabolic pathway. He hypothesized that R. padi, having evolved in the Palearctic region, is not well adapted to C4-grasses that mainly grow in warmer regions. The polyphagous species A. fabae and M. persicae exhibited intermediate performances in comparison to the two specialist aphid species. This is in accordance with Tosh et al. (2003) who found that, during the host plant selection phases 4-6 (see above, Powell et al. (2006)), aphid specialists reject more easily and efficiently non-host plants than generalists do.

Chesnais et al. (2015) evaluated the intrinsic rate of increase of the same four aphid species as the ones tested in this study and it clearly appeared that $M. \times giganteus$ was less suitable for each aphid species than their respective rearing host plants. Accordingly, their feeding behavior was also drastically affected on $M. \times giganteus$ in comparison to what is reported in the literature when they feed on their host plant (Boquel et al., 2012; Gabrys et al., 1997; Powell and Hardie, 2001; Slesak et al., 2001). The unsuitability of $M. \times giganteus$ is also supported by the presence of stress indicators such as a high xylem sap consumption and the occurrence of stylet derailments (Prado and Tjallingii, 1997; Sauge et al., 2002).

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

Two main basic modalities of plant resistance to insects have been defined by Panda and Khush (1995) and Painter (1951): antixenosis affects insect behavior by deterring or reducing the colonization process whereas antibiosis affects insect life history traits (survival, development, and fecundity). Our study revealed that $M. \times \text{giganteus}$ and M. sinensis exhibited a higher resistance level to R. padi than M. sacchariflorus thanks to a combination of both resistance mechanisms.

Indeed, on a susceptible host plant such as *H. vulgare*, the high population rate of increase of *R. padi* was associated with a high

duration of phloem feeding (58% of the 12 h 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-h 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. × giganteus and M. sinensis. Accordingly, Huggett et al. (1999) demonstrated that R. padi was unable to reproduce and exhibit prolonged feeding on M. sinensis. In our study, M. sacchariflorus was a more susceptible host for R. padi than M. × 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 R. maidis was able to develop and reproduce and exhibited long phases of phloem sap ingestion on the three Miscanthus species (23–40% 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 $M. \times giganteus$ 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 perennial plants is crucial for aphid pests that use annual plant crops that are harvested in summer. Moreover, as the four aphid species, studied cannot complete their life cycle on $M. \times giganteus$, they can be considered as non-colonizing or transient species (Fereres and Moreno, 2009; Irwin et al., 2007). In the context of plant virus spreading, transient aphid species alighting on non-host plants are known to transmit non-persistent viruses before taking off in search of a suitable host plant (Boquel et al., 2012; Gray et al., 2010; Radcliffe and Ragsdale, 2002). Our EPG study clearly showed that the four aphid species performed the brief intracellular punctures (potential drop waveforms) which are directly involved in the transmission of non-persistent viruses (Martín et al., 1997). To our knowledge, only the work by Grisham et al. (2012) reported that M. sinensis could be infected by the non persistent Sorghum mosaic virus (SrMV) which is not present in Europe.

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

Thus, the introduction of miscanthus in the north of France could not only have an effect on the spread of phytoviruses related to Poaceae but also on those related to the other conventional crops. Indeed, *Miscanthus* spp. could constitute a tall barrier which may reduce potential virus dissemination by aphid vectors when aphid vectors migrate between crops. Hooks and Fereres (2006) propose that barrier plants may act as a sink for non-persistent 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, $M \times giganteus$, is also the species which exhibited the highest level of resistance toward 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, *S. avenae* (Fabricius) that exhibited different levels of specialization toward 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.

Acknowledgments

We would like to acknowledge the financial support from the Picardie region (research project MISCPIC). Shân Williams (Maison des langues/Université de Picardie Jules Verne) and Alain Corbière (College of Arts/Université de Picardie Jules Verne) are also thanked for their critical proof-readings of the manuscript especially concerning the English language.

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