Bioenerg. Res. (2015) 8:1275–1283
DOI 10.1007/s12155-015-9589-y

Bioenergy Crops and Natural Enemies: Host Plant-Mediated Effects of Miscanthus on the Aphid Parasitoid *Lysiphlebus testaceipes*

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Abstract *Miscanthus* spp. are biofuel crops that are triggering growing interest worldwide due to their numerous agronomic advantages. Though breeding programs take into account usual key plant traits of agronomic interest (e.g., biomass production, adaptation to broader climatic range), they generally overlook plant attributes relating to pest and pathogen resistance and even more those that may favor or improve the combined use of biological control agents of pests. A recent study showed that the parental species, *Miscanthus sacchariflorus* and, to a lesser extent, *Miscanthus sinensis*, were less suitable and acceptable host plants for the corn leaf aphid *Rhopalosiphum maidis*, one of the main pests of *Miscanthus* × *giganteus* in the USA, than the hybrid *M.* × *giganteus*. In the present laboratory study, we investigated the host plant-mediated effects of these three miscanthus species on various life history traits of the aphid parasitoid *Lysiphlebus testaceipes*. A clear host plant effect was shown on aphid size and, consequently, on parasitoid fitness parameters. High plant resistance to aphids was shown to be more detrimental to the parasitoid than partial resistance, with *M. sacchariflorus* being the least suitable host plant to both aphid and parasitoid development. Selection of partial resistance, such as the one exhibited by *M. sinensis*, should then be preferred to support efficient aphid regulation by parasitoids. This study provides the first contribution to the evaluation of bottom-up effects of a biofuel crop on beneficial insects. It also underlines the need to conduct additional research when considering the implementation of new biomass crops.

Keywords Biofuel crop · *Miscanthus* spp · Host plant resistance · Tritrophic interactions · *Rhopalosiphum maidis* · *Lysiphlebus testaceipes*

Introduction

Biofuel crops, being a renewable and sustainable alternative to fossil fuels, represent energy-efficient fuel production systems. They could also play a positive role in moderating climate change as they can help in reducing greenhouse gas emission through C sequestration. For the past decade, second-generation feedstocks, particularly, have been triggering a growing interest in the USA and in Europe as, through their status of dedicated crops, they do not compete with food crops in the context of limited resources. However, large-scale monoculture production of such crops may have substantial negative impacts on ecosystems both at the landscape and field levels [1].

The development and/or conversion of lands for biofuel crops may lead to a loss of areas currently providing other ecosystem services [2, 3], including a loss of floral and faunal biodiversity [4–8]. It is generally suggested that massive introduction of biomass crops,

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through reduction of plant diversity, will also affect arthropod diversity by modifying associated food webs and by inducing community structure changes [9]. Biocontrol is an important ecosystem service, and if the number/diversity of natural enemies is reduced, it may result in increased impact of pest arthropods, whether they are existing pests or species that newly emerge as pests [2]. This reduction may affect not only the bioenergy crops but also those adjacent [1, 3]. The efficacy of beneficial arthropods in limiting pest numbers may also be reduced due to biofuel crops altering their spatial or temporal distribution [10] or failing to provide them with needed shelter or alternative food sources [11–13]. Finally, exotic bioenergy crop species share common features that can potentially lead them to become weeds in sensitive environments [2, 3]. However, if breeding efforts can result in species being less fecund while still remaining productive, as is the case for the sterile triploid *Miscanthus* × *giganteus*, the invasive potential could drop significantly [14].

M. × *giganteus*, a perennial C4 grass originating from East Asia, has seen growing interest due to its numerous agronomic qualities and has been subjected to field trials since the early 1980s. To date, European crops of *M.* × *giganteus* consist of a single clone, which is considered a natural triploid hybrid between a diploid *Miscanthus sinensis* and a tetraploid *Miscanthus sacchariflorus* [15]. In comparison to conventional crops, *M.* × *giganteus* harbors several agronomic advantages such as a high yield potential and water efficiency, low nutritional requirement due to efficient C and N use, and a presumed high tolerance to pests and diseases [16]. Few pests have been reported to directly damage *M.* × *giganteus*, though this may also be due to a lack of field investigations. Several laboratory and field studies indicate that *M.* × *giganteus* is a suitable host plant for some important pests of conventional cereal grain crops. The most notable of these are two main insect pests of maize, the western corn rootworm, *Diabrotica virgifera* (Coleoptera: Chrysomelidae), and the fall army worm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), which successfully develop on *M.* × *giganteus* [17–19]. During an extensive field survey carried out in the USA, large populations of the yellow sugarcane aphid, *Sipha flava* (Hemiptera: Aphididae), and the corn leaf aphid, *Rhopalosiphum maidis* (Hemiptera: Aphididae), were reported on *M.* × *giganteus* with apparent damage at least in youngest stands [20]. Preliminary investigations have also examined the potential conservation value of miscanthus fields for arthropods. Semere and Slater [21] investigated for three consecutive years the biodiversity of the arthropod fauna hosted by *M.* × *giganteus* crops. They showed that arthropod abundance and diversity were positively correlated with the floristic diversity of the miscanthus fields. Stanley and Stout [22] found similar or greater abundance and diversity of beneficial insects (various pollinators and wasps) in *M.* × *giganteus* fields than in conventional food crops. Nevertheless, records on arthropods that are the natural enemies of pests are scarce. The extensive survey by Semere et al. [21] in *M.* × *giganteus* fields in the UK reports the presence of many arthropod species, some of which play an important role in the control of agricultural pests (e.g., hymenopteran parasitoids and predatory ground beetles). The only other data available concern the occurrence of ladybird predators [23, 24] and euryoecious species [25] in *M. sinensis* fields, and to date, nothing is known about the pest-regulating effect of natural enemies in miscanthus crops. Specific studies of pests and pest regulation by natural enemies are therefore crucially needed to assess the overall sustainability of *Miscanthus* spp. as a bioenergy crop.

The development of miscanthus as a biofuel crop has mainly been based on traits related to biomass production. The fact that it lies on a single clone of *M.* × *giganteus* in Europe presents several limitations, including an increased risk of attack by pests and diseases which could be compensated for by breeding new inter-specific hybrids [15]. Such breeding programs should thus select and integrate key plant attributes to allow the combination of pest resistance and biological control [26–28]. In a recent study, we evaluated the ability of *R. maidis* aphids to colonize the three main miscanthus species studied in Europe for biomass production, i.e., the sterile hybrid *M.* × *giganteus* and its two putative parents, *M. sacchariflorus* and *M. sinensis*. In a previous study combining electropenetrography and demographic parameter assessment, *M. sacchariflorus* and, to a lesser extent, *M. sinensis* were shown to be less suitable and acceptable host plants for *R. maidis* than *M.* × *giganteus* [29]. Although such negative host plant effects on aphids may subsequently affect natural enemies, partial plant resistance to aphids can also lead to an efficient biological control [30]. More generally, upper trophic level organisms such as parasitoids can be particularly affected by disturbance events and environmental changes, as predicted by the trophic rank hypothesis, due to cumulative bottom-up effects [31, 32].

The objective of the present study was to evaluate the indirect host plant-mediated effects of the three *Miscanthus* species on the performance of a natural enemy of *R. maidis*, the koinobiont endoparasitoid *Lysiphlebus testaceipes* [33, 34]. We investigated whether and how the host plant could modulate the performance of both the pest and its parasitoid. The conventional food crop *Hordeum vulgare* was used as a reference host of *R. maidis.* The hybrid *M.* × *giganteus* was compared to the two parental species, *M. sinensis* and *M. sacchariflorus*. As *R. maidis* feeding behavior and performances were negatively affected on *M. sinensis* and *M. sacchariflorus* [29], we hypothesized that aphid size would also be impacted and this would in turn affect the fitness of *L. testaceipes* parasitoids.

Materials and Methods

Plants

Plantlets of winter barley (*H. vulgare* var. Cervoise) were obtained from seeds deposited on commercial sterilized potting soil in plastic pots (80×80×90 mm; 40 seeds per pot). Plantlets of the three miscanthus species, i.e., *M.* × *giganteus* (var. GigB, $2n=3x=57$), *M. sacchariflorus* (var. Sac, $2n=2x=38$), and *M. sinensis* (var. Goliath, $2n=4x=76$) [35], were obtained by in vitro multiplication as described by Rambaud et al. [36].

Before they were used for the experiments, all plants were grown and maintained in a growth chamber (SNIJDERS, Economic Premium ECP01E) under 20±1 °C, 60±5 % relative humidity, 4 klx, and 16:8 light cycle. All miscanthus in vitro plantlets were randomly arranged on the same bench (mid-height) in the same growth chamber. Barley plantlets were maintained in another growth chamber, also on a mid-height bench. Plantlets were used for the experiments when they were approximately 20 cm high (2 weeks old for barley and ca. 6 to 8 weeks after multiplication for *Miscanthus* sp.).

Insects

A laboratory colony of the aphid *R. maidis* (Fitch, 1856) (Hemiptera, Aphididae) was initiated from a parthenogenetic aphid population collected on sorghum in 2006. Aphids were reared on plants of winter barley (*H. vulgare* var. Cervoise) in ventilated Plexiglas® cages (240×110×360 mm) which were maintained in a growth chamber (SANYO, Versatile Environmental Test chamber) under 20±0.5 °C, 60±5 % relative humidity, 4 klx, and 16:8 light/dark (L/D) cycle.

Parasitoids *L. testaceipes* (Cresson, 1880) (Hymenoptera, Braconidae) were obtained from Viridaxis (Gosselies, Belgium) as mummies. Upon reception, mummies were individually transferred to a plastic tube (75×13 mm) closed with a cotton plug. Once emerged, parasitoids were sexed and mating was allowed by regrouping in the same tube two to three males with five to six females. They were fed ad libitum with a solution of honey and water (30 % *v*/*v*) impregnated on a small piece of paper towel. Parasitoids were maintained in a climate chamber (SNIJDERS, Economic Premium ECP01E) 20±0.5 °C, 60 ±5 % relative humidity, 4 klx, and 16:8 L/D light cycle.

Direct Effect of the Host Plant on the Development and Size of *R. maidis*

Aphids were submitted to four different treatments (Hvul, MGig, Msac, Msin) depending on the host plant on which they fed (*H. vulgare*, *M.* × *giganteus*, *M. sacchariflorus*, *M. sinensis*, respectively). Cohorts of synchronized *R. maidis* nymphs were regularly reared on cut leaves of each of the four host plants. First, parthenogenetic adult females were placed on artificial diet in feeding chambers for larviposition [37]. Then, on two successive dates, a total of 41 and 37 individual neonates were collected and randomly submitted to one of the four treatments (Hvulg, $n=19$; Mgig, $n=17$; Msac, $n=20$; Msin, $n=22$). Each individual was allowed to develop in a rearing box consisting of a Petri dish (55 mm in diameter) where freshly cut leaves (total surface of ca. 2 cm^2) of one host plant species had been randomly set in 1.5 % agar. Every 2 days, they were transferred onto a newly prepared rearing box containing freshly cut leaves of their host plant. All aphids were maintained in a climate chamber at 20±2 °C, 60±5 % relative humidity, 4 klx, and 16:8 L/D cycle. They were checked every day for mortality until they reached the adult stage, i.e., when they larviposited their first nymph. This allowed measurement of the mean pre-reproductive period (hereafter PRP) and the mean percentage of larval mortality. Each adult aphid was then measured from the tip of the head to the base of the cauda from a picture taken under a stereomicroscope (Leica M165C) using the JMicroVision (1.2.7 version) software. This experiment also allowed selection of the optimal age at which aphid nymphs should be introduced to parasitoid females for oviposition. As only two stages (second and third instars) were represented in 3-day-old aphids, with a majority of second-instar nymphs whatever their host plant species (Hvulg 73.3 %, Mgig 88.2 %, Msin 95.5 %, Msac 95 %), this age was selected for oviposition by *L. testaceipes* females.

Host-Mediated Indirect Effect of the Host Plant on *L. testaceipes* Life History Traits

Preliminary experiments had been conducted in order to evaluate the potential effect of aphid treatment on the probability of egg laying in each attacked host (probability of true oviposition) under experimental conditions of controlled ovipositions. The procedure for controlled ovipositions consisted in placing a single 3- to 6-day-old standardized *L. testaceipes* female (i.e., mated, fed, and without oviposition experience) with a single 3-day-old *R. maidis* nymph in a small Eppendorf tube (0.5 ml). This setup was designed to by-pass the host plant volatiles during the process of aphid acceptance by the parasitoid female and optimize the percentage of true ovipositions. Each parasitoid female was only used once. For each treatment (Hvul, Mgig, Msac, Msin), 21 aphids per treatment were parasitized and then dissected under a stereomicroscope immediately after oviposition to determine the presence or absence of a parasitoid egg. The frequency of true oviposition (ca. 73 %) was not significantly affected by the treatment (Fisher's exact tests, $P>0.7$ for all pairwise comparisons). Therefore, this procedure of controlled oviposition could be applied whatever the treatment of the aphids prior oviposition.

In order to assess the aphid-mediated indirect effect of the host plant on *L. testaceipes* life history traits, aphids were submitted to one of the four treatments and they were allowed to complete their development for 3 days until they were used for parasitization by *L. testaceipes* females. Before parasitization, each aphid nymph was measured as described in "Direct Effect of the Host Plant on the Development and Size of *R. maidis*" and associated results are presented together with those of the said section in "The Host Plant Has Direct Effects on the Development and Size of *R. maidis*." Immediately after measurement, each 3-day-old *R. maidis* nymph was submitted to controlled oviposition by a *L. testaceipes* female. Controlled ovipositions were performed on 16 successive dates. For each date, an equal number of aphid nymphs (three nymphs on average per treatment) were submitted to controlled oviposition. Each stung aphid nymph was then individually placed back onto its host plant in a newly prepared rearing box and observed daily until death or emergence of a F1 parasitoid wasp. All dead aphids (i.e., aphids that had changed their usual color and were not moving under mechanical stimulation) were dissected under a stereomicroscope (Leica M165C) to check for the presence of dead immature parasitoids. Once a mummy was formed, it was measured and transferred to a plastic tube (75×13 mm) closed with a cotton plug. Emerged parasitoids were sexed and fed ad libitum with a solution of honey and water (30 %*v*/*v*) impregnated on a small piece of paper tissue until death. Upon death, parasitoids were stored at −80 °C for further measurements. The tibia length of both males and females was measured using a stereomicroscope. Females were dissected into a drop of NaCl solution (0.9 %) to collect their ovaries, and the total number of mature eggs present in the two ovaries was recorded.

The following parasitoids' life history parameters were computed:

- Parasitism success as the percentage of parasitoids completing total development: (no. of emerged parasitoids/ no. of parasitized aphids)×100
- Sex ratio (%): (no. of females/no. of (females+males))× 100
- Total developmental time (from oviposition to adult emergence) in days
- Adult longevity of male and female parasitoids in days
- Tibia length (in μm) of male and female parasitoids as a proxy for parasitoid size
- Egg load of female parasitoids

Statistical Analysis

We first investigated the effects of host plant species on the size and development of aphids. The effects of host plant species on pre-reproductive period (PRP) and larval survival were tested with generalized linear models using a quasipoisson and binomial distribution, respectively. The effects of host plant species on either aphid size at 3-day-old or adult aphid size were tested with ANOVA. Whenever the effect of host plant species was significant, post hoc Tukey HSD tests were used for pairwise comparisons between host plant species.

The combined effects of host plant and aphid size at oviposition on parasitoid life history traits (parasitism success, sex, developmental time, tibia length, egg load, and longevity) were also investigated using generalized linear models, including host plant species as a factor and aphid size at oviposition as a covariate. Models were fitted using binomial (parasitoid emergence and sex), quasipoisson (developmental time, egg load, longevity), or gaussian distributions (tibia length). Here again, pairwise comparisons between host plant species were performed with Tukey HSD tests whenever the effect of host plant species was significant. Models were validated by inspection of the residuals. All statistical analyses were performed in the R software [38].

Results

The Host Plant Has Direct Effects on the Development and Size of *R. maidis*

Neither the pre-reproductive period (*M.* × *giganteus* 8.35±0.79 (n=19), *M. sinensis* 8.59±0.96 (n=22), *M. sacchariflorus* 8.60±0.75 (n=21), *H. vulgare* 8.16±0.69 (n=20)) nor the larval mortality (*M.* × *giganteus* 10.53 % (n=19), *M. sinensis* 0 % (n=22), *M. sacchariflorus* 4.76 % (n=21), *H. vulgare* 5.00 % (n=20)) of *R. maidis* differed significantly across host plant species (PRP: GLM, $F_{3,77}=1.36$, $P=0.26$; larval mortality: GLM, $F_{3,193}=0.07$, $P=0.98$).

Host plant species significantly affected the size of 3-day-old aphids (ANOVA, $F_{3,176}=4.03$; $P<0.01$). Three-day-old aphids reared on *H. vulgare* were slightly but significantly longer than those reared on either *M. sacchariflorus* (Tukey HSD, $P=0.02$) or *M. sinensis* (Tukey HSD, $P=0.01$) (Fig. 1a).

Upon completion of development, the effect of host plant species on aphid size was notable (ANOVA, $F_{3,77}=35.68$, $P<10^{-4}$). Aphids reared on *H. vulgare* were longer than those reared on the three other host plant species (Tukey HSD, all $P<10^{-4}$). In addition, aphids reared on *M.* × *giganteus* were significantly longer than those reared on either of the two parental plant species (Tukey HSD, *M.* × *giganteus*/ *M. sacchariflorus*, $P=0.003$; *M.* × *giganteus*/*M. sinensis*, $P=0.04$) (Fig. 1b).

The Host Plant Affects *L. testaceipes* Life History Traits via Aphid-Mediated Indirect Effects

The parasitism success of *L. testaceipes* on *R. maidis* was high (*M.* × *giganteus* 88.0 % (n=50), *M. sinensis* 92.7 % (n=41),

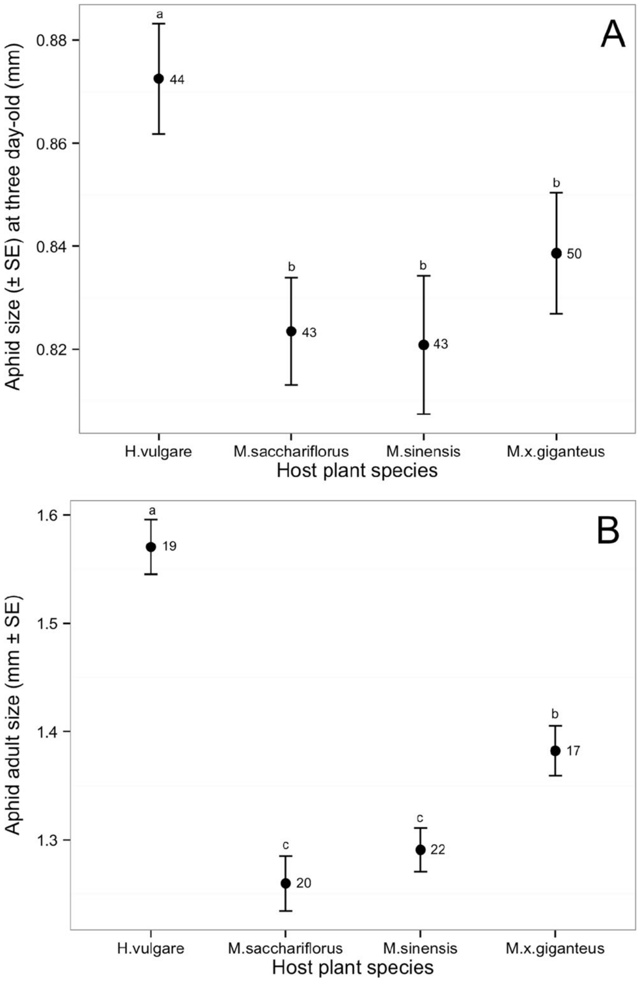

Fig. 1 Mean (± SE) aphid size (mm) of 3-day-old *R. maidis* individuals (**a**) and adult individuals (**b**) reared on the four tested host plant species. Sample sizes are indicated beside each *dot*. *Letters* indicate grouping according to post hoc multiple comparisons (Tukey HSD tests)

M. sacchariflorus 81.0 % (n=42), *H. vulgare* 81.0 % (n=42)). Emergence of *L. testaceipes* adult parasitoids was not significantly related to host plant species (GLM, $F_{6,178}$=1.69, P=0.12) nor to aphid size at oviposition (GLM, $F_{4,176}$=1.93, P=0.10). The interaction between host plant species and aphid size at oviposition was not significant either (GLM, $F_{3,175}$=1.94, P=0.12).

Sex ratio was balanced (% of females; *M.* × *giganteus* 52.0 % (n=44), *M. sinensis* 52.0 % (n=38), *M. sacchariflorus* 59.0 % (n=34); *H. vulgare* 59.0 % (n=34)). Again, sex of emerged parasitoids was not significantly related to host plant species (GLM, $F_{3,137}$=0.04, P=0.99) nor to aphid size at oviposition (GLM, $F_{1,135}$=2.57, P=0.11). The interaction between the two was, however, significant (GLM, $F_{3,134}$=3.33, P=0.02): there tended to be more females with increasing aphid size on *H. vulgare*, *M. sinensis*, and *M.* × *giganteus*, while the opposite trend was found on *M. sacchariflorus*.

Developmental time of *L. testaceipes* parasitoids differed across host plant species (GLM, $F_{6,135}$=7.57, $P<10^{-4}$). It was significantly shorter on *H. vulgare* as compared to the three other plant species (Tukey HSD, all $P<10^{-3}$) and was shorter on *M.* × *giganteus* than on *M. sacchariflorus* (Tukey HSD, P=0.04). In addition, parasitoid developmental time was negatively related to aphid size at oviposition (GLM, $F_{4,135}$=2.95, P=0.02). There was no significant interaction between host plant species and aphid size ($F_{3,134}$=1.67; P=0.18) (Fig. 2a).

Parasitoid size (tibia length) differed across host plant species (GLM, $F_{6,136}$=12.62, $P<10^{-4}$). It was greater on *H. vulgare* as compared to the three other plant species (Tukey HSD, all $P<10^{-3}$) and greater both on *M.* × *giganteus* (Tukey HSD, P=0.03) and *M. sinensis* (Tukey HSD, $P<10^{-3}$) than on *M. sacchariflorus* (Fig. 2b). In addition, parasitoid size was positively related to aphid size at oviposition (GLM, $F_{4,134}$=4.28, P=0.003). There was no significant interaction between host plant species and aphid size ($F_{3,133}$=0.97; P=0.41).

Parasitoid egg load differed across host plant species (GLM, $F_{6,58}$=8.34, $P<10^{-4}$). It was greater on *H. vulgare* as compared to the three other plant species (Tukey HSD, all $P<10^{-3}$) and greater on *M.* × *giganteus* than on *M. sacchariflorus* (Tukey HSD, P=0.03). In addition, parasitoid egg load was positively related to aphid size at oviposition (GLM, $F_{4,56}$=2.56, P=0.05). There was no significant interaction between host plant species and aphid size at oviposition (GLM, $F_{3,55}$=1.35, P=0.27) (Fig. 2c).

Parasitoid longevity did not differ significantly according to host plant species (GLM, $F_{6,137}$=1.40, P=0.22) but was positively related to aphid size at oviposition (GLM, $F_{4,135}$=3.18, P=0.02). The interaction between host plant species and aphid size was not significant (GLM, $F_{3,134}$=2.53, P=0.06) (Fig. 2d).

Discussion

To our knowledge, this is the first report of a beneficial insect performing its whole life cycle on aphid/*Miscanthus* sp. systems. All three species of miscanthus were able to sustain the development of both the aphid *R. maidis* and its endoparasitoid *L. testaceipes* in our laboratory conditions. However, both aphid and parasitoid characteristics differed across miscanthus host species.

Nutritional suitability and environmental conditions of phytophagous hosts are largely determined by the quality of the host plant itself, and they often have a profound effect on sex ratio, size, developmental time, fecundity, and longevity of the parasitoid [28, 30, 39]. Our results indicate that host plant species affected aphid size. There was no significant size difference between 3-day-old aphids from the two parental *Miscanthus* species and the hybrid *M.* × *giganteus*. However, *R. maidis* aphids reared on the two parental *Miscanthus* species were smaller upon developmental completion than those reared on *M.* ×

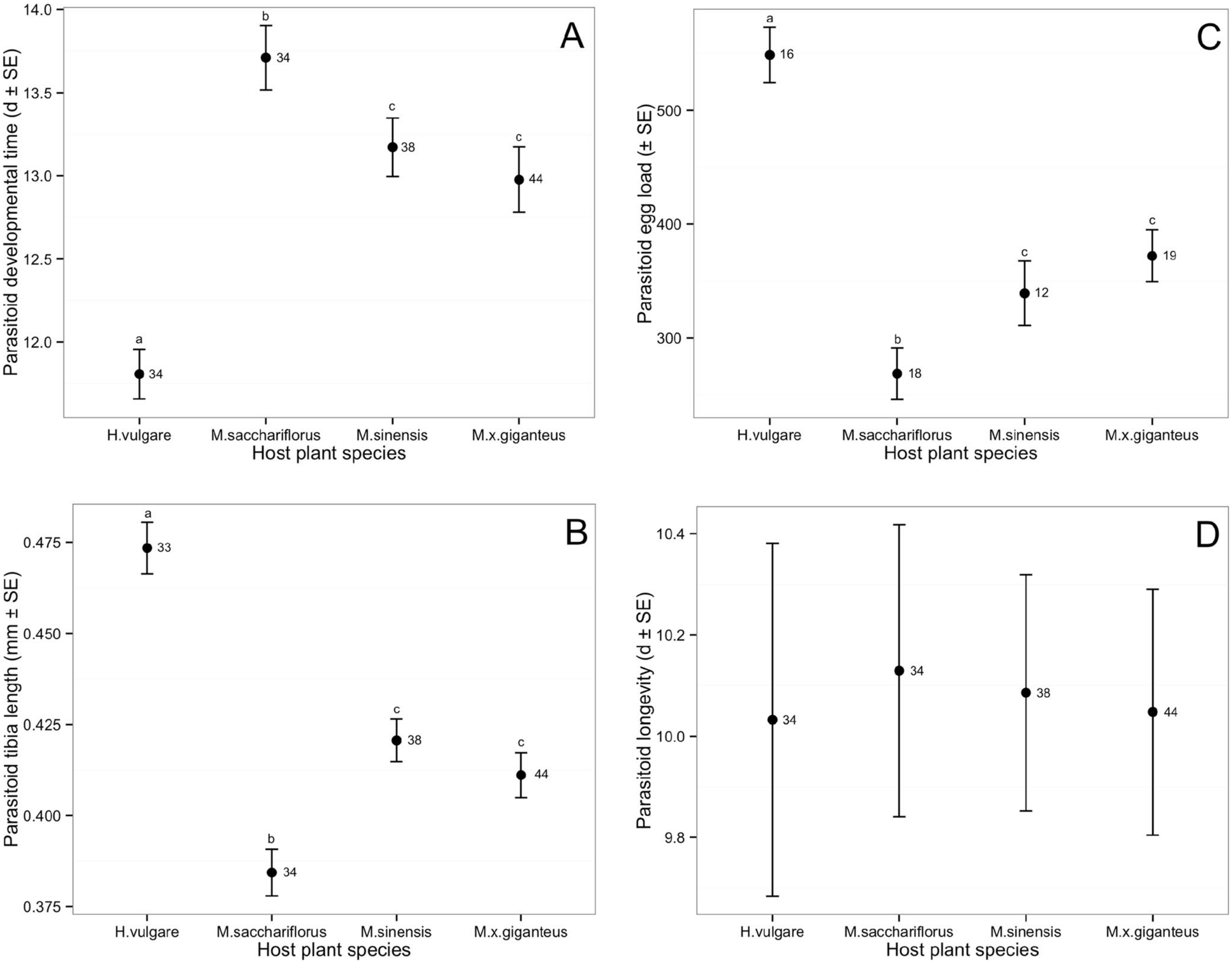

Fig. 2 Mean (± SE) values of *L. testaceipes* parasitoid life history traits parasitizing *R. maidis* aphids reared on the four tested host plant species. **a** Developmental time (days); **b** size, using tibia length as a proxy (mm); **c** egg load (number of eggs); **d** longevity (days). Sample sizes are indicated beside each *dot*. *Letters* indicate grouping according to post hoc multiple comparisons (Tukey HSD tests)

giganteus. As the length of the developmental period (PRP) did not differ across host plant species, it means that aphids grew at a lower rate when reared on the two parental species than on the hybrid *M.* × *giganteus*. This probably reflects differences in aphid trophic behavior, and more specifically a lower sap ingestion rate on the two parental species, as previously demonstrated [29]. Hence, this confirms that *M. sacchariflorus* and, to a lesser extent, *M. sinensis* represent less suitable host plants than *M.* × *giganteus* for *R. maidis*. Aphid size, in turn, appears positively correlated with parasitoid fitness: parasitoids that had developed in bigger aphids exhibited a shorter developmental time, emerged as bigger adults, and lived longer, and females had a greater egg load. These results are in line with studies in other systems where the size of the emerging solitary parasitoid, used as a fitness proxy, is correlated to "host quality" (size, age, stage, and diet) (for a review, see [40–42]). Various studies with koinobionts (i.e., parasitoids of which hosts continue to feed and grow after parasitization) have reported that parasitoid size—which is often correlated with fecundity—is an increasing function of host size or stage at oviposition [43–47]. More generally, a bigger host means more resources for its parasite, and hence, host size is an important selective factor, especially so for those internal parasites that cannot leave the host until their development is completed [48].

Given the observed differences in aphid size across host plant species on the one hand and the positive link between aphid size and parasitoid fitness parameters on the other hand, it is therefore not surprising that, to some extent, *L. testaceipes* performances differed according to host plant species. Parasitoids displayed lower performances when reared on *M. sacchariflorus* as compared to *M.* × *giganteus*: longer parasitoid developmental time, smaller parasitoids (mummy and adult size), and females maturing fewer eggs. This is consistent with the differences observed in aphid size between these two host plants.

Surprisingly, however, the differences observed in aphid size between *M. sinensis* and *M.* × *giganteus* were not reflected in significant differences in parasitoid life history traits. This indicates that, independently from a quantitative effect mediated by aphid size, there is also a qualitative effect of host plant species on parasitoid fitness: although aphid hosts were smaller on *M. sinensis* than on *M.* × *giganteus*, the parasitoids emerging from them had comparable fitness. This might be reflecting subtle differences in the chemical composition of aphids, resulting from variations in phloem composition between *M. sacchariflorus* and *M. sinensis*. This is in accordance with the koinobiont lifestyle of *L. testaceipes*: because hosts continue to feed after parasitization, the amount and/or quality of their diet can in turn influence parasitoid fitness. For example, one might hypothesize that phloem sap composition in *M. sinensis* would be more favorable than *M. sacchariflorus* to the later development of *L. testaceipes. M.* × *giganteus*, being a hybrid species, might contain a phloem sap with an intermediate composition. Therefore, despite being more easily accepted by aphids and hence producing bigger aphids, *M.* × *giganteus* might not be more favorable to the parasitoid *L. testaceipes* than *M. sinensis.* Further work may therefore be needed to better understand the factors affecting parasitoids in this system.

In a multitrophic context, the host plant can affect parasitoids indirectly (via host-mediated effects) or directly (via toxins and/or secondary metabolites) [39, 49–51]. For instance, parasitoids can ingest toxic plant allelochemicals that are present in the hemolymph of their phytophagous host. However, only in few cases have these allelochemicals been identified and their host-mediated or direct effects on parasitoids deciphered. Alkaloids such as nicotine have been well studied. The negative effects of nicotine on the host *Manduca sexta* were shown to subsequently negatively impact various life history traits of its parasitoid *Cotesia congregata*. Nicotine was shown to have direct and indirect effects on the parasitoid, whereas another alkaloid, rutine, only affected the parasitoid larval development via a host quality-mediated effect [52].

Three recent studies have reported different levels of aphid resistance in switchgrass populations [53, 54] and in different miscanthus species [29]. All three studies underlined the importance to take into account pest resistance properties of biofuel crops in the context of sustainable pest management strategies. Pointeau et al. [29] suggested that the reduced sap uptake by aphids on *M. sacchariflorus* and *M. sinensis* was due to a lower phagostimulatory value of their phloem sap and/or the presence of phloem-located phagodeterrents. Furthermore, the aggravated antibiotic properties of *M. sacchariflorus* compared to *M. sinensis* toward *R. maidis* were suggested to be due to different levels of partial resistance to *R. maidis* in *Miscanthus* sp. This is confirmed in the present study by the different effects observed on *L. testaceipes*. The parasitoid fitness parameters were adversely affected on *M. sacchariflorus* but not on *M. sinensis*, compared to *M.* × *giganteus*. Several authors previously reported the negative impacts of aphid-resistant plants on aphid parasitoids. For instance, since the introduction of the soybean aphid *Aphis glycines* in North America (end of the 1990s), many works have been devoted to, on the one hand, developing soybean cultivars resistant to this aphid, and, on the other, evaluating different aphid parasitoid species as potential regulating agents. Chacon et al. [55] were the first to explore the specific interaction between these two pest management strategies in the field using the Rag1-based aphid resistance in soybeans, the Asian endoparasitoid *Binodoxys communis*, commonly released against soybean aphids in North America soybean fields, and their guilds of predators. Aphid-resistant soybeans were shown to protect parasitoids from predation while reducing their reproduction. Further studies showed that the Rag1 resistant soybean cultivar negatively impacted the generalist and cosmopolitan aphid parasitoid *Aphidius colemani*, both directly (increased aphid handling time and reduced preference by parasitoids) and indirectly (decreased body mass and offspring survival) [56]. More recently, Hopper and Diers [57] showed similar results on *Aphelinus certus* (reduced female progeny size) and *Aphelinus glycinis* (reduction in number of mummies).

Although most studies have pointed out incompatibilities between host plant resistance and biological control [51], there are also several reported cases where host plant resistance and biological control can act synergistically. Starks et al. [58] found that successful biological control of the green bug *Schizaphis graminum* by *L. testaceipes* was efficient on both the resistant barley and sorghum varieties. Similarly, a wheat field experiment showed that although both aphid and parasitoid densities were lower on the resistant cultivar, the control of *Sitobion avenae* by *Aphidius* spp. was enhanced on the resistant wheat cultivar [59]. Van Emden [30] underlines the fact that biological control is more likely to be compatible with the use of partially resistant cultivar than highly resistant cultivars. Our work confirms this prediction. It also suggests that in the context of breeding programs for the production of new hybrids of *M.* × *giganteus*, tritrophic effects of *Miscanthus* sp. should be taken into account. Partial plant resistance to aphids should be preferred to high plant resistance to aphids to ensure efficient aphid regulation by parasitoids.

This laboratory experimental work is the first contribution to the evaluation of bottom-up effects of a biofuel crop. Although this work clearly shows that a potential top-down control can be performed by an aphid parasitoid, further studies are needed to assess its efficiency. Both of these bottom-up and top-down approaches should also be performed at a larger scale (greenhouse and field) and ultimately take into account the landscape level [1, 2]. Altogether, our study contributes to filling the gap of knowledge concerning the potential effects of bioenergy crops on beneficial insects such as biocontrol agents. It also illustrates the urge for additional research when considering the implementation of new biomass crop or the conversion of arable lands to new dedicated crops.

Acknowledgments We would like to acknowledge the financial support from the Picardie region (research project MISC PIC). We also thank Gérard Labonne (UMR BGPI, Montpellier, France) for providing the *R. maidis* population. Viridaxis (Gosselies, Belgium) is thanked for providing the *L. testaceipes* mummies for the experiments. Andrew Roots is thanked for critical reading of the manuscript, especially concerning the English language.

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