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Host-dependent larval migration and parasitism risk in a polyphagous moth

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Abstract

Female herbivorous insects are expected to oviposit on the host plant providing the best performance of the offspring. However, in some insects the larvae are mobile and are not totally dependent on the mother's choice. They can change host plant when conditions for development or exposure to natural enemies vary between individual plants within a patch. Here we study larval migration and preference between two host plants, cotton and alfalfa, in the Egyptian leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). Previous studies have shown that although larval performance is better on cotton, females prefer to oviposit on alfalfa, and this preference corresponds to a higher survival of the eggs. In this study, *S. littoralis* larvae showed directed movement between host plants and were found to prefer alfalfa over cotton in field test in Egypt, as well as in laboratory selection of feeding site assays. To determine effects by natural enemies, the parasitism rates and various life-history traits were measured for one larval parasitoid, *Microplitis rufiventris* Kokujev (Hymenoptera: Braconidae), on several host plants including cotton and clover (equivalent host plant to alfalfa). Overall, parasitism was higher on cotton and parasitoid performance (cocoon mass, adult longevity, and female egg load) was better on cotton compared to clover. This fact suggests an enemy-free space on clover and alfalfa, as parasitism rate is higher on cotton, and the parasitoid performance is also better on cotton-fed larvae.

KEYWORDS
Braconidae, dispersal, enemy-free space, feeding, host plant choice, Hymenoptera, Lepidoptera, *Microplitis rufiventris*, Noctuidae, performance, preference, *Spodoptera littoralis*

INTRODUCTION

In herbivorous insects, female selection of a suitable host plant for oviposition can be very important for the survival and fitness of the offspring (reviewed by Refsnider & Janzen, 2010). In species where the progeny has no or very limited ability to change host plant early in life, the mother's choice is particularly crucial. Even for species with mobile larvae, it may still be very important for the female to select a host of the right species and of the right quality that provides the best conditions for offspring performance. Consequently, it has been predicted that this should lead to a strong correlation between female preference and offspring performance (Jaenike, 1978; Thompson, 1988) and there are also many examples where this is the case (Denno et al., 1990; Fox et al., 1994; Barker & Maczka, 1996; Yamaga & Ohgushi, 1999; Dunning et al., 2003; Bourassa et al., 2007; Mphosi & Foster, 2009; Gripenberg et al., 2010; Guo et al., 2021). However, there are also examples where there is not a good match between female preference for host plants and larval performance (Gripenberg et al., 2010).

There may be several reasons behind the mismatch between female preference and larval performance, including both sensory and ecological factors. The lack of correlation between preference and performance could be due to sensory limitations of the female that makes her unable to make

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the right choice (Martel & Boivin, 2011). She may not have access to the appropriate information for her decision and may not be able to detect or discriminate between vital cues indicating host plant quality (Bernays, 2001). In other cases, ecological top-down effects may affect host plant choice, where different host plants can provide different protection against natural enemies (called 'enemy-free space hypothesis'; Jeffries & Lawton, 1984) and this may not be directly connected to larval nutritional quality (Lund et al., 2020). The selective pressure from the natural enemies on herbivores could thus lead them to prefer a plant of lower nutritional quality if it provides benefits such as a protection against natural enemies (Rodrigues & Freitas, 2013).

The pressure from natural enemies may vary over time or with the developmental stage of the offspring. The ovipositing female should then choose the plant offering the best protection for her eggs, as they cannot escape from unfavourable plant conditions and natural enemies. However, that plant might not be the most suitable for the subsequently hatching and feeding larvae. One possibility, for species with sufficiently mobile larvae, would thus be for the female to oviposit on the best host plant species for the eggs, in terms of survival, and for the hatching larvae to migrate to a better plant for their development. For instance, the eggs of *Oreina elongata* Suffrian exhibit the best survival on *Cirsium spinosissimum* Scopoli (Asteraceae), whereas the larval performance on these plants is lower than on *Adenostyles alliariae* (Gouan) (Asteraceae) (Ballabeni et al., 2001). Females of *O. elongata* were even shown to oviposit preferentially on *C. spinosissimum* close to *A. alliariae*, where the larvae can migrate from *C. spinosissimum* to *A. alliariae*, compared to isolated *C. spinosissimum*. Furthermore, the quality of a host plant may change during the larval feeding period and it could be adaptive for the larvae to move to another plant of higher quality. Larval feeding may, for example, activate induced defense mechanisms in the host plant that makes it less suitable as food (Dicke & Baldwin, 2010).

Such larval mobility may be influenced by the diet range of the species. Species that can utilize different host plants, i.e., polyphagous insects, should have a larger possibility of finding alternative host plants nearby a plant chosen by the female and thus a larger selective advantage of having mobile larvae. Accordingly, it has been shown that the correlation between female oviposition preference and larval performance is weaker in generalist species (Gripenberg et al., 2010) and that this also can correspond to the mobility of the larvae (Schäpers et al., 2016). When comparing five species of Nymphalidae butterflies in laboratory experiments, Schäpers et al. (2016) showed that larvae were intrinsically more mobile in the generalist species compared to the specialist ones. If such mobility translates to migration to other plants or plant parts, larvae of generalist species would be more active in choosing host plants for their feeding.

The Egyptian leafworm or cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is a polyphagous moth whose larvae have been found to feed on over 100 plant species from more than 40 families (Brown & Dewhurst, 1975). It is present throughout Africa, but also in a few European countries around the Mediterranean Sea, although low winter temperatures constrain its distribution (Miller, 1976; OEPP/EPPO, 2015; CABI, 2023). *Spodoptera littoralis* is considered as one of the most destructive lepidopteran pests in vegetables and ornamental plants, as it attacks many plant species and many plant parts (leaves, buds, bolls, etc.). It is the most important pest of cotton in Egypt (CABI, 2023).

Ovipositing *S. littoralis* females exhibit an innate hierarchical preference towards different host plant species and larval experience has been found to modify this oviposition innate preference towards the plant experienced (Anderson et al., 2013; Thöming et al., 2013). Using the larval experience could facilitate host plant choice, and could be a way to make host plant choice quicker and reduce risks during host plant search for the female. However, as a consequence, the eggs may not be deposited on the optimal plant in the environment from a larval development and survival point of view, and migration of the larvae from the natal plant toward a more suitable plant could be favourable for their development and survival. Feeding larvae of *S. littoralis* have been found to exhibit migration from induced plants to neighboring uninduced plants (Anderson et al., 2011) and they may detect and use volatiles from plants during feeding behaviour (von Mérey et al., 2013; Rharrabe et al., 2014). *Spodoptera littoralis* thus provides an excellent opportunity to investigate larval migration related to host plant choice.

In this study, we investigated larval host plant preference and movement in *S. littoralis*, both in the field and in the laboratory. We used two host plants showing different characteristics related to preference and performance: cotton, offering the best larval performance (Agrell et al., 2006), and alfalfa or clover, both preferred over cotton for oviposition and offering lower egg parasitism (Sadek et al., 2010). In addition, to test potential top-down effects on larval migration, we also assessed whether larval parasitism rate differs between the host plants, as was found for egg parasitism. Finally, we investigated whether life-history traits of the larval parasitoid *Microplitis rufiventris* Kokujev (Hymenoptera: Braconidae) differ between host larvae fed on different plants. Our hypothesis was that alfalfa and clover could offer larvae better protection against parasitism as observed for eggs and that larvae should thus migrate towards this plant.

MATERIALS AND METHODS

Insects

The *S. littoralis* used in this study was kept in a laboratory culture reared on an artificial diet (Hinks & Byers, 1976) based on potato instead of bean in Sweden, and originated from insects collected in Egypt in 2006. Wild-collected moths from Egypt had been introduced into the culture yearly. All stages of the moths were kept at 25°C, 70% r.h., and L16:D8 photoperiod, and adults were provided with a 10% honey solution. Pupae were collected from the diet, sexed, and the sexes were kept separately until emergence of the adults.

The parasitoids used in this study, *M. rufiventris*, were maintained in a colony (see Hegazi & El-Minshawy, 1979) in a laboratory at Alexandria University, Egypt. The colony originated from field crops, including locally grown cotton, and field-collected parasitoids were added to the rearing twice a year. Parasitized *S. littoralis* larvae were transferred to the laboratory in Alnarp, Sweden, and kept under the same conditions as the *S. littoralis* culture. Emerging adult parasitoids were kept individually and fed ad libitum with a solution of honey and water until used in the experiments.

Plants

For experiments on larval migration, cotton [*Gossypium hirsutum* L. (Malvaceae) var. Delta Pineland 90] and alfalfa [*Medicago sativa* L. (Fabaceae) var. Julus] were used. Experiments on larval parasitism by *M. rufiventris* used cotton, Egyptian clover (*Trifolium alexandrinum* L., Fabaceae), cowpea [*Vigna unguiculata* (L.) Walp., Fabaceae], and maize (*Zea mays* L., Poaceae). Plants were kept in a climatized greenhouse at 25 ± 5°C and 70 ± 10% r.h. Artificial light (Powerstar, HQI-T, 400 W/D, Daylight; Osram, Munich, Germany) was provided in addition to natural light from October until April to obtain a constant L16:D8 photoperiod. The plants were individually grown from seeds in 14-cm-diameter pots. No flowering plants were used.

For the experiments on larval parasitism, clover was used instead of alfalfa, both belonging to the Fabaceae family and thus closely related. In addition, previous studies have shown similar effects of alfalfa and clover on *S. littoralis* behaviour, especially in comparison to cotton; Anderson et al. (2013) have shown that larvae reared on artificial diet, alfalfa, and clover all give females that prefer to oviposit on either clover or alfalfa over cotton plants. Both plants are thus considered as equivalent hosts for *S. littoralis* in this study.

Larval migration

Selection of the feeding site in the field

This experiment was carried out to test the larval preference and migration under field conditions at four dates in 2009: 11 May, 8 and 24 June, and 10 July. Cotton and alfalfa plants were grown as described in Sadek et al. (2010) in two adjacent plots (each ca. 700 m^2) in a field near the city of Assiut, Egypt. Bottomless wire-mesh cages (80 × 80 × 80 cm) were placed on the border between the two crops, so that each cage covered an area of 40 × 80 cm of cotton next to an equal area of alfalfa. The upper sides of the boxes were removable to allow free access to the plants and larvae during the observation. Two batches of 150–200 eggs laid by female *S. littoralis* on paper in the laboratory were stapled onto plant leaves in each cage. Twelve cages were used in total (three cages per date), with egg batches stapled on cotton leaves in six cages and on alfalfa leaves in the other six. Starting from the expected time of hatching, larvae found on cotton and alfalfa were counted every 2nd day until day 10 after hatching. During the first two observation days, a hand-held magnifying lens was used to locate and count the larvae. However, starting from the third observation day, the larvae could be easily seen unaided.

Selection of the feeding site in the laboratory

The selection of feeding site by larvae was also tested under laboratory conditions. To obtain egg masses on the various plants, one male and one female *S. littoralis* randomly taken from the rearing were introduced in a meshed bag fixed around one cotton leaf or one alfalfa branch. When eggs were deposited, generally within the first 2 days, the couple was removed from the plant. The plant with eggs was then placed in a plastic box filled with water (to prevent larvae from escaping), surrounded with four potted plants: two cotton and two alfalfa, in alternate positions. The alfalfa plants were always elevated so that their leaves were at the same level as the cotton leaves. Leaves from neighbour plants touched each other to facilitate larval movement, but the leaf with eggs never touched any other leaves.

After 1 week, the larvae (usually at second or third instar) present on each plant were counted. All experiments (n = 10 of each treatment) were performed in a greenhouse in which temperature varied between 20 and 30°C, and r.h. between 70 and 80%, with both natural and artificial lights from October until April.

Feeding preference

Previous experiments considered the plants as a complex ensemble of aspects: food, chemistry, architecture, etc. It is not possible to distinguish between the preference in food quality with the easiness to move on a specific architecture or the protection offered by the plant structure. A control test was conducted to observe the feeding preference of young larvae when offered cut leaves from both cotton and alfalfa. Ten to 20 first instars were deposited in the center of a plastic container (22.5 × 16.5 × 7.5 cm) with a ventilated lid containing one cotton leaf on one side and two alfalfa branches on the other side, thus offering approximately the same leaf area for each host plant. After 24 h, the larvae on each plant were counted. Contrarily to previous studies looking at feeding preference of older larvae (Agrell et al., 2003), the mass or surface of leaves ingested by larvae could not be measured, as the larvae at that stage do not eat sufficiently to allow precise measures. All experiments (n = 30) were performed at 25°C, 70% r.h., and L16:D8 photoperiod.

Larval parasitism

Larval parasitism on different host species

The parasitism rates of *M. rufiventris* on *S. littoralis* larvae were evaluated both using a choice and a no-choice test. For the choice test, 10 mesh cages (120 × 80 × 60 cm) were prepared, each containing one plant of each of the host plants: cotton, clover, cowpea, and maize. Thirty newly moulted third instar *S. littoralis* were introduced on each potted host plant and three naïve mated 1-day-old *M. rufiventris* females were introduced per cage. After 24 h, the larvae from each host plant were removed and reared until either a parasitoid egressed or the larva pupated. Whenever a female parasitoid was found dead, or the number of recovered larvae was different by more than one at the end of the experiment, the replicate was discarded and another one was conducted.

For the no-choice test, the parasitism rates were obtained on cotton and clover (n = 10 per host plant). Thirty newly moulted third instar *S. littoralis* were introduced on one potted host plant in a nylon and plastic BugDorm-4180F cage (47.5 × 47.5 × 93.0 cm; Megaview Science, Taichung City, Taiwan). Three naïve mated 1-day-old *M. rufiventris* females were introduced per cage for 24 h. The larvae were then removed and reared until either a parasitoid larva egressed or the larva pupated. Whenever a female parasitoid was found dead, or the number of recovered larvae was different by more than one at the end of the experiment, the replicate was discarded and another one was conducted.

Parasitoid life-history traits

Spodoptera littoralis larvae were reared (as described above) on either cotton, clover, cowpea, or maize plants from their hatching, and exposed to *M. rufiventris* females for parasitism when they reached the third instar. One 1-day-old mated female was introduced in a Petri dish (15 × 60 mm) containing host larvae. Oviposition was observed individually and only one oviposition was allowed per host. The parasitized larvae were reared on the same host plant throughout their life, and cocoons were weighed using a Sartorius R200 microbalance (±0.01 mg; Göttingen, Germany). After emergence, adults were sexed and kept at 25°C, 70% r.h., and L16:D8 photoperiod in glass vials, with 80% honey solution, but no hosts, and mortality was noted daily until all individuals were dead, to obtain longevity.

To measure egg load at emergence and after 72 h, third instars reared on either cotton, clover, cowpea, or maize plants were parasitized as described above. Upon emergence, parasitoid females were either killed in 70% ethanol on the day of emergence (n = 10) or kept in glass vials for 72 h (n = 10), with no access to food, before being killed. Females were dissected in saline solution under a stereomicroscope at 40×, the ovaries were extracted, and the mature eggs were counted. The developing eggs of similar size (323–389 μm) were gently teased apart from the reservoir and calyx regions and counted.

Statistical analysis

Statistical tests have been conducted on R v.2.13.0 software (R Development Core Team, 2010). All conditions have been verified before performing the tests.

Larval migration

Selection of feeding site in the field

The proportion of larvae found on alfalfa in the field was tested using a generalized linear mixed model (GLMM) using the *glmer* function from the lme4 package (v.1.1.27.1; Bates et al., 2015) with a binomial distribution. The hatching plant, the time since the beginning of the experiment, and the interaction between hatching plant and time were included as fixed effects and the replicate number was included as a random effect to account for repeated measures. The interaction between hatching plant and time being significant, subsequent models were built to determine the larval distribution among plants at 4, 6, 8, and 10 days. At day 2, the model could not converge due to too many zeros, the larvae being still on their hatching plant. The *drop1* function was used to determine the overall significance of each fixed effect (Zuur et al., 2009).

Selection of feeding site in the laboratory

To evaluate whether the propensity to stay or leave the hatching plant was influenced by the hatching plant, the ratio between the number of larvae that left the hatching plant and the number that stayed was tested with a generalized linear model (GLM) using a binomial distribution, with the hatching plant as a factor.

To evaluate whether the feeding site selected (cotton or alfalfa) was influenced by the hatching plant, the ratio between the number of larvae found on neighbouring alfalfa plants and on neighbouring cotton plants was tested with a GLM with a binomial distribution, with the hatching plant as a factor. To evaluate whether the larvae leaving their hatching plant had a preference between alfalfa and cotton, the number of larvae on each plant species was compared using a GLM with a Poisson distribution.

Feeding preference

The feeding preference was evaluated by comparing the number of larvae found on alfalfa and on cotton leaves after 24 h using a GLM with a Poisson distribution.

Larval parasitism

Larval parasitism on different host plant species

The impact of plant species on the larval parasitism rate under four-choice conditions and under no-choice conditions was tested using a GLM with a binomial distribution. For those tests, the dependant variable was the ratio between the number of larvae parasitized and the number of healthy larvae, with hatching plant included as a fixed effect. Post-hoc pairwise comparison test was performed on the significant plant factor using the *emmeans* function from the emmeans package (v.1.7.2; Lenth et al., 2022).

Parasitoid life-history traits

Using a GLM with a Gaussian distribution, the impact of plant, sex, and their interaction on the cocoon mass and longevity of adults was evaluated. A model by sex was performed when the interaction between diet and sex was significant. The impact of age, diet, and their interaction on the number of eggs produced by female *M. rufiventris* was assessed with a GLM using a Poisson distribution. The *drop1* function was used to determine the overall significance of each fixed effect (Zuur et al., 2009).

RESULTS

Larval migration

Selection of feeding site in the field

Interaction between hatching plant and time since the start of the experiment significantly influenced the proportion of larvae found on alfalfa (Table 1). The subsequent models on the proportion of larvae found on alfalfa at different times indicated that the hatching plant significantly influenced the number of larvae on alfalfa at 4, 6, and 8 days. At 10 days, the model indicated that the hatching plant did not influence the number of larvae on alfalfa – whereas the proportion of larvae found on alfalfa increased with time when larvae hatched on cotton, it was stable when larvae hatched on alfalfa (Figure 1).

TABLE 1 Results of the generalised linear mixed-effect model explaining *Spodoptera littoralis* larval migration over time among host plants in the field test.

Model	Fixed effect	d.f.	F	P
Migration	Hatching plant	1	55.188	<0.0001
	Time	4	732.676	<0.0001
	Plant*time	4	533.714	<0.0001
Migration – 2 days	Hatching plant	1	N/A	N/A
Migration – 4 days	Hatching plant	1	23.596	0.0002
Migration – 6 days	Hatching plant	1	22.310	0.0002
Migration – 8 days	Hatching plant	1	8.155	0.012
Migration – 10 days	Hatching plant	1	0.679	0.44

N/A, at day 2, the model could not converge due to too many zeros.

Selection of feeding site in the laboratory

Hatching plant had an impact on the proportion of larvae leaving it, with more larvae leaving cotton than alfalfa plants (Table 2, Figure 2). The decision to migrate to an alfalfa plant or cotton plant was not influenced by the hatching plant (Table 2) and there was no significant difference between the number of larvae that migrate to an alfalfa compared to a cotton plant (Table 2, Figure 2).

Feeding preference

There were significantly more larvae recovered on alfalfa than on cotton (in total 343 vs. 147) after 24 h (Table 2).

Larval parasitism

Larval parasitism on different host plant species

Under four-choice conditions, the plant species had a significant influence on the number of larvae parasitized (Table 3): more parasitized larvae were recovered from cotton than from any other plants, followed by maize, clover, and cowpea (Figure 3A). In the no-choice test, there was a difference in the number of parasitized larvae depending

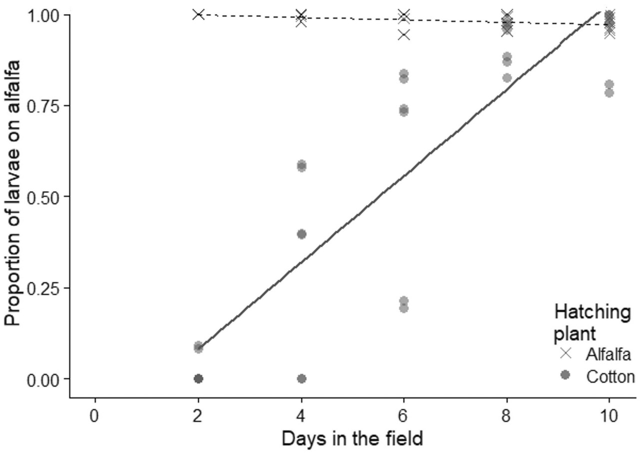

FIGURE 1 Field data showing the proportion of *Spodoptera littoralis* larvae found on alfalfa, every 2nd day until day 10 for neonates that hatched on cotton or alfalfa. The lines indicate the trends based on the raw data.

on their host plant (Table 3): all plants had an equal parasitism rate except for the larvae on maize, which were significantly more parasitized than the ones on cotton (Figure 3B).

Parasitoid life-history traits

The interaction between plant and sex significantly influenced *M. rufiventris* cocoon mass (Table 3): although female cocoons were heavier than males, the impact on cocoon mass depended on the host plant. For both sexes, plant had a significant impact on cocoon mass – the heaviest individuals came from larvae that fed on cotton, followed by clover and cowpea, which were not different, and the lightest came from larvae that fed on maize (Table 3, Figure 4A). The interaction between plant and sex significantly influenced *M. rufiventris* longevity (Table 3). Both sexes lived longer when coming from host larvae fed on cotton and cowpea compared to clover and maize, but only males lived longer when coming from larvae fed on clover compared to maize (Figure 4B). Plant and age significantly influenced females' egg load, but their interaction did not (Table 3): egg load was higher in old females (72 h) compared to younger (0 h) ones, and in females coming from larvae that fed on cotton compared to other plants; females coming from larvae that fed on maize had the lowest egg load (Figure 4C).

TABLE 2 Results of the generalised linear models explaining *Spodoptera littoralis* larval migration and feeding preference among host plants in the laboratory.

Model	Fixed effect	d.f.	z	P
Staying or leaving	Hatching plant	1	−13.840	<0.0001
Migrating on alfalfa or cotton	Hatching plant	1	−0.162	0.87
No. larvae leaving	Host plant	1	−1.076	0.29
Feeding preference	Plant species	1	−6.257	<0.0001

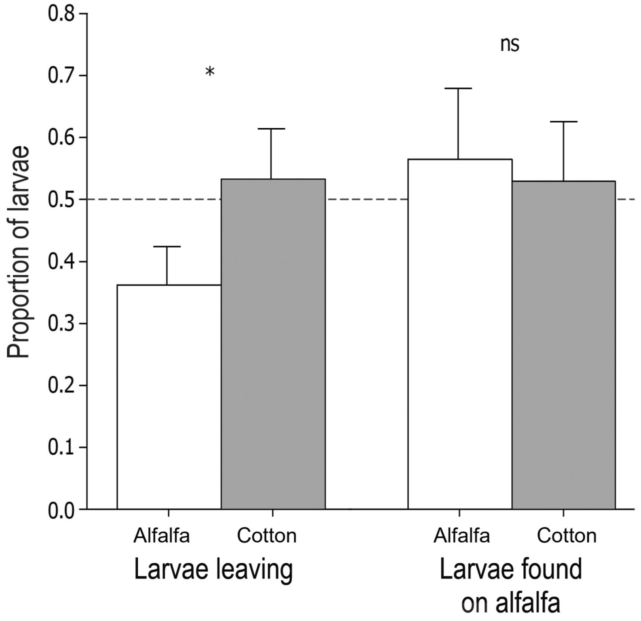

FIGURE 2 Mean (+ SD) proportion of *Spodoptera littoralis* larvae that left their hatching plant, and that are found on the nearby alfalfa plants, after 1 week in the laboratory, that had either hatched on cotton (grey bars) or on alfalfa (white bars). The random distribution of 50% is indicated by the dashed line. The asterisk indicates a significant difference between plants (F-test: $P < 0.05$; ns, $P > 0.05$).

TABLE 3 Results of the generalised linear models explaining *Spodoptera littoralis* parasitism rate and *Microplitis rufiventris* cocoon mass, longevity, and egg production.

Model		Fixed effect	d.f.	F	P
Parasitism rate	Choice	Plant	3	49.646	<0.0001
	No choice	Plant	3	4.823	0.0023
Cocoon mass		Plant	3	114.56	<0.0001
		Sex	1	382.37	<0.0001
		Plant*sex	3	17.279	0.0006
Cocoon mass – Females		Plant	3	158.06	<0.0001
Cocoon mass – Males		Plant	3	32.545	<0.0001
Longevity		Plant	3	166.413	<0.0001
		Sex	1	1441.860	<0.0001
		Plant*sex	3	40.436	<0.0001
Longevity – Females		Plant	3	166.22	<0.0001
Longevity – Males		Plant	3	31.006	<0.0001
Egg load		Plant	3	25.651	<0.0001
		Time	1	814.659	<0.0001
		Plant*time	3	2.310	0.074

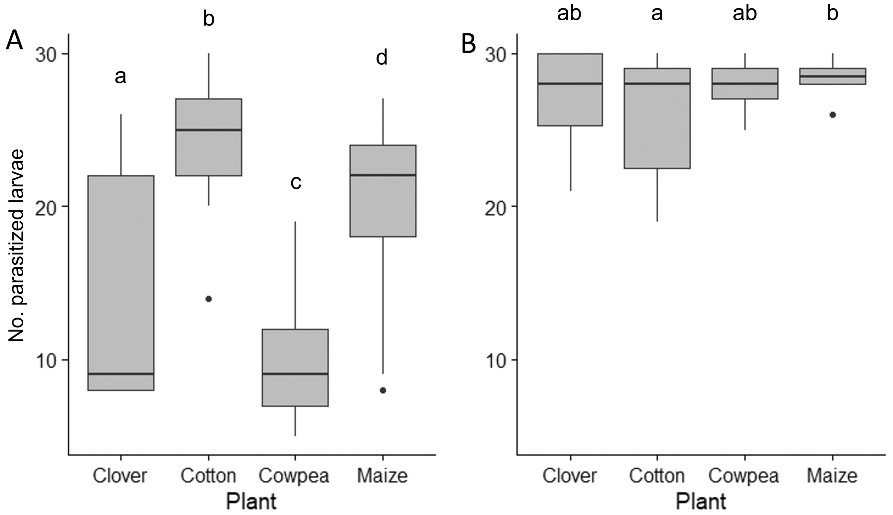

FIGURE 3 Number of *Spodoptera littoralis* larvae parasitized by *Microplitis rufiventris* depending on plant species, offered (A) in a four-choice test or (B) in a no-choice test. Within each boxplot, the line represents the median value and the boxes extend from the 25th to the 75th percentile. The whiskers represent the 10th and 90th percentiles, respectively, and dots denote outliers. Means within a panel capped with different letters are significantly different between host plants (Post-hoc pairwise comparison tests: P < 0.05).

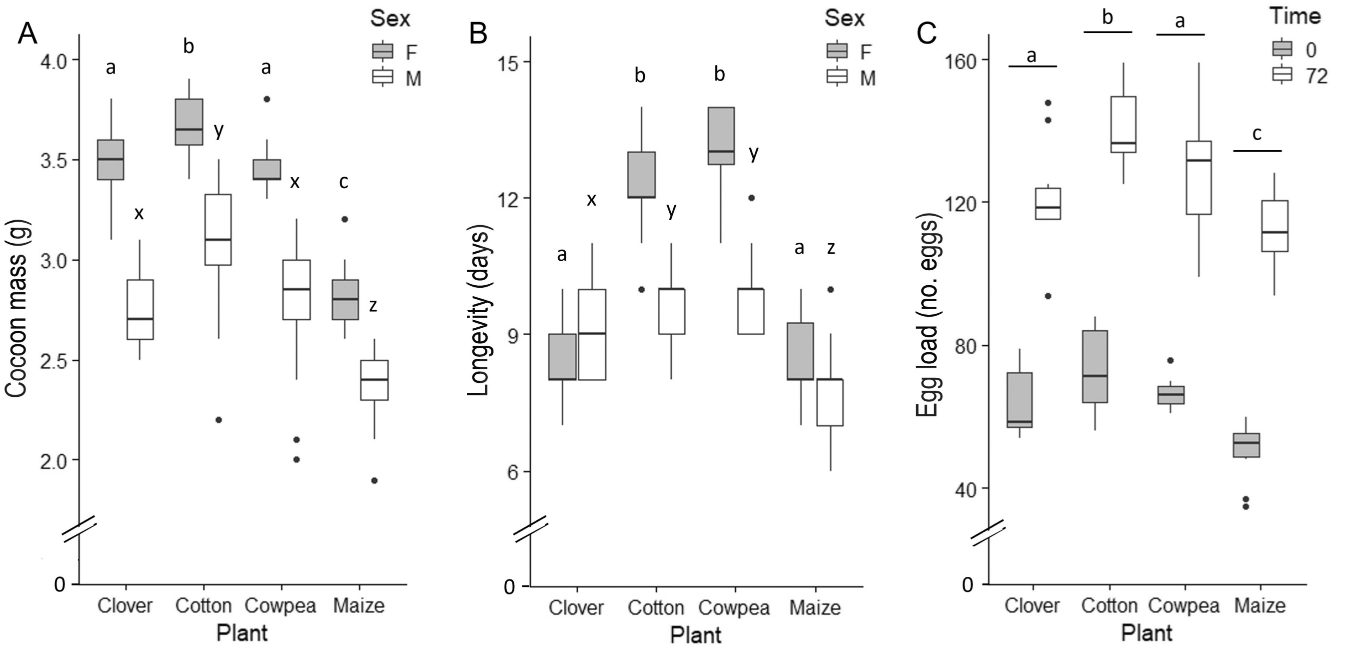

FIGURE 4 Impact of *Spodoptera littoralis* host plant on *Microplitis rufiventris* (A) female and male cocoon mass (g), (B) female and male longevity (days), and (C) egg load at emergence (0 h) and after 72 h. Within each boxplot, the line represents the median value and the boxes extend from the 25th to the 75th percentiles. The whiskers represent the 10th and 90th percentiles, respectively, and dots denote outliers. Means within a panel and within a sex (A, B) capped with different letters are significantly different between host plants (F-test: P < 0.05).

DISCUSSION

Spodoptera littoralis larvae show host-dependent directional migration from one host plant to another both in the field and in the laboratory. We found that the larvae left cotton to a higher proportion than alfalfa plants. After 10 days in the field, most larvae were found on alfalfa independently of whether the hatching plant was cotton or alfalfa. Larvae thus migrate preferentially onto alfalfa if both plants are present but need time to do so. In the laboratory, *S. littoralis* larvae preferred feeding on alfalfa leaves and, when hatching on alfalfa, to a large degree seem to stay on this plant, whereas on cotton plants they move more and migrate away from the plant. These larvae are thus sufficiently mobile to express migration behaviour and, through this non-random migration, preference for host plants.

Even though *S. littoralis* females and larvae display the same general preference for alfalfa when faced with dual choice against cotton (Sadek et al., 2010; current study), female choice of oviposition site does not always provide the best conditions for larvae. The female choice of oviposition site may also depend on host plant availability as well as female safety and experience. The 'optimal bad motherhood' principle predicts that circumstances affecting adult female performance and survival may influence the choice of oviposition site in the individual female (Mayhew, 2001).

It leads to a weaker correlation between female preference and larval performance, which is commonly found in generalist insects (Gripenberg et al., 2010). Females are thus found to deposit eggs on host plants that are not the most suitable for larval survival and larval mobility then enables larvae to choose a different plant than their mother. For example, this has been observed in *O. elongata*: the larvae actively move to their preferred plant species from where they hatch from the eggs (Ballabeni et al., 2001).

Migration is, however, a risky process: predation risk is increased while larvae move from one plant to another (Weisser, 2001). For this behaviour to be more frequent on cotton plants, the protection gained on alfalfa must compensate for increased predation risks encountered and exposure to unfavourable abiotic conditions during migration. The possibility to find enemy-free space on a neighbouring plant could be the driving factor for larvae to leave cotton and migrate to alfalfa, suggesting that predation risk during migration would be lower than that experienced during feeding. The time of exposure to natural enemies while feeding would be much longer than the relatively short time period that is needed to migrate.

Induced responses in host plants could also affect larval movement. Caterpillars of *Parnassius smintheus* Doubleday have been shown to leave their host plant, *Sedum lanceolatum* Torr., because of the plant defences that affect larval performance after a 1–2-day delay (Roslin et al., 2008). Lepidopteran larvae can detect plant volatiles (Rharrabe et al., 2014) and have been shown to be both attracted (Soler et al., 2012) and repelled (Anderson et al., 2011) by plants that are already attacked by other insects. Herbivore-induced plant volatiles (HIPVs) emitted after herbivore damage have been shown to attract parasitic wasps (Turlings et al., 1995) and have a large effect on larval survival rates. Furthermore, natural enemy attraction to HIPVs may differ between host plants and influence predation rates (Khallaf et al., 2023). In their study, ladybird beetles were found to be more attracted to HIPVs from cotton than from alfalfa. Furthermore, volatiles emitted by cotton are more attractive than volatiles from alfalfa to males and females of *Chelonus inanitus* (L.), an egg parasitoid attacking *S. littoralis* (Sadek et al., 2010). Thus, the active migration of *S. littoralis* to alfalfa, even though it is offering a lower performance than cotton (Agrell et al., 2006), could be driven by volatile cues from the host plants and lead to reduced exposure to natural enemies and mortality on alfalfa. It was shown that HIPVs from cotton had a stronger effect on nearby ovipositing *S. littoralis* females than HIPVs from alfalfa or clover (Zakir et al., 2013). Although the impact on parasitoids was not measured, it suggests a difference in HIPVs between those host plants.

The enemy-free hypothesis is also supported by our experiments with respect to the larval parasitoid *M. rufiventris*. Although larvae on cotton and clover plants were equally attacked by *M. rufiventris* in no choice test, the parasitism rate was higher on cotton than on clover in the presence of four different plants. In an environment where both plants are present, larvae would thus benefit from being on clover compared to cotton and contribute to the preference for a plant providing a lower performance. In addition, parasitoids developing in larvae fed on cotton performed better (larger cocoons, higher longevity, and higher egg load at emergence and after 72 h), making the cotton-fed larvae better hosts for the parasitoids than clover-fed larvae. Selection thus probably favours parasitoids attacking larvae on cotton over larvae on clover, creating a differential selective pressure on the larval preference. It is interesting to note that cowpea provided an even safer host plant for the larvae in the four-choice test, and that maize was generally the plant leading to the lowest fitness proxies for the parasitoids, although it was the plant with the second-most attacked larvae in choice tests. Such impacts of host plants on the performance of parasitoids had been previously shown for this system: castor oil and sweet potato leaves were more edible and suitable for *M. rufiventris* developing in *S. littoralis* compared to cotton leaves (Altahtawy et al., 1976).

Other characteristics of the plants could impact the observed higher parasitism rates on clover. For example, the architecture of the plant could also play a role (van Lenteren et al., 1995; Sütterlin & van Lenteren, 1997; Gingras et al., 2002): alfalfa and clover have small, densely stacked leaves that might lower the searching abilities of parasitoids, whereas cotton has fewer, large leaves. It was also shown that the presence of glandular trichomes in alfalfa decreases the searching behavior in the parasitoid *Anagrus nigriventris* Girault (Lovinger et al., 2000). Finally, cotton produces 12× more extrafloral nectar after damage by herbivorous insects (Wäckers & Wunderlin, 1999): such nectar provides additional nutritional resources to parasitoids (Romeis et al., 2005), which may then be attracted by this sugar-rich food. The preference expressed by adults and larvae is thus better explained from a tritrophic rather than a bitrophic perspective.

Our results confirmed that *M. rufiventris* is synovigenic, having some eggs at emergence but producing more during its adult life (Khafagi et al., 2011), although the egg loads at emergence in our study were higher than those in Khafagi et al.'s study. As in most parasitoids, *M. rufiventris* development time, egg load, longevity, and size were previously shown to be affected by the host quality, through host age or plant host (Altahtawy et al., 1976; Hegazi et al., 2007), so the observed impact of host plant on cocoon size, adult longevity, and female egg load was not surprising.

Conclusion

Spodoptera littoralis larvae are sufficiently mobile to migrate from a plant to another, but contrary to our hypothesis, they generally prefer the lower quality plant over the higher quality one, both in the laboratory and in the field. Nutritional quality is then not the major factor selecting for host plant preference in this species. Protection against natural enemies seems to favour the preference for alfalfa over cotton through enemy-free space (Jeffries & Lawton, 1984). Alfalfa offers an enemy-free space not only for *S. littoralis* larvae (this study), but also for eggs (Sadek et al., 2010).

In pest management, such a feeding-site preference could also allow attracting larvae away from the cotton crops by introducing a few rows of alfalfa or clover in a cotton field, for example, a strategy called conventional trap cropping. Trap cropping has gained interest in the last few decades because of concerns surrounding pesticide use (reviewed by Shelton & Badenes-Perez, 2006). Trap cropping in cotton has been used in North and South America, Asia, and Africa against several pest species (reviewed in Javaid & Joshi, 1995). Alfalfa as a trap crop has also been used in California (USA) to attract lygus bug pests away from cotton plants (Godfrey & Leigh, 1994).

AUTHOR CONTRIBUTIONS

Véronique Martel: Conceptualization (equal); data curation (lead); formal analysis (supporting); investigation (lead); methodology (lead); writing – original draft (lead); writing – review and editing (lead). **Fredrik Schlyter:** Conceptualization (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (lead); writing – review and editing (equal). **Medhat M. Sadek:** Investigation (lead); methodology (equal); resources (supporting); writing – review and editing (equal). **Esmat M. Hegazi:** Investigation (equal); methodology (equal); resources (supporting); writing – review and editing (equal). **Valentine Glaus:** Formal analysis (lead); visualization (lead); writing – review and editing (supporting). **Bill Hansson:** Funding acquisition (lead); project administration (equal); writing – review and editing (supporting). **Peter Anderson:** Conceptualization (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); supervision (lead); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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