Parasitoid Community of the Loreyi Leaf Worm *Mythimna (Acantholeucania) loreyi*: Novel Host–Parasitoid Associations and Their Efficiency in the Eastern Mediterranean Region of Turkey

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The loreyi leaf worm, *Mythimna (Acantholeucania) loreyi* Duponchel (Lepidoptera, Noctuidae), is a pest of gramineous crops and causes significant economic damage to maize. In field surveys on maize to determine the parasitoid community and its impact on the pest in the eastern Mediterranean region of Turkey, nine parasitoid species were found associated with immature stages of *M. loreyi:* The hymenopteran (Braconidae and Ichneumonidae) parasitoids *Cotesia (=Apanteles) ruficrus* (Haliday), *Chelonus oculator* Panzer, *Meteorus ictericus* Nees, *Hyposoter didymator* (Thunberg), *Sinophorus* sp.; and the dipteran (Tachinidae) parasitoids *Pseudogonia rufifrons* Wiedeman, *Exorista rossica* Mesnil, *Gonia picea* (Robineau-Desvoidy) and *Linnaemya vulpina* (Fallen) - the last three recorded for the first time as parasitoids of *M. loreyi* in Turkey. *C. ruficrus* was the dominant parasitoid species, being recovered from 38.5% of the larvae collected and was also the most prevalent species, existing in 91.0% of the fields in which *M. loreyi* was found. Total parasitism levels achieved by braconid species was 41.4%, by ichneumonid parasitoids 4.8%, and by tachinid parasitoids 1.9%. In a separate field experiment, seasonal population fluctuations and natural efficiency of *C. ruficrus* on *M. loreyi* were found to be 35.1% and 42.4%, respectively. Population levels of *C. ruficrus* were closely related to fluctuations in the population of *M. loreyi,* with parasitism ranging between 0 and 77.3% during the study.
KEY WORDS: *Mythimna loreyi; Cotesia ruficrus; Hyposoter didymator; Linnaemya vulpine; Exorista rossica; Pseudogonia rufifrons; Gonia picea;* Tachinidae; biological control; parasitoid; maize.

INTRODUCTION

Maize is an important crop in the Mediterranean region of Turkey, where approximately 35% of the total maize crop is grown (*ca* 600,000 ha) (1). In the last 20 years, maize production has increased for both agronomic and economic reasons, providing good income, requiring less labor than other crops, and can be sown after the wheat harvest. Pests of maize are mainly lepidopteran species, particularly the stem borers *Sesamia nonagrioides* (Noctuidae) and *Ostrinia nubilalis* (Crambidae). In addition there are several species of leaf feeders (all Noctuidae), *Spodoptera exigua*, *Spodoptera littoralis*, *Pseudoletia unipuncta* and *Mythimna loreyi,* inhabiting the maize agro-ecosystem. These pests invade maize plants from early growth stages (2–4 leaves) to pollen shedding, with the

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exception of *S. littoralis,* which is a late season pest of maize. They cause severe economic losses, especially on the second crop (late-sown) maize (10).

Current control efforts in Turkey have been focused on suppressing *S. nonagrioides* and *O. nubilalis* and occasionally the other lepidopteran pests by insecticide applications. However, due to the cryptic egg-laying and feeding behavior of these pests, particularly the stem borers, only several days are available for effective insecticide applications before pests bore into stalks and ears. Insecticide applications are not well targeted or timed, and therefore provide poor pest control, resulting in the need for repeat applications and excessive use of chemicals. Some effort has been devoted to exploring the natural enemies of the pests and possibilities of biological control against these pests. This will enable safe and permanent control of the pests and consequently satisfy environmental concerns (11,13).

Among the pest species of maize, *M. loreyi* has been subjected to intensive investigations due to its damage to maize plants in several areas of Turkey (8-10). This is particularly important on second crop maize, because it significantly reduces yields if it attacks the crop just before silking and pollination (10). *M. loreyi* has also been reported as an important pest of gramineous crops in many countries in Asia, Europe, Africa and Australia (2,4,8,18). However, there is little information available about its natural enemies and their efficiency in protecting the crop. The present study was undertaken to determine the natural enemy complex of *M. loreyi* in maize fields and overwintering sites and their role in suppressing the pest population below economic level. Understanding the natural enemy complex of *M. loreyi,* including elucidating their host range and population size in the maize agro-ecosystem, will be very valuable for developing biological control programs against this and other pests of maize.

MATERIALS AND METHODS

Field surveys Random surveys were conducted during the years 2000 through 2002 to identify natural enemies of *M. loreyi* by collecting larvae and pupae from maize fields in the eastern Mediterranean region of Turkey. No egg masses of *M. loreyi* were obtained despite efforts made to collect all preimaginal instars of *M. loreyi* on all checked plants. In each surveyed field an area of $\sim$1 ha was sampled, and all parts of 100 randomly selected maize plants, including leaves, tassels and ears, were visually checked for the presence of *M. loreyi.* All immature stages (larvae and pupae) encountered were brought to the laboratory. Larvae were placed in rearing cups containing fresh maize leaves and ears and held at 25$\pm$1°C, 65$\pm$10% r.h., 16:8 L:D, until pupal formation. Collected pupae were placed individually in plastic cups. Parasitized and unparasitized larvae and pupae were checked regularly, and parasitoids were identified and recorded by taxonomists.

Locations and fields without *M. loreyi* were excluded from the evaluation of the parasitism levels of each parasitoid and their relative abundance. The percent parasitism of each species in total recovered numbers of larvae and pupae, and proportion of each species in total parasitism (relative abundance), were calculated for each year.

Percent parasitism (*PP*) and relative abundance (*RA*) were calculated as follows:

$$PP = (L_p + P_p) \times 100/(L_t + P_t),$$

where *PP* = percent parasitism, *Lp* = parasitized larvae, *Pp* = parasitized pupae, L_t = total number of larvae, P_t= total number of pupae.

RA = (number of larvae or pupae parasitized by each parasitoid species) × 100 / (number of larvae or pupae parasitized by all parasitoid species).

Percent discovery efficiency (DE) of each parasitoid species was estimated as follows:

% DE = (number of fields where each parasitoid was found) × 100 / (total number of fields where *M. loreyi* was found).

These parameters provide information related to natural efficiency, abundance of each parasitoid species in the parasitoid complex, and presence or absence of each parasitoid species with respect to the presence of *M. loreyi.* Data were subjected to analysis of variance using SPSS 10.0. Data on percent parasitism were converted to arcsin $\sqrt{\%}$ to satisfy assumptions of normality. Means were compared by Least Significant Difference (LSD) at $P<0.05$. Percent parasitism of each parasitoid in each year was averaged over the years; thus, mean percent parasitism of *P. rufifrons* and *G. picea* were not included in comparisons, since these two parasitoids were obtained only in 2002.

Seasonal fluctuations and natural efficiency of *Cotesia (=Apanteles) ruficrus* on *M. loreyi* Preliminary results from field surveys in 2000 indicated that *C. ruficrus* was the most common parasitoid species associated with *M. loreyi* at all locations. The seasonal fluctuations and natural efficiency of *C. ruficrus* were evaluated further in a separate experiment in Reyhanlı (36°15' N, 36°30' E) in 2001 and 2002.

Pioneer 3394 hybrid maize was sown on three different dates: 5 June, 20 June and 5 July. Three field plots (7×50 m) and 1750–1800 plants per plot (at a density of 51,000–51,500 plants ha^{-1}) were used at each sowing date. Spacing between plants in a row was 20 cm and 70 cm between rows, with ten rows per plot. At each sowing 15:15:15 N:P:K fertilizer was applied at 500 kg ha^{-1}. When plants reached the 4–6 leaf stage, 500 kg ha^{-1} urea was applied for all plots. No pesticides were used on experimental plots.

Sampling was done once a week from the emergence of maize seedlings until harvesting in all plots. In each plot from five randomly chosen rows, ten adjacent plants per row were visually sampled, for a total of 50 plants per plot at each sampling period. Larvae and pupae of *M. loreyi* on all leaves and tassels of these 50 plants per plot were collected and brought to the laboratory. Larvae were reared as described above. Larvae were checked and supplied with fresh maize leaves and ears daily until pupae formation. Collected pupae were placed individually in plastic cups and checked regularly. Data on emergence of moths and parasitoids from each larva and pupa were recorded.

Tachinid parasitoids were initially identified by Prof. Dr. M. Doğanlar (University of Mustafa Kemal, Hatay, Turkey) and the species were re-identified or confirmed by Dr. Hans Peter Tschorsnig (Stuttgart Museum of Natural History, Stuttgart, Germany). Braconid and ichneumonid species were identified by Prof. Dr. Ahmet Beyarslan (Trakya University, Edirne, Turkey) and Dr. Yasemin Özdemir (PPRI, Ankara, Turkey), respectively. Voucher tachinid parasitoids are deposited in the collection of the second author, and ichneumonids and braconids are deposited in the taxonomists' collections.

RESULTS

During 3 years, a total of 2468 larvae and pupae of *M. loreyi* were collected; of these, 44.4% were parasitized. Nine species (eight in larval stage and one in pupal stage) emerged from immature stages of *M. loreyi* (Table 1). These comprised the braconids *Cotesia (=Apanteles) ruficrus* (Haliday), *Chelonus oculator* Panzer and *Meteorus ictericus* Nees; the ichneumonids *Hyposoter didymator* (Thunberg) and *Sinophorus* sp.; and the tachinids

Linnaemya vulpina (Fallen), *Exorista rossica* Mesnil, *Pseudogonia rufifrons* Wiedemann and *Gonia picea* (Robineau-Desvoidy). *L. vulpina*, *E. rossica* and *G. picea* were recorded for the first time as parasitoids of *M. loreyi* (H. P. Tschorsnig, personal communication), whereas *P. rufifrons* has already been obtained from the pest in Morocco (7). *G. picea* was accidentally recovered from the overwintering generation of the pest at pupal stage in maize stubble at one location on 24 April 2002 (Balcalı-Adana, 37'01" N, 35'21" E). The three other tachinid species are larval parasitoids and were recovered from larvae during the maize growing season.

Among the parasitoid species, there were significant differences in the mean percent parasitism (F=304.132; df= 6, 11; P<0.001). *C. ruficrus* was the most efficient parasitoid species, with parasitism ranging from 34.5% to 40.9%. It was also the most abundant and the most prevalent species, existing in 91.0% of fields from which *M. loreyi* was collected. The relative abundance of braconid parasitoid species was nearly the same in all years. Relative abundance of *C. ruficrus* in total parasitism was very similar throughout the study: 88.7% in 2000, 86.8% in 2001 and 84.6% in 2002 (Fig. 1). *M. ictericus* was less abundant, with a relative abundance of 4.0% in 2000, 5.2% in 2001 and 4.4% in 2002, and was the second most important parasitoid of *M. loreyi*. It was also recorded in 27.6% of fields and had the second highest discovery efficiency. When data were pooled over the years, total parasitism levels achieved by braconid species was 41.4%. Relative abundance of braconids parasitism was 93.0% for *C. ruficrus,* followed by 5.0% for *M. ictericus* and 2.0% for *C. oculator.*

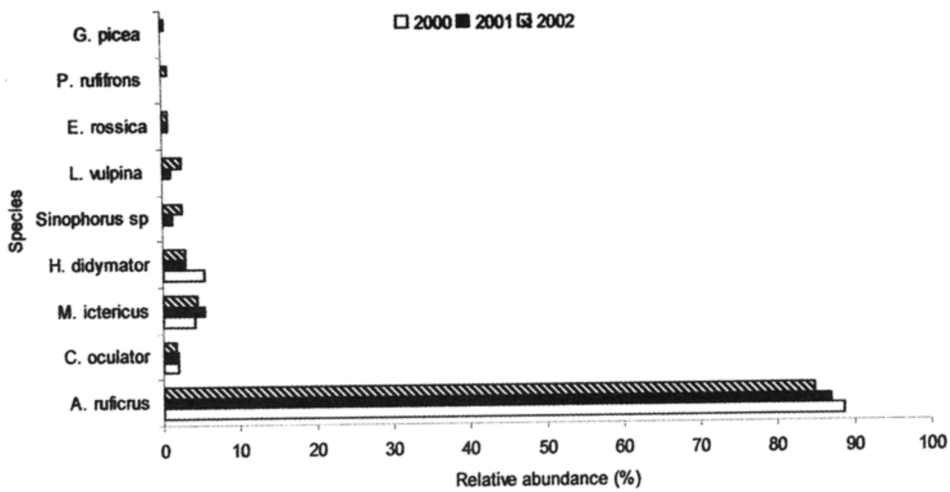

Fig. 1. Relative abundance (%) of the nine parasitoid species reared from *Mythimna loreyi.* Data from all sites were pooled.

Percent parasitism of *H. didymator*, the third most important parasitoid and the most effective ichneumonid parasitoid, ranged from 1.1% to 2.3%. It was collected in 13.8% of fields and found in every year of the study. Its relative abundance over the years was 5.3% in 2000, 2.8% in 2001 and 2.8% in 2002. *Sinophorus* sp. was less effective and abundant

TABLE 1. Summary of parasitoid species recovered from *Mythimna loreyi* on maize collected from the eastern Mediterranean region of Turkey, 2000–2002

Family	Species	Year			Overall		
		2000 (n=714)	2001 (n=970)	2002 (n=784)	% Parasitism (n=2468)	Mean parasitism[z]	DE[x] (%) (n=123)
Braconidae	*Cotesia* (=*Apanteles*) *ruficrus*	39.6	40.9	34.5	38.5	38.2±1.1 a[y] (n=3)	91.0
	Chelonus oculator	0.8	0.9	0.6	0.8	5.1±0.2 c (n=3)	7.3
	Meteorus ictericus	1.8	2.4	1.8	2.0	8.1±0.4 b (n=3)	27.6
Ichneumonidae	*Hyposoter didymator*	2.3	1.3	1.1	1.5	7.2±0.8 b (n=3)	13.8
	Sinophorus sp.	0	0.6	1.0	0.5	5.1±0.6 cd (n=2)	6.5
Tachinidae	*Linnaemya vulpina*	0	0.5	1.0	0.5	4.9±0.8 cd (n=2)	7.3
	Exorista rossica	0	0.3	0.2	0.2	3.0±0.2 d (n=2)	2.4
	Pseudogonia rufifrons	0	0	0.2	0.08	2.8 (n=1)	1.6
	Gonia picea	0	0	0.1	0.04	2.0 (n=1)	0.8

[z] Rates of each parasitoid species during the study (arcsin $\sqrt{\%}$ transformed).
[y] Means followed by a common letter do not differ significantly at $P<0.05$ (LSD test).
[x] Discovery efficiency.

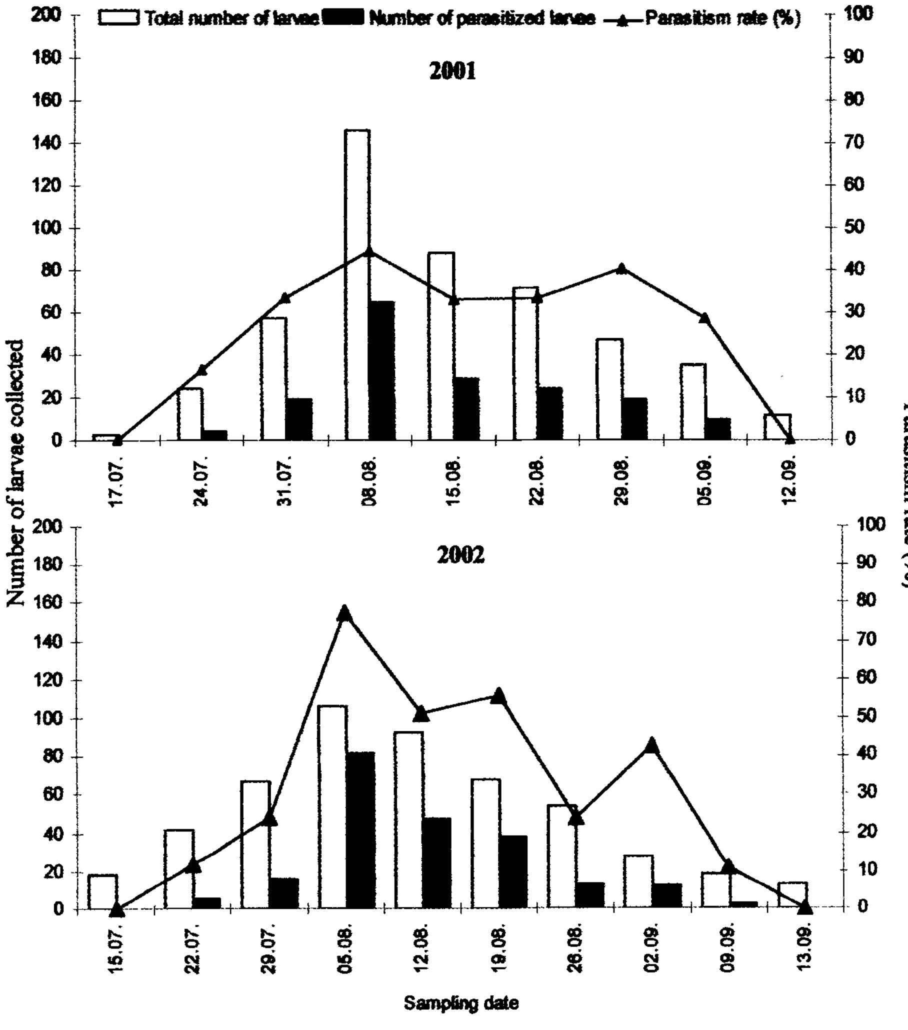

Fig. 2. Number and parasitism rates of *Mythimna loreyi* larvae collected from the experimental maize field in 2001 and 2002.

on larvae of *M. loreyi*: its parasitism rate ranged from 0.6% to 1.0%, and it was collected from 6.5% of the fields sampled. Its relative abundance in total parasitoids of *M. loreyi* larvae in 2001 and 2002 was 1.3% and 2.5%, respectively. The abundance of ichneumonid parasitoids was lower than that of braconid species, the contribution of the former in overall parasitism rate being 4.8% (Fig. 1, Table 1).

Among the observed tachinid parasitoid species, *L. vulpina* showed the highest parasitism: 0.5% and 1.0% in 2001 and 2002, respectively (it was not recorded in 2000), with a relative abundance in total parasitism of 1.0% and 2.0%, respectively; it was present in 7.3% of the fields in which *M. loreyi* was found. In respect to abundance and percent parasitism, *E. rossica* was second. It parasitized 0.3% and 0.2% in 2001 and 2002, respectively, and was recovered from 2.4% of the fields. The other two tachinid parasitoids, *P. rufifrons* and *G. picea*, were found only in 2002. They parasitized 0.08% and 0.04% and were present in 1.6% and 0.8% of the fields, respectively. The contribution of tachinid parasitoids in overall parasitism was 1.9% (Fig. 1, Table 1).

Seasonal population fluctuations and natural efficiency of *C. ruficrus* on *M. loreyi*
Natural efficiency was defined as the number of larvae parasitized by the target parasitoid species under natural conditions; it was used to understand levels of parasitism and how species interact with progressing seasonal changes. In 2001, a total of 484 larvae were found, 170 of which were parasitized, resulting in 35.1% parasitism. After the first discovery of parasitized larvae on 24 July, the parasitism rate rose to 44.5% on 8 August, when the pest populations were the most abundant. The parasitism continued until 5 September (Fig. 2).

A total of 506 *M. loreyi* larvae were collected in 2002, 42.4% of which were parasitized by *C. ruficrus*. A similar population trend was observed between *M. loreyi* and *C. ruficrus* in 2002. The first larvae parasitized by *C. ruficrus* were recorded on 22 July and the highest parasitism (77.3%) by this species was observed on 5 August, when *M. loreyi* was most abundant in the field. Later in the same season, the parasitism rate declined with the host populations until 9 September, when no parasitized larvae were found. The parasitism rate ranged from 0 to 77.3% during the course of the study. A total of 990 *M. loreyi* larvae were collected, 385 of which were parasitized by *C. ruficrus*; overall parasitism level was 38.8%.

DISCUSSION

In this study, nine parasitoid species were recovered from *M. loreyi*, resulting in 44.4% parasitism. The contribution of braconids to parasitism was much higher than that of ichneumonids and tachinids. Among braconid parasitoids, the gregarious endo-parasitoid *C. ruficrus* had the highest parasitism levels and was thus considered the most important species for natural biological control. It is a polyphagous species attacking several lepidopteran pests of maize, including *Mythimna* spp., *S. exigua*, *S. nonagrioides* and *O. nubilalis*, as well as lepidopteran pests of other crops such as alfalfa and cotton. It can survive unfavorable climatic conditions during winter by attacking different lepidopteran hosts (2,11,14,15). In addition, each female parasitoid can lay up to 250 eggs per generation (5). Due to these features, *C. ruficrus* was introduced against *Mythimna separata* in New Zealand in 1971, and since then complete biological control of *M. separata* has been achieved (12,17). It parasitized up to 95% of *M. separata* in the field, and contributed about US$4.5 million–10 million annually to New Zealand's economy (3). Likewise, *C. ruficrus* was efficiently utilized against *M. separata* in India (15).

The solitary endoparasitoid *M. ictericus* was the second most effective parasitoid species in the region, even though its parasitism rate was low (2.0%). It is a polyphagous larval parasitoid, attacking a wide range of lepidopteran hosts. This wasp was suggested as a potential natural enemy of *S. exigua* in maize (14). However, it did not provide high parasitism of *M. loreyi* in the current study.

Hyposoter didymator was the most prevalent ichneumonid species emerged from field-collected *M. loreyi* and was the third most common parasitoid species found. This solitary endoparasitoid has been used in biological control of *Helicoverpa* spp. in several crop systems. Recent developments in beneficial insect rearing technology facilitated mass rearing of *H. didymator* for release against noctuid pest species on other crops (6,19).

A total of four tachinid parasitoids were obtained from *M. loreyi* larvae and pupae. Among them, *L. vulpina, E. rossica and G. picea* are new records of the parasitoid complex of *M. loreyi*, although negligible parasitism levels were achieved by each parasitoid (<1%). *P. rufifrons* was also obtained (parasitism level of 0.08%); it was recorded previously from *M. loreyi* in Morocco (7).

Our experiments suggested that parasitism levels in each year fluctuated, reaching >75% and being closely related to variations in the host populations. Mortality levels of *M. loreyi* larvae caused by *C. ruficrus* were significant and higher in 2002 (42.4%) than in 2001 (35.1%).This could be due to many factors: the parasitoid can also exploit other lepidopteran pests of maize which appear on other crops and alternative wild host plants around the experimental fields that are also sources of nectar and pollen for natural enemies. This consequently facilitated an increase in *C. ruficrus* populations when *M. loreyi* was at low densities, and led to a higher accumulated population of the parasitoid in the following year. The impact of weeding regimes on natural enemy composition and the abundance of *M. separata* in pearl millet was adversely affected by herbicide usage, suggesting that a minimum of weeding, which does not affect the crop adversely, should be undertaken to promote the biological control of *M. separata* (16).

In our surveys and on station experiment we found no egg masses of *M. loreyi*, probably due to the fact that eggs are laid in the leaf sheaths and stems of maize plants and therefore were difficult to notice on sampled plants. The cryptic egg-laying sites provide temporal protection for *M. loreyi* from searching parasitoids. In contrast, the higher diversity of parasitoids associated with only larval stages of *M. loreyi* might be related to the feeding habits of the larvae. Larvae openly feed on leaves and tassels and are exposed to parasitoid attacks.

A high level of biological control by the complex of parasitoids was observed on *M. loreyi*. Our study suggests that reduction in insecticide applications against maize stem borers may, in fact, improve biological control efforts. For example, some of the parasitoids recovered from *M. loreyi* are polyphagous and attack other lepidopteran pests in the absence of *M. loreyi* or maize. This enhances biological control of lepidopteran pests, enables them to survive in the absence of *M. loreyi* and presumably persist throughout the whole year in crops such as maize, cotton and soybean, which are the main crops grown in the region. Furthermore, several noctuid and pyralid stem borers, such as *S. exigua, S. nonagrioides, P. unipuncta* and *O. nubilalis,* are identified in the maize agro-ecosystem during both growing seasons. These species may possibly serve as secondary hosts for either *C. ruficrus* or *H. didymator* (10,11,14) when the *M. loreyi* population is scarce.

Although *M. loreyi* has caused significant crop losses to maize in Turkey (9-11), recently it has not been considered an economically important pest and/or a target of insecticide applications, which may be thanks to the natural biological control achieved by the diverse parasitoid complex. Our findings suggest that an integrated pest management approach including natural conservation, augmentative releases of parasitoids, proper and selective insecticide usage, would enhance the efficiency of natural enemies inhabiting the maize agro-ecosystem. Further research improving biological control of maize stem borers is urgently needed, in addition to the current efforts on plant breeding for resistance or tolerance mechanisms, and development of economic damage levels for timely insecticide applications. Mass production and utilization of the stem borers' egg parasitoids in biological control of the pests by augmentative releases may result in reduced insecticide use that would in turn enhance the efficiency of the parasitoid community inhabiting maize. These are targets of our future work.

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