Field Evaluations of Parasitoids of Larval Stalkborers Against *Eoreuma loftini* (Lepidoptera: Pyralidae) in Rice[1]

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ABSTRACT Five species of parasitoids of larval stalkborer were evaluated in Texas in 1987 against the Mexican rice borer, *Eoreuma loftini* (Dyar), in field enclosures in rice, *Oryza sativa* L. The braconids, *Alabagrus stigma* (Brullé) and *Allorhogas pyralophagus* Marsh, and the bethylid, *Goniozus natalensis* Gordh, each parasitized >5% of the available *E. loftini*. The braconids, *Apanteles minator* Muesebeck and *Macrocentrus prolificus* Wharton, parasitized <1% of the available hosts. *Allorhogas pyralophagus* parasitized the highest percentage of borers, including 65.6% of the fifth- and sixth-instar *E. loftini*. *Alabagrus stigma* and *G. natalensis* parasitized fewer borers overall, although parasitism by *A. stigma* averaged 21.2% of small *E. loftini* in the rice leafsheaths. *Alabagrus stigma, A. pyralophagus,* and *G. natalensis* are candidates for further evaluation in the event that *E. loftini* becomes a pest of rice in Texas.

KEY WORDS Parasitoid, biological control, stalkborer, Braconidae, Bethylidae.

The Mexican rice borer, *Eoreuma loftini* (Dyar), recently expanded its geographic range from western Mexico (van Zwaluwenburg 1926, 1950, Box 1953) into eastern Mexico and southern Texas (Johnson 1981). Since its discovery in the Lower Rio Grande Valley of South Texas, *E. loftini* has been a major pest of sugarcane (interspecific hybrids of *Saccharum*) and a minor pest of corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* Moench) (Browning et al. 1989). *Eoreuma loftini* adults and immatures were discovered during surveys near Corpus Christi, in the coastal bend of Texas, in 1985. This indicted that range expansion was continuing northward and eastward in Texas. In 1987, *E. loftini* adults were first recorded from pheromone traps in Victoria and Jackson counties, at the southwestern edge of the Texas rice (*Oryza sativa* L.) belt. Although the range expansion of *E. loftini* has since slowed, it still represents a threat to the rice-producing areas of Texas and Louisiana (Browning et al.

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1989). Insecticides applied for control of *E. loftini* in sugarcane are ineffective, typically providing <60% damage reduction at labelled rates (Johnson 1985, Pfannenstiel et al. 1990a,b, Meagher et al. 1994). In view of these problems, studies were conducted to discern whether several exotic parasitoids of larval stalk borers would parasitize *E. loftini* in rice under field conditions. Successful parasitoid species could then be targeted for more intensive studies as potential biological control agents of *E. loftini* should it become a pest in rice.

Materials and Methods

Parasitoids of larval stalkborers (Table 1) were obtained from laboratory cultures used for field release and evaluation against *E. loftini* in sugarcane in Texas (Browning et al. 1985, Pfannenstiel et al. 1990). All parasitoids were reared in the laboratory at Weslaco, TX on E. loftini or the sugarcane borer, *Diatraea saccharalis* F. (Lepidoptera: Pyralidae), using methods similar to those described by Smith et al. (1993). All host insects were reared at the USDA, APHIS facility in Mission, TX using the methods of Martinez et al. (1988).

A 0.6-ha plot of flooded rice (cv. 'Lemont') was maintained in Hidalgo Co., TX, during the summer and fall of 1987, for field studies. After maturation of the first crop, a ratoon crop was allowed to grow without harvesting the first crop to ensure high densities of *E. loftini.* Field enclosures (0.4 × 0.4 × 0.85 m, organdy with wood frame) were placed over ratoon rice containing *E. loftini* larvae, and mated female parasitoids of each species were released into the enclosures. One species of parasitoid was released per enclosure with the number of female parasitoids released varying from 10 to 40 depending on species (Table 2). The number of parasitoids released per cage was determined by the lifespan, size, and availability of the parasitoids. *Apanteles minator* Muesebeck (Hymenoptera: Braconidae) and *Macrocentrus prolificus* Wharton (Hymenoptera: Braconidae) adults live about 3 days and 2 days, respectively, so the number of females per cage was increased from 20 to 40, and these females were placed in the cages within several hours of emerging. Fewer females of the large *Alabagrus stigma* (Brullé) [=*Agathis stigmatera* (Cresson)] (Hymenoptera: Braconidae) were available, so only 10 females were released per cage. Twenty females of both *Allorhogas pyralophagus* Marsh (Hymenoptera: Braconidae) and *Goniozus natalensis* Gordh (Hymenoptera: Bethylidae) were released per cage. Adults of *A. stigma, A. pyralophagus,* and *G. natalensis* are long-lived and had either a preoviposition period to complete or needed time for mating. Therefore, only 5- to 10- day-old individuals were released. All parasitoids (except G. natalensis) were reared in the laboratory at two to five times the density used in the field cages, with no signs of adult interference or superparasitism affecting reproduction.

Enclosures were randomly assigned a location in a 10 × 10 m area within the rice field as parasitoid females were available for release. Each enclosure was removed 1 wk after parasitoid release, and the rice stems within were destructively sampled. *Eoreuma loftini* larvae were dissected from rice stems, placed in 30-ml plastic cups containing a modified soybean-wheatgerm diet (Shaver and Raulston 1971) and held at 26 ± 1° C. Borer size and location in

Table 1. Larval parasitoids evaluated against *E. loftini* in rice, 1987.

Parasitoid species	Original Host	Country of Origin
Braconidae		
Alabagrus stigma (Brullé)	*Diatraea* spp.	Bolivia
Allorhogas pyralophagus Marsh	*Eoreuma loftini*	Mexico
Apanteles minator Muesebeck	*Diatraea* spp.	Bolivia
Macrocentrus prolificus Wharton	*Diatraea* spp. *E. loftini*	Mexico
Bethylidae		
Goniozus natalensis Gordh	*Eldana saccharina* Walker	South Africa

the plant at time of collection were recorded. Larval size was defined as small (1st and 2nd instars), medium (3rd and 4th instars), or large (5th and 6th instars). The proportion of larvae that was parasitized, died from unknown causes (i.e., including handling damage and parasitoid-induced mortality), or that eclosed as adults was determined after 8 wk. Field enclosure studies were conducted from October to December 1987. A cold front which caused dieback of the rice prevented completion of the four replicates planned for all parasitoid species. More was known about *A. pyralophagus* and its ability to parasitize its natural host, *E. loftini,* in the field; therefore, it was given the least priority in replicating field cages and had only two replicates completed when the rice was killed.

The relationship between *E. loftini* larval size and location within the rice plant was analyzed using the chi-squared test for independence (SAS Institute 1985). Proportional survival, parasitism, and mortality caused by each species and the number of hosts parasitized per female per day were analyzed using an unbalanced analysis of variance due to unequal replication among parasitoid species, with means separated by a protected Fisher's LSD test (Proc GLM, SAS Institute 1985). For analysis, all proportions were arcsine transformed and then backtransformed for presentation. The number of hosts parasitized per female per day was analyzed to account for the varying number of females per cage and differences in female lifespan. Non-age related mortality of adult parasitoids within the cages was assumed to be negligible. Effect of host size and location on parasitism was analyzed for each parasitoid causing >5% parasitism using Maximum-Likelihood Analysis of Variance for categorical data to examine main effects and interactions (Proc CATMOD, SAS Institute 1985). Medium and large size classes were combined for *A. pyralophagus* and *G. natalensis* to eliminate zero counts for medium and large borers in the model.

Table 2. Parasitism of *E. loftini* larvae in rice by five exotic parasitoid species.

Parasitoid Species	Females per Cage	Cages	Hosts per Cage	Percent			Hosts parasitized
				Survival	Parasitism	Mortality**	no. / female / day
Alabagrus stigma	10	4	50.5 ± 17.1	66.0 ± 6.2 b	8.6 ± 3.0 b	25.4 ± 7.2 a	0.057 ± 0.011 b
Allorhogas pyralophagus	20	2	54.0 ± 18.3	22.8 ± 15.6 a	45.2 ± 27.6 a	32.0 ± 12.0 a	0.146 ± 0.015 a
Apanteles minator	40	3	65.0 ± 21.6	74.3 ± 8.7 bc	0 c	25.7 ± 8.3 a	0 d
Goniozus natalensis	20	3	32.7 ± 12.1	70.5 ± 9.2 bc	11.6 ± 3.2 b	17.9 ± 6.8 a	0.026 ± 0.008 c
Macrocentrus prolificus	40	3	24.0 ± 10.1	84.7 ± 13.0 c	0.80 ± 1.3 c	14.5 ± 14.7 a	0.006 ± 0.009 d

* Means (± SD) within a column followed by the same letter are not significantly different by protected Fishers' LSD, $P > 0.05$.
** Mortality is in addition to verified parasitism.

Although parasitoid-induced mortality and mortality caused by handling could not be separated, the effect of parasitoid species on borer mortality can be inferred because background mortality should be similar across all cages.

Results and Discussion

The location of *E. loftini* larvae on rice plants varied with larval size (χ^2 = 56.5, df = 2, $P < 0.001$). The proportion of larvae found between the plant stalk and a leafsheath was 37.5% for small larvae, compared to only 5% for large larvae. Parasitoid success may vary with the availability of appropriately sized hosts in suitable locations. *Alabagrus stigma* and *M. prolificus* probe within the leafsheaths and damaged areas of plants to lay eggs on smaller host larvae; movement into the stalk may protect larvae from these parasitoids while exposing them to parasitism from others.

Four of the five species of parasitoids successfully parasitized and developed to adulthood on *E. loftini* in rice, although these species differed in the level of parasitism observed (F = 18.24, df = 4, 10, $P < 0.001$) (Table 2). *Apanteles minator* was the only unsuccessful species, although there was only one case of successful parasitism by *M. prolificus*. The other three parasitoid species, *A. stigma, A. pyralophagus,* and *G. natalensis,* were generally more successful, each parasitizing >5% of all available hosts. In addition, approximately 2% of the hosts recovered were parasitized by a naturally-occurring egg-larval parasitoid, *Chelonus sonorensis* Cameron (Hymenoptera: Braconidae); these individuals were not included in the analysis. Larval mortality from factors other than successful parasitism was not significantly different among borers exposed to the five parasitoid species examined (F = 1.22, df = 4, 10, P = 0.361). Analysis of larvae parasitized per female per day indicated only a slightly different pattern than overall percent parasitism with *A. stigma* parasitizing significantly more hosts per female per day than *G. natalensis* (F = 85.36, df = 4, 10, $P < 0.001$) (Table 2). Survival of the *E. loftini* larvae to adulthood was significantly different across parasitoid species (F = 7.17, df = 4, 10, $P < 0.01$). Only 22.8% of all larvae survived to become adults when exposed to *A. pyralophagus* as compared to 66.0 to 84.7% of larvae exposed to the other four parasitoid species.

Allorhogas pyralophagus, a gregarious external parasitoid, parasitized 45.2% of all available hosts, significantly more than any other parasitoid tested (Table 2). *Allorhogas pyralophagus* reached its host by drilling through either the leafsheath or the stalk and attacked all host instars. Parasitism by *A. pyralophagus* was significantly higher in medium and large borers, and was independent of location (χ^2 = 6.97, df = 2, $P < 0.05$) (Table 3). A large proportion of the mortality of smaller borers (about 30% of observed mortality) within the *A. pyralophagus* cages appeared to be parasitoid-induced without parasitoid progeny production even though overall mortality was not significantly higher. Paralysis of hosts by *A. pyralophagus* without apparent oviposition was commonly observed when the borers were collected, particularly in the smaller hosts. Paralyzed hosts were identified by their flaccid condition typical of that which has been observed in laboratory studies (Melton and Browning 1986). *Allorhogas pyralophagus* is established in the Lower Rio Grande Valley

Table 3. Effect of larval size and location on percent parasitism and mortality of *E. loftini* exposed to three parasitoids in rice.

Eoreuma loftini		*Agathis stigmateris*			*Allorhogas pyralophagus*			*Goniozus natalensis*		
Size	Location*	n	Parasitism	Mortality	n	Parasitism	Mortality	n	Parasitism	Mortality
Small	1	51	9.8	29.4	30	10.0	46.7	12	16.7	16.7
	2	33	21.2	30.3	12	8.3	66.7	13	0	30.8
Medium	1	51	3.9	27.5	32	50.0	28.1	32	15.6	18.8
	2	28	3.6	21.4	2	0	100.0	7	0	0
Large	1	35	2.9	28.6	32	65.6	15.6	34	11.8	8.8
	2	4	0	0	0	–	–	0	–	–

* Location: Either (1) inside rice stem or (2) between rice stem and leafsheath.

(Hawkins et al. 1987, Smith et al. 1987), but percent parasitism in sugarcane has generally been <5% (H. W. Browning, unpublished data). In sugarcane, Hawkins et al. (1987) showed that some *E. loftini* were unavailable for parasitism due to the short ovipositor length of *A. pyralophagus* and large diameter of the sugarcane stalk. In rice, however, *A. pyralophagus* may parasitize a much larger proportion of the borers present due to the small stem diameter. This hypothesis was supported by the high level of parasitism observed in this study.

Macrocentrus prolificus is the only parasitoid in this study, other than *A. pyralophagus,* which has been recovered from *E. loftini* in Mexico; however, only one of 72 potential hosts recovered in this study was parasitized (Table 2). *Macrocentrus prolificus* is a polyembryonic internal parasitoid which attacks primarily first and second instars feeding in the whorl and leafsheaths of gramineous plants (Smith et al. 1993). Adult *M. prolificus* only live about 2 days, limiting the time in which a female must find a host. Therefore, the number of acceptable hosts in suitable locations encountered by the *M. prolificus* females within the cages may have been limited. In an unenclosed situation, females will be able to forage farther, so although this study suggests that *M. prolificus* will not be effective under field conditions, further study should be considered.

Goniozus natalensis and *A. stigma* also were successful, parasitizing 11.6 and 8.6%, respectively, of *E. loftini* larvae within test enclosures (Table 2). All *E. loftini* parasitized by the gregarious external parasitoid *G. natalensis* were inside the rice stalk (Table 3). There was no significant difference in parasitism by *G. natalensis* due to host size or location (χ^2 = 1.86, df = 2, P = 0.40), although this may be the result of relatively low sample sizes. In sugarcane, G. *natalensis* entered host tunnels by digging through the frass left by the host (R. S. Pfannenstiel, personal observation). In rice, it is unknown how *G. natalensis* females entered the stalks.

Alabagrus stigma was most successful in parasitizing early instars in the leafsheaths (21.2%) (Table 3). There was a significant interaction between host size and location and their effects on parasitism (χ^2 = 24.03, df = 4, P < 0.001). *Alabagrus stigma* searched by probing between the leafsheaths and the stalks or into damaged areas of the stalk to locate hosts. Once a host was located, a single egg was laid inside the host and the parasitoid developed internally until exiting the host just before pupation. Searching by *A. stigma* is similar to that of *M. prolificus*, although *A. stigma* has a longer ovipositor than *M. prolificus* (15-20 mm vs 5-7 mm, respectively) (Smith et al. 1993). *Alabagrus stigma* already occurs in rice-producing areas of Texas on *D. saccharalis*, where it has been recovered from both rice and corn (Authors' unpublished data).

Apanteles minator was not recovered from any *E. loftini* during this study. *Apanteles minator* reaches potential hosts by travelling up tunnels produced by the host larvae. Unlike the natural hosts of *A. minator,* which push frass from their tunnels, *E. loftini* does not clear its tunnels of the moist frass. Frass-filled tunnels left by *E. loftini* may be a barrier to *A. minator* and other parasitoids of stalkboring lepidoptera which use larval tunnels to reach potential hosts (Smith et al. 1993).

Three of the five species examined, *A. pyralophagus, A. stigma* and *G. natalensis*, demonstrated potential as biological control agents of *E. loftini* in rice. Of these, the coevolved parasitoid, *A. pyralophagus,* appears most promising, attacking all stages of the host throughout the rice plant. Parasitism by *A. pyralophagus* was 65.6% on large *E. loftini* larvae, in addition to an undetermined amount of additional parasitoid-induced mortality. The two other successful parasitoids, *A. stigma* and *G. natalensis*, have no previous evolutionary history with *E. loftini,* although their coevolved hosts occupy similar ecological niches. In the event that *E. loftini* becomes a pest in rice, these three parasitoid species are good candidates for further research and colonization attempts.

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