Seasonal changes in infestation level of sugarcane by the pink borer, *Sesamia inferens* (Lepidoptera: Noctuidae), in relation to a parasitoid, *Cotesia flavigena* (Hymenoptera: Braconidae), on Okinawa Island

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(Received 22 January 1999; Accepted 7 June 1999)

Abstract

Infestation of sugarcane by the pink borer, *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae), parasitism of the pink borer by *Cotesia flavigena* (Cameron) (Hymenoptera: Braconidae) and host-parasitoid relationships were examined on Okinawa Island over a period of 4 years. Bimonthly surveys indicated that infestation by the pink borer had two peaks, one in April/May (20.5 to 29.3%) and the other, in August (22.2 to 29.5%), whereas parasitism of the pink borer had only one peak in August (61.2 to 80.1%). The density of *C. flavigena* at time *t* depended most strongly on the density of the pink borer at time *t* (r = 0.77 to 0.84), whereas, the density of the pink borer depended most strongly on the density of *C. flavigena* at time *t* - 3, i.e., 1.5 months earlier (r = -0.29 to -0.44). It was notable that the mean number of parasitoids that emerged from a single host varied seasonally, being smallest in February (17.6 to 26.8) and largest in August (48.8 to 59.2). A positive correlation between the mean number of emerged parasitoids and the mean temperature in each month (r = 0.87) suggested that the number of eggs oviposited by a female per host was influenced by temperature.

Key words: *Sesamia inferens, Cotesia flavigena*, infestation level, parasitism level, host-parasitoid relationship

INTRODUCTION

The pink borer, *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae), has been reported as a borer pest of sugarcane, *Saccharum officinarum* L. in Pakistan, India, China, Taiwan, The Philippines, Indonesia, Solomon Islands (Rao and Nagaraja, 1969) and in the southern part of Japan (Nagatomi, 1972) including Okinawa (Takara and Azuma, 1968; Azuma and Oshiro, 1969; Azuma, 1977). When the pink borer attacks young plants, the characteristic “dead-heart” (destruction of central meristem leading to desiccation of the youngest leaf) symptom appears (Rao and Nagaraja, 1969); in older plants, impaired growth and desiccation of the attacked stems occur (Metcalf and Breniere, 1969). Although infestation levels of sugarcane during different seasons have been reported (Azuma, 1977), no population studies on this pest have been carried out throughout an entire year. Azuma (1977) also reported a 42-day larval period of the pink borer using 2 sugarcane varieties, *Yomitanzan* and *NCo 310*, without mentioning any seasonal variation in the larval period. Thus, no detailed information about the life cycle of this insect, especially the number of generations per year in Okinawa, has been available.

*Cotesia (=Apanteles) flavigena* (Cameron) (Hymenoptera: Braconidae) has been reported as a dominant parasitoid of the pink borer in Okinawa (Takara and Azuma, 1968; Azuma and Oshiro, 1969). However, its role in the population dynamics of the pink borer remains unclear because of the lack of data on seasonal changes in parasitism of the pink borer by *C. flavigena*. In order to exploit *C. flavigena* in control programs against the pink borer, information on the infestation levels of sugarcane by the pink borer and host-parasitoid relationships is a prerequisite. Thus in this study,
we first evaluated the economic importance of the pink borer based on the infestation levels in sugarcane throughout the year and the potential of *C. flavipes* as a bio-control agent. Second, we analyzed the relationships between the density of the parasitoid and the infestation of the sugarcane in light of host-parasitoid relationships. Finally, we examined the seasonal differences in the numbers of emerged parasitoids per host and provide some explanation for shifts in the density of parasitoids.

**MATERIALS AND METHODS**

The field survey was carried out in two sugarcane (var. NCo 310) fields on Okinawa Island from 1994 to 1997. Field 1 (42 m × 38 m) was located on the campus of the University of the Ryukyus. It was surrounded by fields of other crops and was composed of 36 lines, each containing 86-149 sugarcane stalks/tillers. Field 2 (32 m × 30 m) was located outside the campus, surrounded by residential areas as well as fields of other crops. It was ca. 1 km away from Field 1 and was composed of 28 lines, each containing 69-132 sugarcane stalks/tillers. Infestation of sugarcane by the pink borer occurs throughout the year (Azuma, 1977). Consequently, sampling was carried out twice a month. However, we suspended the survey in January each year when the sugarcane was harvested, and resumed in February when new tillers emerged from the remaining portion of the ratoon stalks. Available sugarcane stalks or tillers (>50 cm in height) in each odd- or even-numbered line were counted alternately in both fields, and thereafter all the plants showing dead-heart symptom and/or those which had a larval hole were removed from the base. They were split open with pruning scissors and categorized into 4 groups based on the following criteria: (1) fresh frass with larva(e); (2) fresh frass with no larva; (3) old and decomposed frass with no larva; and (4) infestation scars only. A stalk/tiller with criterion 1 or 2 was considered as being newly infested, i.e., infestation had occurred since the last survey. Those with category 3 or 4 were considered as older infestations; i.e., infestation had occurred prior to the last survey. To avoid over-estimation of the percent infestation of sugarcane for each survey, we used only the newly infested stalks/tillers (criterion 1 and 2). The infested portion of the stalk/tiller with the larva(e) was separated from the healthy part of the plant with a knife and brought to the laboratory. The larvae were reared individually in a plastic jar (10 cm × 6 cm) with a piece of fresh sugarcane stem under constant conditions of 27.5 ± 0.5°C and 12L:12D photoperiod. Each larva was checked once a day until the parasitoid larvae emerged from the pink borer or the host insect pupated. When all the emerged parasitoid larvae formed cocoons, the host was dissected under a microscope and dead final stadium parasitoid larvae were counted. Thus, percent infestation of the sugarcane by the pink borer, percent parasitism of the pink borer by *C. flavipes*, and the numbers of emerged and dead parasitoids per host were determined.

Because the number of available tillers of the sugarcane in each field varied during each survey, ranging from 435 to 846, the numbers of pink borer and *C. flavipes* in each survey were transformed into numbers per tiller, i.e., density of the host and the parasitoid. Although, well known prey-predator models, such as Nicholson and Bailey (1935), are based on discrete generations of a prey and its predator, the generations of the pink borer are overlapping (Kalra and Srivastava, 1964). Thus, such models are not applicable for analyzing host-parasitoid relationship in the present study. Therefore, host-parasitoid relationship was analyzed by Pearson correlation, *r*, between the density of *C. flavipes* at survey time *t* and the density of the pink borer at *t* + 1. Surveys were carried out twice a month so that the number of surveys was 22 (11 months) in each year. We changed *i* from −6 to 6 so that a minimum sample size of 16 was obtained.

To determine the effects of weather conditions on the number of adult *C. flavipes* that emerged per host, the relationship between the mean numbers of emerged parasitoids and the mean values of each factor of weather from the previous survey to the present one was examined. Weather data from the Monthly Weather Report 1993-1997 (*Kishou Geppou*) by the Japan Weather Association were used.
RESULTS

Seasonal fluctuations of infestation of sugarcane by the pink borer, parasitism of the pink borer by *C. flavipes*, density of the pink borer, and density of *C. flavipes* were not significantly different between Field 1 and Field 2 (Kolmogrov-Smirnov's test, for infestation: $\chi^2=2.16$, $p=0.68$; for parasitism: $\chi^2=1.77$, $p=0.37$; for density of the pink borer: $\chi^2=2.07$, $p=0.51$; for density of *C. flavipes*: $\chi^2=2.35$, $p=0.54$). Therefore, data from the 2 fields were combined and are shown in Fig. 1. A total of 546 tillers were available, and 5.5% (30 tillers) were infested by the pink borer in the first survey of 1994 (Fig. 1A). It reached the 1st peak in April (28.7%) and the 2nd in August (29.7%). A similar pattern was recorded in the subsequent 3 years, where a first peak occurred in April 1995 (24.4%)/May 1996-1997 (29.3% and 20.5%) and the second peak in August 1995-1996 (26.1% and 29.5%)/July 1997 (22.15%). Changes in the density of the pink borer per tiller (Fig. 1B) had almost the same trend as the percent infestation, and the 2 parameters were strongly correlated ($r=0.95$, $p<0.0001$).

Figure 1C shows seasonal changes in percent parasitism of the pink borer by *C. flavipes*. A total of 56 pink borer larvae were obtained from the 30 infested tillers in the first survey in 1994, and 35.7% (20) of the larvae were parasitized. Parasitism decreased to 9.1% in March, increased up to 79.6% in August, and then decreased until December. Similar patterns were obtained in the following 3 years. Thus, the minimum level of parasitism (12.3-16.4%) was observed in March, and the maximum (61.2-80.1%) in August. Figure 1, A and C, also shows that the rate of parasitism had only one distinct peak despite two peaks in the level of infestation by the pink borer. Figure 1D shows that the density of the parasitoid reached a peak in April-June (5.1-5.9) and another in August (7.8-9.9). It is notable that the density of the parasitoid decreased sharply after August except in 1997.

Figure 2 shows the correlation between the density of *C. flavipes* at survey time $t$ and the density of the pink borer at $t+i$, when $i$ changed from $-6$ to 6. The left side of the

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**Fig. 1.** Seasonal changes in percent infestation of sugarcane by the pink borer (A); mean number of pink borer per tiller of sugarcane (B); percent parasitism of pink borer by *C. flavipes* (C); and mean number of *C. flavipes* per tiller of sugarcane (D) on the Island of Okinawa. Arrows indicate harvest of sugarcane.
host with time delay \((i>0)\). In 3 out of the 4 years of the study, the correlation was negative at \(i=2\) and all became negative from \(i=3\) to 5. The strongest negative correlation was obtained at \(i=3\) \((r=-0.29\) to \(-0.44, p<0.0001)\). Consequently, \(C.\ flavipes\) is considered to be an important factor in the control of the pink borer.

Figure 3 shows the seasonal changes in the mean number of adult parasitoids that emerged per host. The mean (±SD) number was recorded as 18.9 (±1.9) during the 1st survey in February, increased to 59.2 (±4.3) in June, and then decreased to 31.9 (±3.7) in November 1994. Such seasonal changes in the mean number of emerged adults also occurred in the following 3 years; that is, a minimum level in February or March (17.6-26.8), and a maximum level in June, August or September (48.8-51.1). In contrast to the sharp decrease in the density of the parasitoid after the peak in August (Fig. 1D), the decreases in the mean numbers of emerged adults were gradual throughout the survey.

It is notable that the ratio of the maximum to the minimum number of emerged adults per host in each year ranged from 2.4 to 3.1. Thus, we examined the relationship between the mean number of emerged parasitoids and some weather factors, i.e., relative humidity, precipitation, wind velocity and temperature. We obtained a strong positive correlation between the mean number of emerged parasitoids \((Y)\) and temperature \((X)\) \((Y=-1.56+1.67X, r=0.87, p<0.0001)\) (Fig. 4, top). However, no significant correlation was obtained with the other factors.

The number of emerged parasitoids may change when the number of eggs deposited per host changes with host density. If a parasitoid oviposits fewer eggs when the host density is high, and more eggs when it is low, we expect a negative correlation between host density and the mean number of emerged parasitoids. However, a positive correlation \((r=0.20, p<0.0001, n=88)\) was obtained. Another possibility may be that mortality in the immature
stage changes with temperature. In this case, we also expect a negative correlation between temperature and mortality. However, it is impossible to record the mortality of each immature stage of an endoparasitoid such as *C. flavipes*, because it requires dissection of the host, and the parasitoids can not survive within a dead host. Consequently, we used the percent of dead final stadium parasitoid larvae, which could not emerge from the hosts, as a mortality parameter. As expected, a strong negative correlation was obtained ($r = -0.91, p < 0.0001, n = 44$) between mean temperature and the percent of dead parasitoid (Fig. 4, bottom) larvae. However, the mortality was very low and never exceeded 5% irrespective of the temperature.

**DISCUSSION**

Tillers, which are infested by the pink borer before May, die and new tillers emerge to grow as millable sugarcanes. However, when the tillers grow to more than 1 m in height and turn into stalks after May, the newly emerged tillers can not grow into millable stalks because of competition for light and/or space with the surrounding older tillers/stalks. Consequently the stalks, which are infested after May, persist until harvesting time (Mia, personal observation). Azuma (1977) conducted a survey in Okinawa at the time of sugarcane harvest and reported a mean (SD) of 11.3 (2.9)% infestation by the pink borer. Thus, the infestation level reported by Azuma (1977) is considered to be a result of cumulative infestation of the sugarcane for at least the previous 6 months. While in the present study we removed all the infested tillers/stalks in each survey, a mean infestation of 5.7 (3.5)% was obtained for the last surveys, i.e., 1 month before the harvest, over the 4 year period. Therefore, our results are not directly comparable to the previous infestation level reported by Azuma (1977). Thus, we estimated the cumulative percent of infestation of the sugarcane, $CI$, at the last survey in each year as follows,

$$ CI = \frac{1}{t-12} \sum_{t=-12}^{-1} \left( \frac{NI_t}{\sum_{t=-12}^{-1} (NI_t + NH)} \right) 100, $$

where $NI_t$ is the number of infested stalks at survey time $t$ and $NH$ is the remaining number of healthy stalks at the last survey; note that $t=0$ denotes harvesting time and $t=-12$ denotes 6 months before the harvest. As a result, a mean (SD) cumulative infestation of 41.6 (3.8)% for the 4 years was obtained, indicating that this pest has become more economically important in Okinawa recently than in previous years.

The surveys conducted in this study showed that the density of *C. flavipes* decreased sharply after August except during 1997. This observation might be due to the over-utilization of the pink borer host by *C. flavipes* during August (Fig. 1A). The density of the parasitoid was strongly dependent on the density of the host at $i=0$, with the effect of the parasitoid on the host being strongest at $i=3$, i.e., after three surveys (1.5 months). This duration (45 days) corresponds with one generation time of the pink borer, 40–52 days in Okinawa under field conditions (Azuma, 1977) and 49 days under laboratory conditions of 28°C (Mia and Iwashashi, unpublished data), suggesting that the decreased density of the pink borer was partly due to increased density of *C. flavipes* during the previous generation of the pink borer. The duration of 40–52 days would indicate that the pink borer passes through about 7 to 9 generations in a year in Okinawa, and accordingly the corresponding number of peaks in the infestation levels should appear if the generations are discrete. However, Fig. 1A shows at most 4 to 5 peaks even if we include 2 or 3 smaller peaks in addition to the 2 larger peaks in each year. Because all of the immature stages of the pink borer were found in the sugarcane throughout the year in the present survey, there may be overlapping generations of this species in Okinawa. Thus, the number of peaks in Fig. 1A would not necessarily correspond to the number of generations.

The mean numbers of emerged *C. flavipes* per host varied seasonally by as much as 3.1 fold. Two factors may contribute to such a variation, i.e., number of eggs deposited per host and mortality in the immature stages. These factors would play a different role under different host conditions, e.g., density and body size. A weak positive correlation between the mean number of emerged parasitoids and the density of the pink borer ($r=0.20, p<0.0001$) indicates that
density was not responsible for the variation. Under laboratory conditions, percent egg hatch, based on more than 500 eggs from 20 female C. flavipes, was 100%, and a mean mortality in the larval stage was estimated as 2.6% at a temperature of 17°C (Mia and Iwahashi, unpublished data). Moreover, the very low mortality of 0.4–4.4% in the final instar stadium recorded in the present study indicates that the effect of mortality would also be negligible. Thus, the number of eggs per host appears to be the primary factor.

C. flavipes attacks several lepidopteran insects which differ in body size and large numbers of parasitoids tend to emerge from larger host species (Moutia and Courtois, 1952; Kalra and Srivastava, 1964; Gifford and Mann, 1967; Kajita and Drake, 1969). It predominantly parasitizes the 4th stadium larvae of the pink borer (Mia and Iwahashi, unpublished data). Thus, body size variation in the 4th larval stadium of the host could result in variation in the number of parasitoids that emerge. However, the ratio of a maximum to minimum body size of the 4th instar larvae under the laboratory conditions was at most 1.2 (162–187 mg) (Mia and Iwahashi, unpublished data). These results strongly suggest that the number of eggs per host changed during the different seasons irrespective of host condition. Because females discriminate and avoid pink borers, which have already been parasitized (Mia and Iwahashi, 1997), it is likely that all of the eggs in a host would have been oviposited by a single individual of C. flavipes. The strong correlation between the mean number of emerged parasitoids and temperature ($r=0.87$, $p<0.0001$) suggests that the number of eggs oviposited in a host by a female parasitoid would vary with temperature.

ACKNOWLEDGEMENTS

We thank N. Kamata for his critical reading of the manuscript and for his invaluable comments. We also thank K.Y. Kaneshiro for correction of the English. Thanks also are due to S. Azuma for his useful suggestions during the course of the study, and Y. Ishimine for allowing use of the sugarcane field.

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