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Biological Control Agents and Native Parasitoids in the Population System of the Butterfly *Hypolimnas bolina* (L.) (Lepidoptera: Nymphalidae)

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Abstract—Over 100 species of parasitic and predacious insects have been deliberately introduced for biological control of pests on Guam. A population of the butterfly *Hypolimnas bolina* was studied to determine mortality factors and to see if there were serious effects on this non-target butterfly. Only two species introduced for biological control, *Trichogramma chilonis* and *Brachymeria lasus*, were found attacking *H. bolina*. Both species are known generalists that attack a wide variety of hosts. *H. bolina* failed to reproduce in the study site in 17 of 22 months. This failure resulted from a combination of predation by ants on eggs, larvae and pupae, and parasitisation of eggs by two species of native parasitoid. The native parasitoids caused density dependent mortality. Ant predation was inversely density dependent. Populations of *H. bolina* were little influenced by *T. chilonis*, but *B. lasus* parasitized 24% of the pupae and was an important mortality factor in two of the months when reproductive efforts failed. Generalist parasitoids such as *T. chilonis* and *B. lasus* should not be imported for biological control purposes.

Introduction

Biological control is an effective, useful and inexpensive control technique. Out of about 300 target pests around the world, complete biological control has been achieved in about 100 cases including the duplication of previously successful cases (Ehler 1990). Unlike chemical control techniques, biological control does not need frequent reapplication. Once the natural enemies become established, they become a permanent part of the ecosystem, continuously helping check pest population growth and reducing need for insecticides. This decreases pollution and pesticide interference with natural enemies of other pests, restoring normal population controls in the ecosystem. Observations like this led DeBach (1974) and others to consider biological control a safe strategy without environmental problems. Recently, Howarth (1983, 1991) has challenged this assumption and pointed out that there may be serious environmental problems caused by biological control introductions if they do not confine themselves to the target pest. He suggested that deliberate biocontrol introductions may have caused extinction of species native to islands.

On Guam, over 100 species of entomophagous arthropods, four vertebrates, three snails and one nematode have been introduced for biological control of 41 pest species (Nafus & Schreiner 1989). Of these, two species of insect, *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) and *Brachymeria lasus* (Walker) (Hymenoptera: Chalcidae), and one vertebrate, the cane toad *Bufo marinus* L., attack nontarget butterflies, *Hypolimnas anomala* (Wallace) and *H. bolina* (L.) (Nymphalidae) (Nafus 1993). These biocontrol agents were of little importance in the population dynamics of *H. anomala*, which was periodically abundant enough to defoliate its host plant. The primary mechanisms controlling *H. anomala* were viral disease, ants and food shortages caused by defoliation. *H. bolina* suffered higher mortality from exotic enemies, mainly from *B. lasus*, but the importance of this mortality on the population dynamics of the species was not clear. The purpose of this paper is to examine the role of mortality caused by ants, biocontrol introductions and native natural enemies.

Materials and Methods

The study was conducted in Barrigada, Guam on a population of the small tree *Pipturus argenteus*. This species is successional, occupying disturbed habitats and cliff lines. In the study area, eleven trees were scattered along a hillside within 300 m of each other. Six trees were chosen for the study. Once a week from August 1987 to May 1989, 100 leaves on each tree were examined for eggs of *H. bolina*. If few eggs were found, additional leaves were searched to increase the sample size for determining mortality factors. Such additional eggs were not counted in the density census. To determine mortality factors, all eggs were circled using permanent black ink, and assigned numbers. The eggs were examined daily until they hatched. Eggs that failed to hatch were collected to rear parasitoids. Eggs attacked by predators were examined in the laboratory to determine which predator attacked it. Determinations were based on comparisons with the type of damage found to eggs where the predator had been observed attacking the egg. Eggs were only counted as ant predated if the ants (*Monomorium floricola* (Jerdon) and *Tapinoma minutum* Mayr.) were observed removing the eggs, or if distinct cut marks were present

All larvae that hatched were checked daily until they died, disappeared, or pupated. A record of all mortality agents that could be determined was kept. All pupae produced by the larvae being investigated, and any additional pupae that could be found, were checked daily until they emerged. Pupae failing to emerge within two days of the normal developmental period were collected and incubated to see if they were parasitized. Pupae not producing parasitoids were dissected to see if the cause of mortality could be determined.

Guam is tropical, with distinct wet and dry seasons, but temperature conditions are relatively uniform as mean monthly temperature vary only about 2°C over the year. Seasonal differences in *H. bolina* populations, and predation and parasitization rates were compared in the wet and dry seasons using t-tests. For the analysis, the months August through November were considered the wet season, February through May the dry season, and December, January, June and July transitional months. Linear regression was used to examine mortality rates caused by ants and parasitoids in relation to the population density of *H. bolina*. Months in which 10 or less eggs were found were omitted from the analysis.

Results

The number of eggs of *H. bolina* found in the density sample fluctuated with season (Table 1). Counts were distinctly lower in the dry season compared with the wet season ($t = 4.6$; d.f. $= 14$; $p < 0.001$). A monthly average of 6.6 eggs per 100 leaves was found in the dry season compared with an average of 46.8 eggs in the wet season. Oviposition rates in transitional months were intermediate, but more like the wet season (36.4 eggs per 100 leaves). Differences in the percentage of eggs hatching ($t = 1.07$; d.f. $= 14$; $p < 0.3$), eaten by ants ($t = -0.07$; d.f. $= 14$; $p < 0.5$), or parasitized ($t = 0.38$; d.f. $= 14$; $p < 0.5$) were not significant between the wet and dry seasons. Survival of the eggs was related to the number present (Fig. 1).

The only introduced biological control agent to attack the eggs was *Trichogramma chilonis*. Parasitization rates by *T. chilonis* were erratic and low except for September and October 1987 when the parasitoid attacked over 20% of the eggs (Table 1). In September, *T. chilonis* was the most important mortality factor, but a high percentage of eggs hatched despite this. In October, *T. chilonis* was replaced by the native parasitoids *Telenomus* sp. and *Ooencyrtus* sp. as the major mortality factor, and fewer eggs hatched. Subsequently native parasitoids remained at high levels in most months, and *T. chilonis* was rare or absent. Overall, *T. chilonis* appeared to have little impact, being most abundant when numbers and survival percentages of *H. bolina* eggs were highest, and rare or absent when there were few eggs. The two native parasitoids were consistently present and exerted density dependent parasitization. Parasitization rates were highest when there were many eggs, and low when there were few (Fig. 2).

Ants, predominantly *Monomorium floricola*, *Solenopsis geminata* (F.), and *Tapinoma minutum*, were consistent and important mortality factors (Table 1). Mortality was inversely density dependent with predation rates being highest when there were few eggs and lowest when there were many (Fig. 3).

Other mortality factors found were eggs eaten by other herbivores, storm removal of leaves, leaves or limbs breaking off the tree, and fungal infection. Some eggs failed to develop for unexplained reasons, and a fair number disappeared without leaving evidence of the mortality factor. In many cases this was probably due to ant predation. Several ants including *S. geminata* were observed removing whole eggs.

Larval mortality was high and in most months no larvae survived to pupate (Table 1). Few causes of larval mortality could be determined. No parasitoids were ever reared from larvae, nor was any evidence of parasitisation (external

Table 1. Mortality factors found affecting eggs, larvae, and pupae of the butterfly *Hypolimnas bolina* in relation to the season. Exotic parasitoids only include deliberately introduced biological control species.

	Eggs							Larvae				Pupae				
				% killed by						% killed by				% killed by		
					parasitoids											
	no. per 100 leaves	total No.	% hatched	ants	exotic	native	other factors	No.	% sur-vived	ants	other factors	No.	% sur-vived	ants	exotic parasi-toids	other factors
1987																
Aug	17.7	170	43	35	0	1	21	not followed				1	100	0	0	0
Sep	58.0	246	26	18	28	8	21	31	13	0	79	3	0	33	33	33
Oct	27.8	111	6	19	22	31	22	3	0	0	100	6	0	33	67	0
Nov	28.6	208	6	56	0	30	8	2	0	0	100	0				
Dec	39.0	177	6	62	0	24	8	10	0	40	60	0				
1988																
Jan	34.5	187	7	33	0	17	43	14	0	19	81	0				
Feb	1.4	7	0	14	0	57	29	0	0	0	100	0				
Mar	1.8	7	71	0	0	0	29	6	0	67	33	0				
Apr	2.0	14	43	29	0	29	0	6	0	0	100	0				
May	0.2	10	0	0	0	100	0	1	0	0	100	0				
Jun	5.3	31	81	13	0	0	6	33	24	0	76	3	100	0	0	0
Jul	55.5	106	7	2	12	49	30	7	14	14	71	14	21	7	29	43
Aug	81.0	444	1	2	6	76	16	2	50	0	50	12	0	0	0	100
Sep	68.3	205	0	6	0	76	18	1	0	0	100	0				
Oct	29.4	161	2	12	0	53	32	1	0	0	100	0				
Nov	63.8	316	9	35	0	37	18	46	2	0	98	13	31	15	31	23
Dec	65.5	359	22	29	2	25	22	152	6	9	86	3	100	0	0	0
1989																
Jan	30.4	158	23	47	0	20	10	49	0	18	82	0				
Feb	3.3	28	14	57	0	18	11	3	0	0	100	0				
Mar	18.3	81	2	56	7	29	6	3	0	0	100	0				
Apr	17.0	68	21	47	0	0	32	19	0	0	100	0				
May	9.0	18	72	22	0	0	6	9	0	0	100	0				

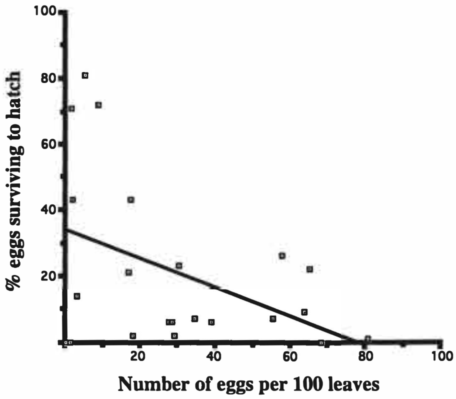

Figure 1. Number of *H. bolina* eggs hatching in relation to the number of eggs laid. Mortality is density dependent.

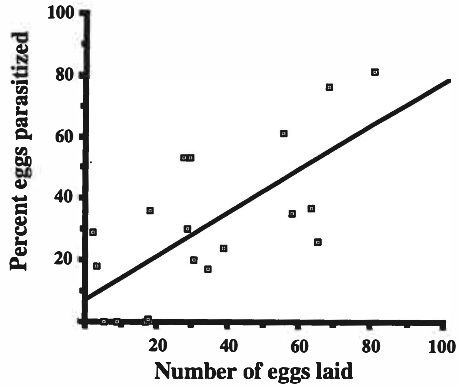

Figure 2. Number of *H. bolina* eggs parasitized in relation to the number of eggs laid. Parasitoids are density dependent.

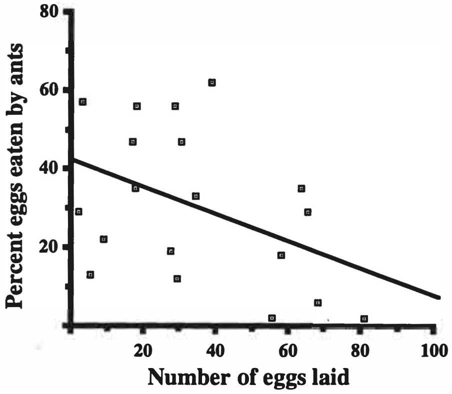

Figure 3. Number of *H. bolina* eggs eaten by ants in relation to the number of eggs laid. Ant predation is inversely density dependent.

parasitoids, cocoons, mummified skins, deflated larval skins, etc.) found in the field. Two larvae were found that appeared to have a viral or bacterial disease. Ant predation was the most common mortality factor observed. Of the attacks observed, most involved first instars being carried away, or were on molting larvae. Occasional predation by the exotic, accidentally introduced mantid *Hierodula patellifera* (Serville) and the pentatomid bug *Eocanthecona furcellata* (Wolff) were seen, and a few larvae were observed to starve after typhoons or *H. anomala* defoliated the trees. Most larvae disappeared without a trace. Of the 388 larvae followed, 36% disappeared during the period when cohorts were molting (mostly at the end of the first or second instar), and an additional 28% vanished early in the first instar.

The only introduced biological control agent found attacking pupae was *Brachymeria lasus.* Parasitization rates ranged up to 66%. Overall, of the 55 pupae followed, 25% emerged, 24% were parasitized by *B. lasus,* and 11% were killed by ants. Most of the rest died of unknown causes.

Discussion

Eggs were found in every month during the study period, indicating that generations are continuous on Guam. Despite the continuous oviposition, *H. bolina* failed to reproduce in the study site in 17 of the 22 months the study lasted. Much of the reason for this was a combination of predation by ants on eggs, larvae, and pupae, and parasitisation of eggs by two species of native parasitoid.

The egg parasitoids including *T. chilonis* exerted density dependent population responses. Their populations fell along with those of *H. bolina,* relaxing pressure during critical times. Ants responded in the opposite way, and exerted higher pressures when populations were low. Their population levels are not dependent on the resources provided by *H. bolina,* and they continue to search leaves for other prey even when few *H. bolina* eggs are present. Because of this, mortality rates went up when *H. bolina* populations fell, increasing the likelihood of local extinction. Potentially, they could be a severe problem for species lacking defenses against ants. Several species of Lepidoptera on Guam have adaptations to avoid ant predation. *H. anomala* remains with its eggs and defends them against ants but not parasitoids (Nafus & Schreiner 1988, Schreiner & Nafus 1991). Several other species of Lepidoptera lay eggs on spider webs, a place that is relatively safe from ants (Nafus & Schreiner 1991).

The vast majority of biological control agents released on Guam did not attack *H. bolina.* The two species that were present are known to be generalists with a wide host range. In this case, it seems that *T. chilonis* did not have much impact, but this cannot be generalized to other species. *T. chilonis* may regulate populations of the hawk moth *Agrius convolvuli* L. on Guam (Nafus & Schreiner 1986), and thus could affect other native species. *B. lasus* contributed to the failure of *H. bolina* to reproduce in two months, and parasitized about 30% of the pupae in two months when reproduction did occur. Although it is obviously not threatening *H. bolina* with extinction, it has the potential to exert negative effects on nontarget species and represents a class of biological control agents that should require rigorous review before introduction. These generalized species should be assessed for potential negative impacts, and there must be very cogent reasons for introducing them. Specialized species should not require this intensive review process unless there are native species that are closely related to the target species. Host specificity tests may be indicated in these cases.

Ants should never be imported for biological control purposes. Because they can maintain high population levels, and have very general feeding habits, their effects are more likely than those of parasitoids to be density independent or inversely density dependent. These characteristics give them vast potential for seriously harming a wide variety of species in native ecosystems. Serious consideration of potential negative effects should also be given when considering augmenting ant populations for biological control purposes in agroecosystems.

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