Movement of Introduced Biological Control Agents onto Nontarget Butterflies, *Hypolimnas* spp. (Lepidoptera: Nymphalidae)

DONALD M. NAFUS

Agricultural Experiment Station, College of Agriculture and Life Sciences, University of Guam, Mangilao, GU 96923

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ABSTRACT Since 1911, over 100 species of exotic organisms have been deliberately introduced for biological control of various pests on Guam. Of these, 27 species were released against seven lepidopterous pests including one butterfly and one skipper. Recently, concern has mounted about the negative effects of these introduced biocontrol agents on nontarget species, particularly endemic island species. Guam may have suffered extinction of as much as 20–25% of its butterfly fauna. To determine the extent of movement and possible impacts of biocontrol agents on nontarget butterflies, mortality factors affecting juvenile stages of *Hypolimnas anomala* (Wallace) and *Hypolimnas bolina* (L.) were studied. Both species were attacked by the same parasitoids and predators, which included native, accidentally introduced, and deliberately introduced species. Parasitoids killed 2.4% of the eggs of *H. anomala*, and ants removed about 25% of the eggs. Parasitization was about 40% in *H. bolina*, and ants killed about 35% of the eggs. One species introduced for biological control, *Trichogramma chilonis* Ishii, was found parasitizing the eggs, but it caused little mortality. No larval parasites were reared for either species. *H. anomala* suffered heavy mortality from a disease. Both species were attacked by ants and two exotic, self-introduced predators. The major pupal parasite was *Brachymeria lasus* (Walker), a deliberate biological control introduction. *B. lasus* parasitized 24.6% of the pupae of *H. bolina* and 2.9% of those of *H. anomala*. Ants attacked 17% of *H. anomala* pupae and 7% of those of *H. bolina*. *B. lasus* and *T. chilonis* are polyphagous parasitoids that have the potential to affect a variety of nontarget species. This type of parasitoid, and highly polyphagous predators like ants, should require more intensive evaluation and review for use in classical biological control programs than more specialized species.

KEY WORDS nontarget species, biological control, *Hypolimnas*

BIOLOGICAL CONTROL has been considered a relatively safe method of pest control with few negative impacts. Recently, Ehler (1990, 1991) and Howarth (1991) pointed out some environmental problems caused by biocontrol introductions, and Howarth suggested that deliberate biocontrol introductions have caused over 100 extinctions. Among these are extinction of endemic island species such as the coconut moth, *Levuana iridescens* Bethune-Baker (Lepidoptera: Zygaenidae), which was driven to extinction by the deliberately introduced tachinid *Ptychomyia remota* Aldrich (Robinson 1975).

Guam, the southernmost and largest island in the Marianas chain, has 20 species of butterflies and skippers recorded on it (Swezey 1942; I. Schreiner & D.M.N., unpublished data). Most of these are widely distributed Asian species, but there are also two endemic species and one endemic subspecies. Since 1945, four or possibly five species of butterflies have become extremely rare or extinct. These include the endemic butterflies *Euploea eleutho* (Latreille & Godart) (Danaidae) and *Vagrans egistina* (Latreille & Godart) (Nymphalidae) and the widespread species *Appias paulina* Cramer (Pieridae) and *Papilio xuthus* L. (Papilionidae). In addition, the endemic subspecies of the nymphalid *Hypolimnas octocula* (Butler) is extremely rare.

P. xuthus was once abundant, clustering by the hundreds at road pools or muddy spots (Swezey 1942), but has not been seen since 1968. *A. paulina, E. eleutho*, and *V. egistina* were all uncommon in 1936, although *V. egistina* was widely distributed (Swezey 1942). *A. paulina* and *E. eleutho* have not been collected since the 1940s, but *V. egistina* was collected as late as 1978.

Reasons for the possible extinction or rarity of these butterflies are unknown. One possible contributing factor is deliberately introduced biological control agents. Guam has had a long history of biological control that began in 1911 (Nafus & Schreiner 1989). Over 100 species of entomophagous arthropods, four vertebrates, three snails, and one nematode have been introduced to control 41 pest species. Against Lepidoptera, 27 species of parasitoids and predators were introduced, including three species, *Apanteles papilionis* Viereck (Hymenoptera: Braconidae), *A. erionotae* Wilkinson, and *Pteromalus luzonensis* Gahan (Hymenoptera: Pteromalidae), specifically targeted for butterflies or skippers. The purpose of this study was to determine if deliberately introduced biological control agents were moving onto nontargeted hosts. Because of the rarity, or nonexistence, of the butterflies listed above, I could not study them, so I chose two species in the family Nymphalidae, *Hypolimnas anomala* (Wallace) and *Hypolimnas bolina* (L.), as indicator species of possible environmental problems. Mortality factors affecting these butterflies were examined.

Materials and Methods

The study was conducted on nine host plants of the small tree *Pipturus argenteus* (Forster filius) Weddell in Barrigada, Guam. *H. anomala* lays its eggs in a large cluster that it guards (Nafus & Schreiner 1988). Each week, egg clusters on each tree were located, marked according to the methods of Nafus & Schreiner (1988), and the number of eggs present was counted. All marked clusters were examined daily until the eggs hatched. The number of eggs hatched, destroyed by predators, or parasitized were counted and the species of predator or parasitoid was recorded if present. After the eggs hatched and the larvae had moved off the leaf, the remaining eggs were followed to see if they were parasitized. Eggs whose fate could not be determined in the field were taken to the laboratory and examined. Parasitized eggs were incubated to determine the species of parasitoids. On each host plant, from one to six clusters that produced larvae were followed. Each day the larvae were checked and counted. Any predators or parasitoids observed feeding on them were recorded along with observed mortality. Samples of all instars were periodically removed from a few clusters and reared in the laboratory to check for parasitoids. Ten of each instar were taken for each sample. Larvae leave the host plant and disperse into the surrounding vegetation to pupate. In consequence, most pupae could not be found. The location of any pupa that was found was marked, and the pupa was checked daily. Pupae not emerging after the normal developmental period were collected and incubated to rear parasitoids. Pupae not producing parasitoids or butterflies were dissected to see if the mortality factor could be determined.

H. bolina lays its eggs singly or in small clusters of <20 eggs. Each week 100 leaves on each of six hosts were searched for new eggs. Each egg found was rechecked daily until it hatched, and a record of any predation or parasitism was kept. Parasitized eggs were collected and reared to determine the species of parasitoid. Any larvae that hatched were followed throughout their life cycle. Because few eggs hatched, additional leaves were searched for eggs. Larvae hatching from these eggs and any other larvae found were also followed. Pupae were monitored as in the case of *H. anomala,* except that because *H. bolina* pupates on the host plant near the feeding site, the surrounding vegetation was not searched. Mortality factors were studied from August 1986 to May 1989 for *H. anomala* and from August 1987 to May 1989 for *H. bolina.*

For the purposes of this paper, I use the terms native, exotic, endemic, and biocontrol introduction as follows. Endemic refers to species with distributions restricted to Guam or the Marianas Islands. Native indicates that the species was present in the first major collections on Guam in 1911 and 1936 (Swezey 1942), and that it has a widespread Asian distribution continuous across various islands to Guam. No inferences are made as to mode of entry. Residence time on Guam of these species is unknown. Exotic indicates that the species was not found in the first major collections that took place on Guam, but it is now common. Introduction dates are the first collection recorded in the literature or in the University of Guam insect collection. Biological control introduction refers only to species that were deliberately introduced to control a pest on Guam.

Results

Egg Parasitoids and Predators. Eggs of both species of butterflies were attacked by the same natural enemy complex (Table 1). Ants were the most important mortality factor of the eggs of *H. anomala* (Table 2). *H. bolina* was heavily attacked by both parasitoids and ants. *Monomorium floricola* (Jerdon), *Solenopsis geminata* (F.), and *Tapinoma minutum* Mayr were the most important species. *M. floricola* was present on all of the host plants and was responsible for most of the ant predation on *H. anomala* and *H. bolina.* It opened the eggs in situ, removed the contents, and left the chorion. *S. geminata* was second in importance, but was present only on portions of two host plants. It removed entire eggs. *T. minutum* was present on two host plants, but had difficulty opening eggs. It primarily attacked eggs near hatching or removed larvae that had just cut through the chorion and were emerging from the egg.

Eggs of *H. bolina* were heavily parasitized, but those of *H. anomala* were rarely attacked. Three species of parasitoids were commonly found (Table 3). Two of these, *Telenomus* sp. (Hymenoptera: Scelionidae) and *Ooencyrtus* sp., appear to be undescribed native or possibly en-

Table 1. Species that consumed juvenile *Hypolimnas anomala* or *H. bolina*

Species	Family	Species attacked	Stage attacked	Type	Status
Hemiptera					
Pseudoloxops signatus (Usingor)	Miridae	a	E	Predator	Endemic (Carvalho 1956)
Eocanthecona furcellata	Pentatomidae	a,b	L	Predator	Exotic, 1973
Lepidoptera					
Chrysodeixis sp.	Noctuidae	a,b	E	Herbivore	Exotic, 1936 (Swezey 1946)
Hypolimnas anomala	Nymphalidae	a,b	E	Herbivore	Native (Swezey 1942)
Hypolimnas bolina	Nymphalidae	a,b	E	Herbivore	Native (Swezey 1942)
Orthoptera					
Valanga excavata	Acrididae	a,b	E	Herbivore	Endemic (Swezey 1946)
Mantodea					
Hierodula patellifera	Mantidae	a,b	L	Predator	Exotic, before 1971
Hymenoptera					
Monomorium floricola	Formicidae	a,b	E, L	Predator	Native, before 1911 (Swezey 1942)
Solenopsis geminata	Formicidae	a,b	E, L, P	Predator	Native, before 1911 (Swezey 1942)
Tapinoma spp.	Formicidae	a,b	E, L, P	Predator	Native, before 1911 (Swezey 1942)
Technomyrmex albipes	Formicidae	a,b	E, L	Predator	Native, before 1911 (Swezey 1942)
Anoplolepis longipes (Jerdon)	Formicidae	a,b	L, P	Predator	Native, before 1911 (Swezey 1942)
Telenomus sp.	Scelionidae	a,b	E	Parasitoid	Native
Ooencyrtus sp.	Encyrtidae	a,b	E	Parasitoid	Native
Trichogramma chilonis	Trichogrammatidae	a,b	E	Parasitoid	Biocontrol, 1971 (Nafus & Schreiner 1989)
Trichogrammatomyia tortricis	Trichogrammatidae	a	E	Parasitoid	Exotic, 1986
Brachymeria lasus	Chalcidae	a,b	P	Parasitoid	Biocontrol, 1973 (Nafus & Schreiner 1989)
Vertebrate predators					
Bufo marinus		a	L	Predator	Biocontrol, 1937 (Nafus & Schreiner 1989)

NOTE: *H. anomala, H. bolina, Chrysodeixis* sp., and *Valanga excavata* are herbivores, but ate eggs of *Hypolimnas anomala* or *H. bolina* along with leaf tissue if encountered. Spiders, unidentified viruses, and other diseases are not included. Species considered exotic are those not reported in early surveys but now common. Native species are those present in early surveys that have widespread, continuous distributions in the Oriental region suggesting long residency. Endemic refers to species restricted to the Mariana Islands. Abbreviations: a, *H. anomala;* b, *H. bolina;* E, egg; L, larva; P, pupa.

demic species. The *Telenomus* species was distinctly different from *T. remus* Nixon or *T. nawai* Ashmead, which had been introduced for control of *Spodoptera litura* (F.) and *S. mauritia* (Boisduval). The third species was *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), a species introduced for biocontrol of *Ostrinia furnacalis* (Guenée).

Telenomus sp. was the dominant egg parasitoid, accounting for 66% of the parasitized eggs of *H. bolina* and 47% of those of *H. anomala*. *Ooencyrtus* sp. and *T. chilonis* varied in importance depending on the species of butterfly (Table 3). *Trichogrammatomyia tortricis* Girault was also reared from a few eggs of *H. anomala*.

Minor losses of eggs were caused by leaves being blown off during typhoons, limbs breaking and falling, incidental ingestion by herbivorous insects, predacious bugs, desiccation, infertility, and so forth. The grasshopper *Valanga excavata* (Stål) (Acrididae), and three species of Lepidoptera (Table 1) ate eggs on the leaf tissue they were consuming.

Larval Predators and Parasitoids. No parasitoids were reared from larvae of either species nor was any evidence of parasitization (larval mummies, external parasitoids, wasp cocoons, etc.) observed in the field. Most larval mortality could not be determined; the larvae disappeared (Table 4). In some cases, predation events or other mortality factors were observed. Predators found attacking larvae are listed in Table 1. The most commonly observed predators were the ants *T. minutum* Mayr, *S. geminata*, and *Technomyrmex albipes* (Fr. Smith) (Table 4). Predation by ants ranged from 8 to 14% in *H. bolina* and from 1 to 18% in *H. anomala*. Most attacks were observed on recently hatched first instars or on molting first and second instars. For *H. bolina*, 64% of the total first and second instars followed disappeared during the period when the molt was expected, suggesting that much of the unknown mortality was probably due to ants. An additional 7% disappeared at other times during the first two instars. Mortality for *H. anomala* was also highest during the first two instars.

The major mortality factor affecting *H. anomala* was an unidentified disease (Table 4). About 20% of the larvae were found dead on the plant, but this count is low because many diseased larvae fell and were not found or were removed by scavengers. Dead larvae were examined in the laboratory, but no bacteria or polyhedral inclusion bodies were found. The larvae often remained attached to the leaves by a pair of prolegs, hanging downward in an inverted V. The skin was filled with fluid and easily broken. These symptoms are characteristic of some viral diseases (Vaughn 1974). The disease was most prevalent during periods of rainy weather when

Table 2. Mortality factors of eggs of *Hypolimnas anomala* and *H. bolina*

Yr	Total eggs per site		Mean no. per site or % of total eggs		Mean no. per site or % of total eggs killed or eaten by					
			Hatched	Unhatched	Parasitoids	Ants	Other predators	Herbivores	Fungus	Other factors
H. anomala										
1986	142,816	$\bar{x} \pm$ SD[a]	9,272 ± 4,579	1,147 ± 1,401	498 ± 721	4,900 ± 5,482	1 ± 2	0 ± 0	4 ± 13	47 ± 142
		%	58.4	7.2	3.1	30.9	0.0	0.0	0.0	0.3
1987	103,364	$\bar{x} \pm$ SD	8,694 ± 4,465	746 ± 441	195 ± 155	3,016 ± 2,604	20 ± 37	233 ± 338	0 ± 0	16 ± 35
		%	67.3	5.8	1.5	23.3	0.2	1.8	0.0	0.1
1988	147,577	$\bar{x} \pm$ SD	11,497 ± 6,901	1,347 ± 1,145	460 ± 467	3,047 ± 1,487	1 ± 2	20 ± 37	142 ± 306	1,934 ± 1,303
		%	62.3	7.3	2.5	16.5	0.0	0.1	0.8	10.5
1989	45,403	$\bar{x} \pm$ SD	3,407 ± 3,487	249 ± 293	81 ± 101	1,699 ± 1,630	0 ± 0	9 ± 26	0 ± 0	230 ± 406
		%	60.0	4.4	1.4	29.9	0.0	0.2	0.0	4.1
H. bolina										
1987	916	$\bar{x} \pm$ SD	29.8 ± 9.9	5.2 ± 3.5	42.3 ± 31.6	58.5 ± 50.5	0.2 ± 0.4	9.7 ± 5.8	0.8 ± 1.6	6.2 ± 7.1
		%	19.5	3.4	27.7	38.3	0.1	6.3	0.5	4.0
1988	1,848	$\bar{x} \pm$ SD	53.2 ± 66.2	13.0 ± 9.8	154.7 ± 185.5	55.0 ± 54.5	1.2 ± 1.0	9.0 ± 17.7	2.8 ± 13.0	19.1 ± 6.0
		%	17.3	4.2	50.2	17.9	0.4	2.9	0.9	6.3
1989	353	$\bar{x} \pm$ SD	14.5 ± 13.9	2.0 ± 4.4	10.8 ± 9.9	28.7 ± 26.8	0.0 ± 0.0	0.5 ± 1.2	0.0 ± 0.0	2.3 ± 3.6
		%	24.6	3.4	18.4	48.7	0.0	0.8	0.0	4.0

[a] Sample mean ± 1 SD.

Table 3. Parasitoids attacking eggs of the nymphalid butterflies *H. anomala* and *H. bolina*

Attack by parasitoids	*H. anomala*	*H. bolina*
Overall % eggs parasitized	2.4	39.5
% of total parasitized by		
Telenomus sp.	47	66
Ooencyrtus sp.	17	21
Trichogramma chilonis	36	12
Other species	<1	1

larval populations were high, but once present, the disease persisted on a tree for several months and destroyed most of the *H. anomala* clutches. *H. bolina* suffered little mortality from disease during the larval stage, even when intermingled with infected *H. anomala* larvae, although a few larvae died, and some developed more slowly.

Exotic predators, primarily the mantid *Hierodula patellifera* (Serville) and the pentatomid bug *Eocanthecona furcellata* (Wolff), killed about 1 to 2% of *H. anomala* larvae and up to 9% of *H. bolina*. Both the mantids and the bugs tended to remain in the area where they found larvae for several days afterwards. Mantids followed clusters of *H. anomala* from leaf to leaf.

The only biocontrol agent that attacked the larval stage of *H. anomala* was the cane toad, *Bufomarinus* L. The toad fed on older instars that were dispersing from the tree to pupate. Toads were observed on only two occasions in 3 yr, both times at the base of the tree feeding on processions of larvae moving away from the host plant. I did not see the cane toad eat *H. bolina*.

Pupal Mortality. From 25 to 40% of the pupae of *H. anomala* and up to 28% of those of *H. bolina* emerged (Table 5). Ants and disease were the major mortality factors observed for *H. anomala* pupae. Mortality from disease was much higher than is listed, because much of the mortality listed in the "Other" category was ultimately due to disease, although the proximal causes of death were failure to pupate properly or falling from pupation sites. Pupating larvae from clusters that were infected with disease were sluggish and used little silk to secure themselves to the substrate. Movements during molting loosened the silk and left the pupa dangling by a few strands of silk. These subsequently fell within a day or two after pupation. The parasitoid *Brachymeria lasus* (Walker) (Hymenoptera: Chalcidae), introduced for control of *Pericyma cruegeri* (Butler) (Lepidoptera: Noctuidae), was a minor source of mortality. It attacked <4% of the pupae.

H. bolina pupae were much less common than those of *H. anomala*, because mortality was extremely high for eggs and larvae. Pupae were found in only 11 mo during the study, and often only one or two eggs survived to pupate despite many hundreds being laid. *B. lasus* was a major mortality factor in 1987, but was less important in

Table 4. Mortality factors of larvae of *Hypolimnas anomala* and *H. bolina*

Yr	Total larvae found		Larvae per site	Larvae surviving to pupate	Mean no. or % killed by class of predator: Introduction status: Accidental	Biocontrol	Ants	Native	Mean no. or % killed by: Disease	Other factors	Mortality factor not determined
H. anomala											
1987	44,654	$\bar{x} \pm$ SD[a]	5,582 ± 2,256	186 ± 143	67 ± 97	8 ± 24	69 ± 74	1 ± 1	1,096 ± 491	1,196 ± 343	3,959 ± 1,700
		%		3.3	1.2	0.2	1.2	0.0	19.6	3.5	70.9
1988	46,982	$\bar{x} \pm$ SD	6,712 ± 2,912	487 ± 443	49 ± 69	0 ± 0	50 ± 634	1 ± 14	1,344 ± 1,051	157 ± 151	4,224 ± 1,841
		%		7.3	0.7	0	6.7	0.0	20	2.3	62.9
1989	19,166	$\bar{x} \pm$ SD	2,738 ± 2,381	128 ± 108	79 ± 154	0 ± 0	98 ± 1,036	2 ± 54	514 ± 853	127 ± 182	1,390 ± 1,267
		%		4.7	2.9	0	18.2	0.1	18.8	4.6	50.8
H. bolina											
1987	54	$\bar{x} \pm$ SD	10.8 ± 7.0	0.2 ± 0.4	1.0 ± 2.2	0.0 ± 0.0	0.8 ± 1.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	8.8 ± 4.8
		%		1.9	9.3	0.0	7.4	0.0	0.0	0.0	81.5
1988	420	$\bar{x} \pm$ SD	70.0 ± 74.4	8.0 ± 11.2	0.2 ± 0.4	0.0 ± 0.0	4.2 ± 7.9	0.0 ± 0.0	0.3 ± 0.5	1.0 ± 1.1	56.3 ± 64.1
		%		11.4	0.2	0.0	6.0	0.0	0.5	1.4	80.5
1989	87	$\bar{x} \pm$ SD	14.5 ± 9.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 3.6	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 4.0	10.5 ± 7.3
		%		0.0	0.0	0.0	13.8	0.0	0.0	13.8	72.4

[a] Sample mean ± 1 SD.

1988 and was not observed in 1989, a year that was characterized by a large amount of ant predation on eggs and larvae. Of 353 eggs followed in 1989, only two survived to pupate, and both pupae were opened by ants. For about one-third of the *H. bolina* pupae, the mortality factor could not be determined. Most of these pupae failed to emerge. Possibly the disease that affected *H. anomala* killed *H. bolina* pupae even though it had little effect on the larvae. In the laboratory, Lepidoptera larvae that are not infected with enough inoculum of virus die during the pupal stage and show no particular symptoms other than poor emergence (Vaughn 1974).

Discussion

Neither species of *Hypolimnas* is threatened on Guam. *H. anomala* is sufficiently abundant that it occasionally defoliates its host. *H. bolina* is uncommon, but not rare or threatened. Obviously, a study of these species during one point in time at one location does not delineate how much impact the various mortality factors had on species that went extinct at a different point in time. It should, however, provide clues as to the species that might be problems, even if these are rare on the species chosen as indicators. Three biological control species were found attacking the two *Hypolimnas* butterflies. One of these, the cane toad, is one of the classical mistakes in biological control. The cane toad was introduced to Guam in 1937 as a general predator. It may have controlled the slug *Veronicella leydigi* Simroth (Nafus & Schreiner 1989), but it also became a nuisance in its own right (Eldredge 1988, Nafus & Schreiner 1989). Densities of 185 to 225 toads per ha have been recorded in areas near water or human habitation (Chernin 1979), and it has probably affected ground-dwelling native insects and mollusks, although impact studies have not been done.

Of the 27 species of insects released for biocontrol of Lepidoptera, two, *T. chilonis* and *B. lasus*, parasitized the butterflies. Both species were introduced in the early 1970s (Nafus & Schreiner 1989) and are generalists that parasitize a diverse array of Lepidoptera. *T. chilonis* was introduced to control the Asian corn borer, *Ostrinia furnacalis* (Guenée) (Lepidoptera: Pyralidae), a serious pest of corn on Guam. It is ineffective at controlling the corn borer (Nafus & Schreiner 1986) and also was not a significant source of mortality for the *Hypolimnas* species. On Rota it has coexisted with *V. egistina* since the late 1930s (Nafus & Schreiner 1989), but this does not mean that it could not have adverse effects in other places or on other species. On Guam, from 50 to 100% of the eggs of the sphingid moth *Agrius convolvuli* (L.) are parasitized by *T. chilonis* (Nafus & Schreiner 1986).

Table 5. Mortality factors affecting pupae of *Hypolimnas anomala* and *H. bolina*

Yr	Total pupae		Pupae per site	Emerged	Mortality factor				
					Ants	Disease	Biocontrol	Other	Unknown
H. anomala									
1986	109	$\bar{x} \pm SD^a$	15.6 ± 14.8	6.3 ± 8.0	3.3 ± 7.0	1.4 ± 1.8	0.3 ± 0.5	3.0 ± 4.8	11.3 ± 1.5
		%		40.4	21.1	9.2	1.8	19.3	8.3
1987	238	$\bar{x} \pm SD$	29.8 ± 21.1	7.5 ± 9.4	1.8 ± 2.3	5.6 ± 5.0	1.0 ± 1.1	8.9 ± 7.9	5.0 ± 4.9
		%		25.2	5.9	18.9	3.4	29.8	16.8
1988	158	$\bar{x} \pm SD$	26.3 ± 4.9	11.2 ± 3.3	3.5 ± 3.4	2.3 ± 3.1	1.0 ± 0.6	6.3 ± 1.8	2.0 ± 1.7
		%		42.4	13.3	8.9	3.8	24.1	7.6
1989	29	$\bar{x} \pm SD$	4.8 ± 9.0	1.3 ± 2.8	0.5 ± 0.5	0.0 ± 0.0	0.2 ± 0.4	0.5 ± 1.2	2.3 ± 5.7
		%		27.6	10.3	0.0	3.4	10.3	48.3
H. bolina									
1987	14	$\bar{x} \pm SD$	2.3 ± 3.9	0.3 ± 0.5	0.2 ± 0.4	0.5 ± 1.2	1.3 ± 2.4	0.0 ± 0.0	0.0 ± 0.0
		%		14.3	7.1	21.4	57.1	0.0	0.0
1988	47	$\bar{x} \pm SD$	7.8 ± 7.8	2.2 ± 2.1	0.7 ± 1.6	0.2 ± 0.4	1.5 ± 1.5	0.5 ± 0.8	2.8 ± 3.3
		%		27.7	8.5	2.1	19.1	6.4	36.2
1989	2	$\bar{x} \pm SD$	0.3 ± 0.8	0.0 ± 0.0	0.3 ± 0.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		%		0.0	100.0	0.0	0.0	0.0	0.0

[a] Sample mean ± 1 SD.

B. lasus was not a serious mortality factor for *H. anomala,* but it parasitized a high percentage of pupae of *H. bolina* and had an adverse effect on that species. In the study area, few adults of *H. bolina* were produced, and often several months passed in succession when no eggs survived to adults. Most of the mortality was caused by ants and native parasitoids early in the life cycle of the butterfly. *B. lasus* tended to be an important mortality factor at times when pupae were most abundant and pressure from ants and egg parasitoids was lowest. This probably lessened its impact on *H. bolina,* but mortality levels were sufficiently high that it played a role in the population regulation system. For other species of butterflies, particularly rare species confined to small or restricted habitats, the possibility exists that the additional mortality could cause extinction if the timing were critical. The nymphalid *V. egistina,* for example, was a rare butterfly on Guam even early in the century when its populations were healthy. Recent losses of habitat associated with human population growth and the development of a tourism industry on Guam have almost certainly reduced populations and restricted its distribution. The addition of a parasitoid like *B. lasus* could easily have been the final step to push the butterfly to extinction, although there is no direct evidence to support or reject this idea. In the case of other butterflies, *A. paulina, P. xuthus,* and *E. eleutho,* neither *B. lasus* nor *T. chilonis* had any impact, because all three species disappeared before either parasitoid was introduced. Other biocontrol introductions could have been involved, but most of the other species introduced had narrower host ranges, and they were not found on the indicator species. Their involvement is less likely. Similarly, the two parasitoids introduced to control the swallowtail *Papilio polytes* L. could not have affected *P. xuthus* because that species was already extinct before their introduction. More likely, other factors were responsible. *P. xuthus* and *P. polytes* share the same hosts and are ecological equivalents. *P. polytes* was introduced to Guam sometime in the late 1940s or early 1950s (Peterson 1957). Before that, *P. xuthus* was abundant (Swezey 1942) and had continued to be abundant enough to be classified as a serious pest by Peterson (1957). By the 1960s, however, it had declined and the last specimen was collected in 1968. It has not been seen since then, and everywhere in the Marianas that *P. polytes* has appeared, *P. xuthus* is no longer present.

Exotic predators like *H. patellifera* and *E. furcellata* caused some mortality of the *Hypolimnas* species, but the most important predators were ants. Ants preyed on all juvenile stages of both butterflies. All of the ants are tramp species that were already on Guam when the first survey took place in 1911 (Swezey 1946). Howarth (1991) suggested that biological control introductions were the major factor in the extinction of 15 species of Lepidoptera in Hawaii, one of which was an *Agrotis* species. He did not comment on the role of introduced ants, although he did recognize the serious problems caused by ants in the native ecosystems in Hawaii. No ants are native to the Hawaiian Islands, and the native fauna has not developed defenses against them (Loope et al. 1988). In Haleakala National Park, the Argentine ant, *Iridomyrmex humilis* (Mayr), has dramatically reduced populations of several native species including a species of *Agrotis* (Loope et al. 1988). The *Agrotis* species was nearly eliminated from areas where the ant was present. It is easy to find parasitized insect life stages because they often remain in the field long after unparasitized cohorts have developed. Ant predation takes place quickly and leaves little evidence. Thus, it is easy to underestimate the role of ant predation while overestimating that of parasitization. Without detailed life history data, the roles of ants, deliberately introduced biocontrol agents, and accidentally introduced generalists (Funasaki et al. 1988) cannot be disentangled, and claims of causation for extinction should not be assigned.

The majority of insect biocontrol agents that were introduced to Guam were specialists that did not attack the indicator species. The two species, *T. chilonis* and *B. lasus,* that did cause mortality were known generalists and are examples of the kind of parasitoid that should require intensive review and discussion before introductions are made. Both species have very wide host ranges, thus having the possibility of affecting a variety of nontarget species. *B. lasus* was brought in from Papua New Guinea to control *P. cruegeri.* In Papua New Guinea, Szent-Ivany (1960) reported that a complex of parasitoids parasitized 10 to 37% of *P. cruegeri* pupae, of which *B. lasus* was the most common, but he also reported that severe defoliation of poinciana still took place. No studies were done before introduction to indicate that parasitoid effectiveness was being hampered by local mortality factors, and thus it is questionable as to whether there was any indication that this species would be effective. Although the species established on Guam, it is not effective and the looper continues to be a serious pest (Nafus & Schreiner 1989).

Highly polyphagous predators like ants are another class of organisms that should require serious review before use in a biological control program. Predacious ants frequently take a wide range of prey and have high population levels, giving them the potential to create a variety of nontarget effects. Historically, ants have not been widely used in biological control because the more specialized parasitoids have been preferred, but there are examples of their use in inoculative, augmentative, and even classical biological control. As early as the ninth century in China, ants were used to control pests of citrus (Simmonds et al. 1976, Coppel & Mertins 1977). In Europe, periodic inoculation with *Formica* spp. has been used to protect forests against a variety of Lepidoptera and Diptera species (Waters et al. 1967, Turnock et al. 1976), and the imported fire ant has been experimentally manipulated to reduce boll weevil damage in cotton in the United States (Jones & Sterling 1979). In at least two cases, ants have been imported in classical biological control programs. In 1902, a botanist, O. C. Cook, imported the ponerine ant *Ectatomma tuberculatum* (Olivier) from Coahuila, Mexico, and released it in Texas for control of boll weevil in cotton, but it did not establish (Cate 1985). Finnegan (1975) felt that areas of mixed coniferous forests in Canada would benefit by addition of the ant *Formica lugubris* Zetterstedt, an important predator in European forests. In 1971, this ant was imported from northern Italy and released in eastern Canada. It established and provided economic benefits by reducing the need for insecticide use (Finnegan 1980), but assessments for possible environmental problems have not been reported. In Europe, *F. lugubris* alters the structure of insect communities in birch (Fowler & MacGarvin 1985), bracken (Heads 1986), and pine plantations (Sudd & Lodhi 1981), and it almost certainly has affected insect communities in North America. Potentially, nontarget effects may have been greater because the ant is not native to North America and some food species may lack specific adaptations to avoid predation by *F. lugubris.* Heads (1986) indicated that adaptations to avoid predation by one species of ant are not necessarily effective against a different species. In addition, ants may interfere with other important beneficial species (Jones & Sterling 1979). In consequence, some herbivore species may decline, but others, particularly Homoptera and Hemiptera, may increase, offsetting any yield gains. In birch, *F. lugubris* lowered populations of external leaf chewers, but populations of ant-tended aphids increased to damaging levels, making it questionable as to whether there were actual benefits to the tree (Fowler & MacGarvin 1985).

Given the potential for environmental damage, I suggest that precautionary measures be taken before introducing predators or parasitoids with very broad host ranges. Ideally, to be considered for introduction, these species must have demonstrated control potential for the target pest, must be screened for significant negative impacts on native species, and must have sufficient economic or environmental benefits to offset any negative environmental costs. In practice, these conditions may be difficult or impossible to fulfill or prohibitively expensive. Less environmental problems would be expected from the more specialized predators and parasitoids (Cumber 1960, Funasaki et al. 1988), and less review is needed. Current guidelines are probably adequate for these species.

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References Cited

Carvalho, J.C.M. 1956. Heteroptera: Miridae. Insects Micronesia 7: 1–100.

Cate, J. R. 1985. Cotton: status and current limitations to biological control in Texas and Arkansas, pp. 537–556. *In* M. A. Hoy & D. C. Herzog, Biological control in agricultural IPM systems. Academic, New York.

Chernin, M. I. 1979. Population dynamics and reproductive strategy of *Bufo marinus* (L.) on Guam. M.S. thesis, University of Guam, Mangilao.

Coppel, H. C. & J. W. Mertins. 1977. Biological insect pest suppression. Springer-Verlag, New York.

Cumber, R. A. 1960. The interaction of native and introduced insect species in New Zealand. Proc. N.Z. Ecol. Soc. 8: 55–60.

Ehler, L. E. 1990. Environmental impact of introduced biological-control agents: implications for agricultural biotechnology, pp. 85–96. *In* J. J. Marois & G. Bruening [eds.], Risk assessment in agricultural biotechnology. University of California Division of Agriculture and Natural Resources Publication 1928.

1991. Planned introductions in biological control, pp. 21–39. *In* L. R. Ginzburg [ed.], Assessing ecological risks of biotechnology. Butterworth-Heinemann, Boston.

Eldredge, L. G. 1988. Case studies of the impacts of introduced animal species on renewable resources in the U. S.-affiliated Pacific Islands, pp. 118–146. *In* Topic reviews in insular resource development and management in the U.S.-affiliated islands. University of Guam Marine Laboratory Technical Report 88. University of Guam, Mangilao.

Finnegan, R. J. 1975. Introduction of a predacious red wood ant, *Formica lugubris* (Hymenoptera: Formicidae), from Italy to eastern Canada. Can. Entomol. 107: 1271–1274.

1980. La fourmi rouge des bois. For. Conserv. 43: R3–R4.

Fowler, S. V. & M. MacGarvin. 1985. The impact of hairy wood ants, *Formica lugubris*, on the guild structure of herbivorous insects on birch, *Betula pubescens*. J. Anim. Ecol. 54: 847–855.

Funasaki, G. Y., P.-Y. Lai, L. M. Nakahara, J. W. Beardsley & A. K. Ota. 1988. A review of biological control introductions in Hawaii: 1890–1985. Proc. Hawaii. Entomol. Soc. 28: 105–160.

Heads, P. A. 1986. Bracken, ants and extrafloral nectaries. IV. Do wood ants (*Formica lugubris*) protect the plant against herbivores? J. Anim. Ecol. 55: 795–809.

Howarth, F. G. 1991. Environmental impacts of classical biological control. Annu. Rev. Entomol. 36: 485–509.

Jones, D. & W. Sterling. 1979. Manipulation of red imported fire ants in a trap crop for boll weevil suppression. Environ. Entomol. 8: 1073–1077.

Loope, L. L., A. C. Medeiros & F. R. Cole. 1988. Effects of the Argentine ant on the endemic biota of subalpine shrubland, Haleakala National Park, Hawaii, pp. 52–62. *In* L. K. Thomas, Jr. [ed.], Management of exotic species in natural communities, vol. 5. Proceedings of the Conference on Science in the National Parks, 13 July 1986. Fort Collins, CO.

Nafus, D. M. & I. H. Schreiner. 1986. Parasitoids of the corn borer *Ostrinia furnacalis* (Lep.: Pyralidae) in the Mariana Islands. Entomophaga 31: 219–224.

1988. Parental care in a tropical nymphalid butterfly, *Hypolimnas anomala*. Anim. Behav. 36: 1425–1431.

1989. Biological control activities in the Mariana Islands from 1911 to 1988. Micronesica 22: 65–106.

Peterson, G. D. 1957. An annotated check list of parasites and predators introduced into Guam during the years 1950–1955. Proc. Hawaii. Entomol. Soc. 16: 199–202.

Robinson, G. S. 1975. Macrolepidoptera of Fiji and Rotuma. E. W. Classey, Oxon, U.K.

Simmonds, F. J., J. M. Franz & R. I. Sailer. 1976. History of biological control, pp. 17–39. *In* C. B. Huffaker & P. S. Messenger [eds.], Theory and practice of biological control. Academic, New York.

Sudd, J. H. & A. Q. Lodhi. 1981. The distribution of foraging workers of the wood ant *Formica lugubris* Zetterstedt (Hymenoptera: Formicidae) and their effects on the numbers and diversity of other Arthropoda. Biol. Conserv. 20: 133–145.

Swezey, O. H. 1942. Insects of Guam-I. Bernice P. Bishop Mus. Bull. 172: 1–218.

1946. Insects of Guam-II. Bernice P. Bishop Mus. Bull. 189: 1–237.

Szent-Ivany, J. H. 1960. Observations on the distribution, ecology, and ethology of *Pericyma cruegeri* Butl. (Lepidopt., Noctuidae) in Papua. Trans. Papua New Guinea Sci. Soc. 1: 7–15.

Turnock, W. J., K. L. Taylor, D. Schröder & D. L. Dahlsten. 1976. Biological control of pests of coniferous forests, pp. 289–311. *In* C. B. Huffaker & P. S. Messenger [eds.], Theory and practice of biological control. Academic, New York.

Vaughn, J. I. 1974. Virus and rickettsial diseases, pp. 49–85. *In* G. E. Cantwell [ed.], Insect diseases. Marcel Dekker, New York.

Waters, W. E., A. T. Drooz & H. Pschorn-Walcher. 1967. Biological control of pests of broad-leaved forest and woodlands, pp. 313–336. *In* C. B. Huffaker & P. S. Messenger [eds.], Theory and practice of biological control. Academic, New York.

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