

Agricultural Experiment Station



1989 Annual Report
College of Agriculture and Life Sciences
University of Guam

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This productive orchid is called *Aranda Mariana Lila*.

Photo by Dr. James McConnell



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FROM THE DIRECTOR

In 1989, much applied research was done in collaboration with scientists in the American Pacific. AES scientists participated in four regional research projects and continued work beneficial to Guam, the region, and the Tropics in general.

Work at the Yigo agricultural experiment station, which covers almost 50 acres, was completed with the first experiment laid out in November. Yigo Station is now self-contained, cleared, fenced, and with power and water. A poultry experimental building at the Inarajan station was started. The fencing and start of dam construction was initiated at the Ija station. Guam now has four experiment stations representing the major agricultural soils of Guam.

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Small Landholders of Guam: Production Analysis and Risk-Avoiding Behaviors

The agricultural economics program was restarted in late July, 1989. One of the first projects initiated was a study of the market and production risks in growing vegetable crops on Guam. The project is intended to examine both production and price risk as an impediment to the development of agricultural production on Guam.

Risks to the farmers of Guam can be divided into four general categories. Price risks occur when there is an over-abundance of a crop, and the price falls below production costs. Production risks fall into three groups: weather, diseases and insects. A 32-year histogram of maximum sustained wind velocities is shown in Figure 1. The relative frequency can be considered to be an estimate of the probability of the maximum sustained winds reaching a specified range in any given month.

By developing a better understanding of the risks involved in fresh vegetable and fruit production on Guam, we hope to be able to offer methods of compensating for these risks. The long-run objective of the project is to aid in increasing local production of fruits and vegetables.

Since the project is just beginning, there have been no impacts to date. Current efforts are focusing on upgrading the information available to the farmers and marketers of Guam's produce. The current method of delivering this information, the Crop and Price Report, is being upgraded. A joint memorandum of understanding between the

Guam Department of Agriculture and the College of Agriculture and Life Sciences is being negotiated. Historical data on prices is in the process of being compiled and keypunched for analysis. Figures 2 and 3 compare the prices of locally-produced cabbage and tomatoes with the prices of the equivalent products imported from the Continental United States. In Figure 2, it can be seen that head cabbage produced on Guam brings about the same price in the market as head cabbage, imported from the U.S. mainland, currently between \$0.60 and \$0.80 per pound. This can be interpreted as indicating that the two items are viewed as being essentially the same by consumers. Cabbage is easily shipped, and it stores well. Thus, there is little in the way of natural transportation and shipping cost barriers to encourage the local farmers to increase their production of it.

Figure 3 compares the prices of slicing tomatoes grown on Guam with the imported product. Locally grown tomatoes almost always sell at a discount to the imported tomato. This indicates that the locally-produced tomato is not considered by the market to be equal in quality to the imported tomato. However, tomatoes do not ship or store well. Fresh slicing tomatoes are generally air-freighted to Guam. The higher shipping costs and storage losses cause fresh tomatoes to retail for a considerable premium over the U.S. mainland price as can be seen in Figure 4. Thus, there is room to improve the marketability of locally-produced tomatoes by improving the quality, and the natural trade barriers will serve to protect the premium that Guam's farmers receive over the prices received by U.S. mainland farmers.

Figure 1

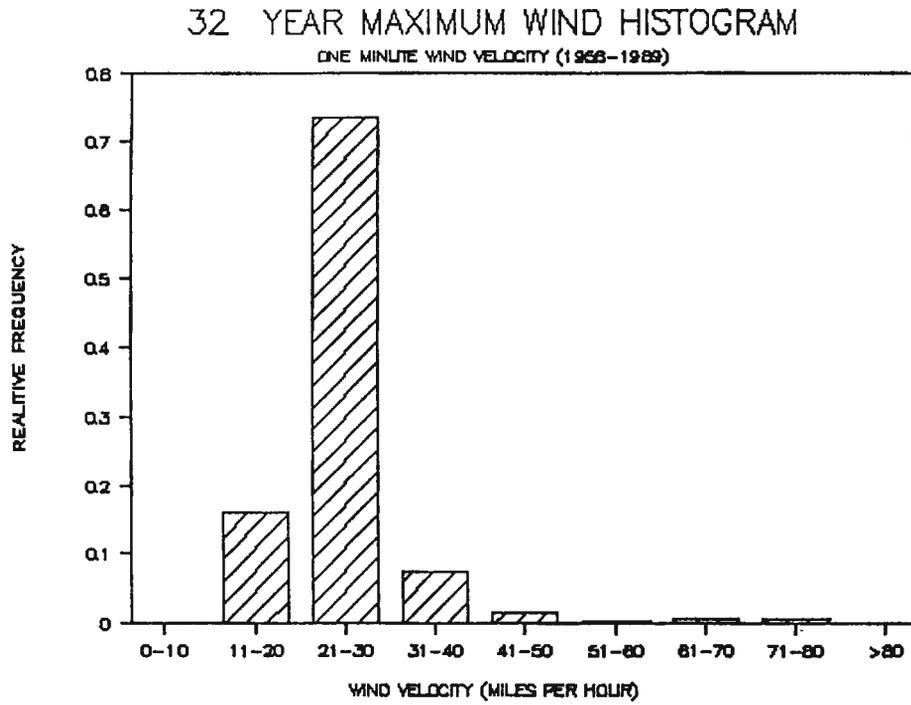


Figure 2

HEAD CABBAGE PRICES

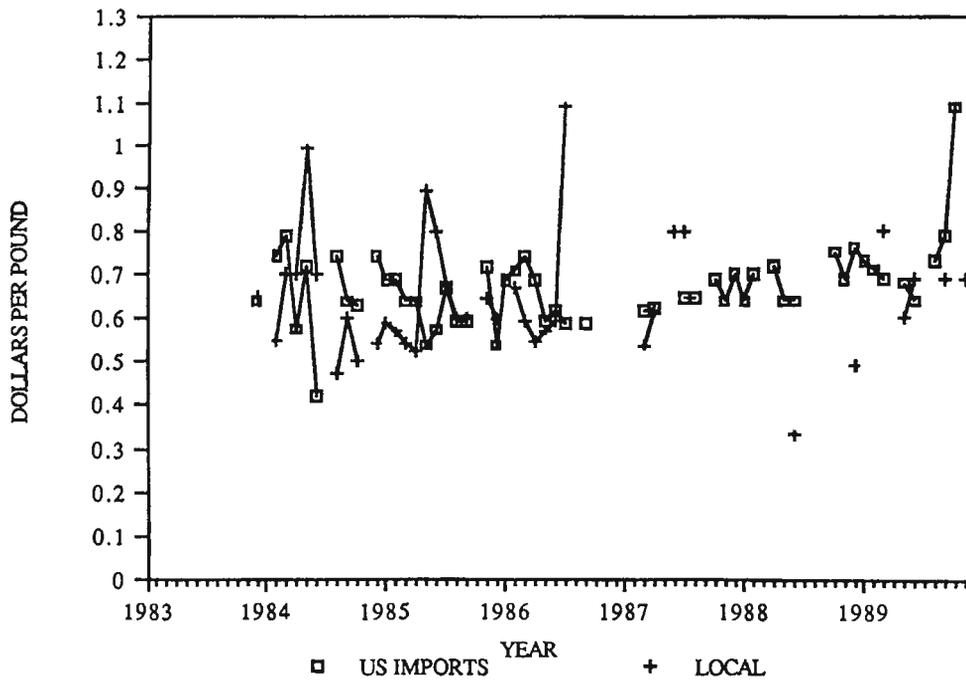


Figure 3
TOMATO PRICES

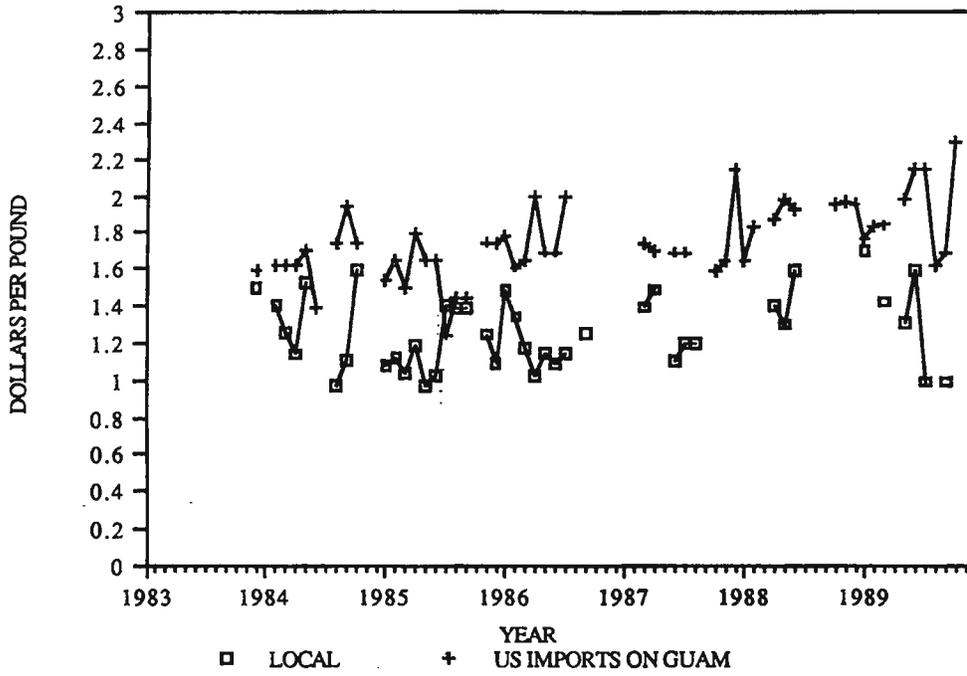
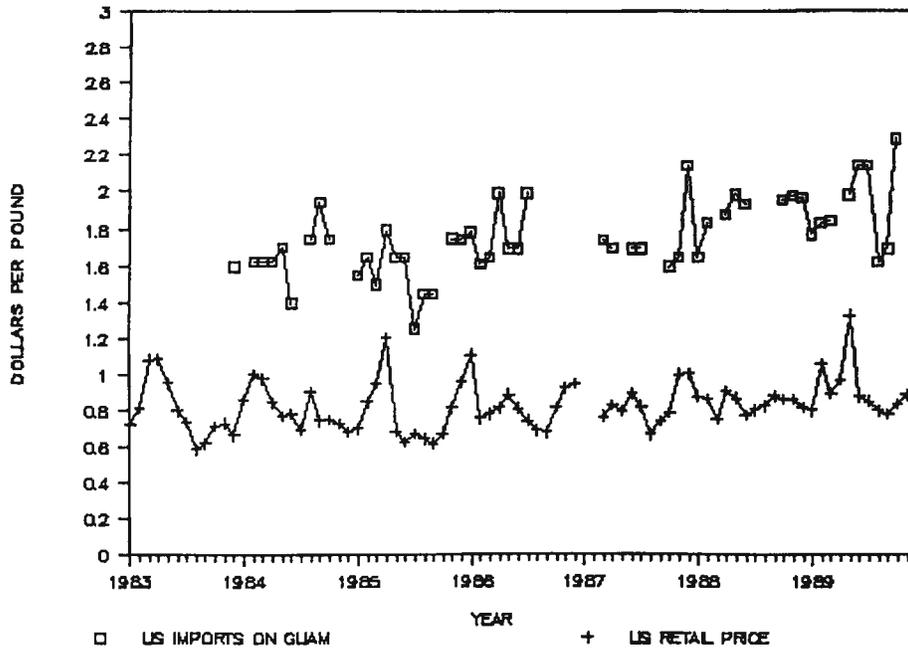


Figure 4

TOMATO PRICES





Introduction

Research continued in agricultural engineering to study the application of trickle irrigation to vegetable crops. Two experiments were conducted to study the effects of various irrigation water application rates on the production of radish and Chinese cabbage.

A) Experiment 1 was conducted in a screenhouse at the Agricultural Experiment Station. Radish seeds (*Raphanus sativa* L., variety: Minowase Summer Cross No. 3) were planted into 12-inch pots containing Guam clay soil with one ounce of 10-20-20 fertilizer per pot. Foliar 60 fertilizer was applied as solution by spraying to the leaves and stems once weekly 2 weeks after planting seeds. The seedlings were thinned to one plant per pot. The micro-irrigation emitter (Submatic button emitter) was placed 12 inches away from the plant with one emitter per pot. The experiment had four water application rates replicated four times. A complete randomized block design was used. The water application ranged from 0.6 to 1.2 ET (0.6, 0.8, 1.0, and 1.2 estimated potential evapotranspiration) of approximately 0.088, 0.119, 0.147 and 0.175 gallons (0.34, 0.46, 0.57 and 0.68 liters) per day. The discharge rate of the Submatic button emitter is 3.243 liters per hour (1.85 minutes/100 ml).

Irrigation times for the treatments were as follows:

Treatment 1: 6.25 minutes (1.82 minutes/100 ml)

Treatment 2: 8.25 minutes (1.77 minutes/100 ml)

Treatment 3: 11.00 minutes (1.92 minutes/100 ml)

Treatment 4: 13.00 minutes (1.90 minutes/100 ml)

Data collection were diameter of root, length of root, weight of top, weight of root and total fresh weight. The result of effect of various water application rates on growth and production of radish is presented in Table 1. Treatment 1.0 ET produced the largest diameter of root and the highest amount of water (1.2 ET) produced the longest root. Higher amount of water (1.0 ET AND 1.2 ET) produced higher yield of root and total plant (top and root). While there were no significant differences in fresh weight of top among the four treatments.

B) Experiment 2 was also conducted in the screenhouse at the Agricultural Experiment Station. Chinese cabbage seed (*Brassica chinensis*) L., variety: Green Petiole Pai-Tsai) were planted into 12 inch pot. All procedures and treatments were the same as described in Experiment A except one ounce of 16-16-16 fertilizer per pot and seedlings were thinned to 3 plants per pot.

The result of effect of various water application rates on production of Chinese cabbage is shown in Table 2. Highest and lowest amount of water treatments (0.6 ET and 1.2 ET) produced the lowest yields in tops, roots and total plants. Treatments of 0.8 ET and 1.0 ET produced the highest yields in tops, roots and total plant.

Usefulness of finds

On a tropical island like Guam, the most serious problems facing vegetable crop production are pests and water, either too much or too little, under traditional farming system. Growing vegetables in the greenhouse with trickle irrigation could prove to be an alternate method for crop production especially during the wet season. It was found that the problems with insects and diseases were minimum during the period of these two experiments.

Table 1. Effect of Various Water Application Rates on Growth and Production of Radish

Water Application rate	Diameter root (cm)	Length of root (cm)	Fresh weight of (top/plant (g)	Fresh weight of root/plant (g)	Total Fresh (top & root) /plant (g)
0.6 ET	5.71 ab*	34.14a	414.5a	362.8a	777.3a
0.8 ET	5.57 a	33.64a	418.0a	366.7a	784.7a
1.0 ET	6.17 b	37.77ab	413.2a	511.2b	924.4b
1.2 ET	5.75 ab	40.24b	401.7a	517.0b	918.7b

*Means followed by the same letter within same measurement in a column do not differ significantly at the 5% probability level using Duncan's Multiple Test.

Table 2. Effect of Various Water Application Rates on Production of Chinese Cabbage

Water Application Rate	Fresh Weight of tops/pot (g)	Fresh Weight of roots/pot (g)	Total Weight (tops & roots) (g)
0.6 ET	313.0*	65.7a	378.7a
0.8 ET	346.6b	74.4b	421.0b
1.0 ET	341.5b	74.0b	415.5b
1.2 ET	300.5a	62.7a	363.2a

*Means followed by the same letter within same measurement in a column do not differ significantly at the 5% probability level using Duncan's Multiple Test.



Yield Response of Head Cabbage Plant to Varying Nitrogen and Potassium Levels

Head cabbage, a shallow-rooted vegetable, needs an ample supply of nutrients to sustain its rapid growth during its entire growing cycle. A pot culture study was conducted to investigate applied nitrogen and potassium on the effect on head cabbage yield.

Head cabbage K-K cross plants were transplanted into 30cm high x 25cm in diameter plastic pots filled with soil. The soil used has been classified as Yigo series (clayey, gibbsitic, isohyperthermic, Tropeptic Eustrustox). The soil pH was 6.9

and nutrient levels were 2.8 ppm for bicarbonate soluble phosphorous, 37 ppm for acetate extractable potassium, 1733 ppm exchangeable calcium, and 126 pm exchangeable magnesium, indicating low phosphorous and potassium supply for normal growth. Determined organic matter was 5.8%, generally indicating sufficient supply of nitrogen for normal growth.

The treatments of five levels of N and five levels of K were combined in a 5 x 5 factorial arrangement according to a randomized complete block design with four replications. Rates of added ammonium sulfate (N) and potassium chloride (K) were 0, 60, 120,

180 and 240 kg ha⁻¹. These rates of N and K were applied five days after transplant. The rate of 180 Kg ha⁻¹ treble superphosphate (P) was applied to all pots at transplanting time. Cabbage heads were harvested in one picking at sixty days as the heads reached marketable maturity. Each head was trimmed to the first leaf folding the head and weighed on a gram balance.

It was observed that increasing rates of N and K interacted in their effects on the head cabbage yields. At high levels of applied N the yield increase with the increased rates of K. The results showed that the yield increased significantly as both the N and K levels were increased to 240 kgha⁻¹.

Table 1. Yield of Head Cabbage as Affected by Five Nitrogen and Five Potassium Rates

Treatment Kg ha-1	Yield Kg per plot			Treatment	
	RI	RII	RII	RIV	Mean*
0-180-0	1.350	1.300	1.350	1.375	1.344 j
0-180-240	1.450	1.475	1.400	1.375	1.425 ij
60-180-180	1.450	1.425	1.400	1.400	1.431 ij
0-180-120	1.450	1.450	1.425	1.425	1.438 i
60-180-60	1.425	1.350	1.500	1.500	1.444 i
60-180-60	1.425	1.450	1.450	1.475	1.450 hi
60-180-120	1.450	1.425	1.475	1.475	1.456 hi
0-180-180	1.400	1.500	1.525	1.425	1.463 hi
120-180-240	1.475	1.450	1.525	1.450	1.475 hi
0-180-60	1.475	1.500	1.525	1.550	1.513 ghi
60-180-240	1.600	1.450	1.550	1.450	1.513 ghi
180-180-180	1.625	1.525	1.425	1.600	1.544 ghf
120-180-120	1.450	1.550	1.550	1.625	1.544 ghf
120-180-60	1.500	1.500	1.650	1.650	1.575 gef
180-180-60	1.625	1.625	1.475	1.575	1.575 gef
180-180-120	1.625	1.475	1.675	1.550	1.581 gef
120-180-180	1.600	1.625	1.600	1.575	1.600 gef
180-180-240	1.600	1.600	1.675	1.575	1.613 def
120-180-0	1.525	1.750	1.600	1.725	1.650 de
180-180-0	1.650	1.600	1.700	1.675	1.656 de
240-180-0	1.775	1.700	1.625	1.700	1.700 dc
240-180-60	1.825	1.675	1.725	1.775	1.750 bc
240-180-120	1.825	1.875	1.775	1.825	1.825 ba
240-180-180	1.900	1.875	1.975	1.800	1.888 a
240-180-240	1.900	1.975	1.850	1.850	1.894 a

* Means with the same letter are not significantly different.



Due to lack of poultry facilities, much of 1989 was devoted to extension service advising poultry and cattle producers. A poultry research complex with brooder, layer and broiler cage systems was designed. The building is due to be completed in July of 1990.

A research paper under title: "Interaction of vitamins A,D,E and K in the diet of broiler chicks" was published in the Poultry Science Journal.

During the summer of 1989, under USDA Apprenticeship program, student training was provided and island's imported and local feed samples were chemically analyzed.

During the year a comprehensive review of literature on tropical feeds was undertaken and a grant proposal under section 406 was submitted for funding.



Biological Control of Red Coconut Scale

The parasite *Adelencyrtus oceanicus* was introduced for the third time in April 1989 into Guam. Since May 7, 1988 to April 28, 1989, a total of 217 females and 406 males were released on Guam. In June 1989, several males and females of *A. oceanicus* were recovered for the first time. The initial spread of the

parasite was slow. A summary of *A. oceanicus* releases on Guam are given in Table 1.

Publications

Marutani, M. and R. Muniappan. 1989. Incidence of the red coconut scale, *Furcaspis oceanicus* (Homoptera:Diaspididae) and its parasites in Micronesia. J. Pl. Prot. Tropics 6:61-66.

Marutani, M. and R. Muniappan, 1989. Use of *Adelencyrtus oceanicus* (Hym:Encyrtidae) for controlling the red coconut scale, *Furcaspis oceanica* (Hom: Diaspididae) in Guam. Paper presented at the International Seminar on "The Use of Parasitoids and Predators to Control Agricultural Pests", Tsukuba, Japan. Oct. 1989.

Table 1. Summary of *A. oceanicus* releases on Guam

Release site	Origin	No. of <i>A. oceanicus</i> released		Dates of Release	Date of first recovery of <i>A. oceanicus</i> near release site
		Female	Male		
1. Mangilao	Ulithi Atolls	22	30	May 7-June 7, 1988	June, 1989
2. Barrigada	Palau	27	83	Oct. 31-Nov. 22, 1988	Dec. 1989
	Palau	157	274	April 12-28, 1989	
3. Mangilao	Palau	11	19	April 10, 1989	
	Total	217	406		

Biological control of Fruit Piercing Moth, *Othreis fullonia*, in the American Pacific

Assessment of Pest Status

Surveys were continued to assess the pest-status of the fruit piercing moth, *Othreis fullonia*, throughout Micronesia. Islands other than Guam which have been visited to date are: Rota, Tinian, Saipan, Yap, Palau, Truk, Pohnpei and Kosrae.

The species was found to be very common on Rota, Tinian, Saipan, Pohnpei and Kosrae. However, it is currently only of economic importance on those islands producing fruit for the export market, namely Tinian and Kosrae.

Relatively low levels of *O. fullonia* occur in Palau, and the species appears to be absent from Yap and Truk.

On Guam, the population density appears to be highly seasonal with highest numbers occurring during the wet season. At this time of the year local growers frequently experience heavy damage to a variety of fruits including banana, guava, mango, papaya and various kinds of citrus.

Food Preference Studies

Preliminary experiments were conducted to determine the food preference of *O. fullonia*. To date, 14 fruits have been tested of which banana is the most preferred followed, in decreasing order of preference, by guava > mango > papaya > pear > starfruit > grape > tomato > plum > orange > apple > calamansi > lemon > eggplant and pomegranate. The data thus far accumulated indicate a definite preference for the sweet aromatic types of fruits over those with lower sugar content.

Life Cycle Studies

Experimental populations of *O. fullonia* were laboratory reared on Guam to determine the life cycle and duration of developmental stages. The eggs were found to take between 2-4 days to hatch. The larvae molt 4-5 times over a period of 15-17 days before pupating for a further 10-11 days. The adult female requires a pre-oviposition period of 4-10 days and lives for a further 30-40 days.

Whilst the total development time (28-40 days) is similar to that reported for this species from other areas of the world, this is the first record of it having a variable number of instars. Life cycle studies for this species are currently underway on Saipan and Pohnpei to determine possible inter-island differences in instar number and developmental times.

Biological Control Studies

Studies of the natural enemies of *O. fullonia* are continuing on Guam and the other islands of Micronesia listed above. The hymenopteran egg parasitoids are known to be among the most effective agents of biological control and, so far, efforts have largely been focused on these.

Biweekly egg collections from six sites in Guam, have revealed at least 10 different species of wasp parasitoids. Two of these wasps are very common and have been identified as *Telenomus* sp., and *Ooencyrtus* sp. The former species, *Telenomus* sp., is the dominant egg parasitoid of both single eggs and egg masses especially during the dry season.

At this time of the year, it frequently accounts for 95-100% of all parasitized eggs collected. During the wetter months, however, the incidence of egg parasitization by *Ooencyrtus* sp. increases markedly to levels which are similar, and occasionally even greater than the former species. Data collected over the last nine months indicate that the percentage parasitization by both species averages around 71% and 88% for single eggs and egg masses respectively.

A third wasp parasitoid, *Trichogramma* sp, accounts for approximately 1% all parasitized *O. fullonia* eggs collected from Guam and evidence is emerging which suggests that, whilst this wasp is common on the island, its efficiency is limited, at least in part, by its low foraging level.

The other seven hymenopteran species isolated to date, show a very low incidence of egg parasitization (<0.1). Two of these, e.g., *Cheilonneurus* sp.(Encyrtidae) and *Marietta* sp.(Aphelinidae) are known hyperparasites of *Trichogramma* sp.. The remaining wasps await identification.

Of those eggs which escape parasitization, in Guam, only a small fraction produce larvae. The remainder fall foul to ants, predatory bugs, and fungal attack or, are simply infertile and fail to hatch. It is estimated that between 1-2% of the total number of eggs laid produce larvae. In spite of this, the species still attains populations that are troublesome at certain times of the year and additional biological con-

trol measures are, therefore recommended to augment and improve the effectiveness of the existing natural enemies already present in Guam.

Extensive surveys conducted on other islands have revealed *Telenomus* sp., *Ooencyrtus* sp. and *Trichogramma* sp. to be the most common egg parasitoids of *O. fullonia* although there are clear inter-island differences in the relative abundance and effectiveness of each genera. For example, in Rota, Tinian and Saipan, the pattern is similar to that found on Guam although *Ooencyrtus* sp. from Guam and Rota (Type 1) clearly differ from that found in Saipan and Tinian (Type II). Interestingly, *Ooencyrtus* sp. was not found in Pohnpei and the incidence of egg parasitization by *Telenomus* sp. was also very low. Surprisingly, *Trichogramma* sp. was the dominant egg parasitoid here and accounted for >90% of all parasitized eggs collected.

A relatively high incidence of egg parasitization by *Trichogramma* sp. also appears to be the case in American Samoa

Related Fruit Piercing Species

Field surveys were continued to determine the presence of other fruit piercing moth species in Micronesia. Three additional primary and 17 secondary fruit-piercing species (all Noctuids) have so far been collected from Guam. All are common, locally, and many have been found on the other islands visited. Several of these species are already known and the rest are currently awaiting identification.

Biological Control of *Lantana camara*

Surveys to assess the status of lantana in Micronesia were continued in 1989. A total of 10 islands were visited viz., Pohnpei, Truk, Kosrae, Palau, Yap, Guam, Rota, Aguijan, Tinian and Saipan. *Lantana* was found widespread on all islands except Rota and Kosrae.

The surveys revealed that 7 of the 13 species of exotic insects introduced into the area over the last forty years to curb the spread of lantana had established and achieved an acceptable level of control of the majority of the islands.

Several different colored varieties of lantana were observed during the inter-island surveys ranging from pink and yellow through pale orange to deep red.

Caged experiments with the Philippine turtle doves, *Streptopelia bitorquata* have shown that a small proportion of ingested lantana seeds survive the action of the crop and pass through the alimentary canal intact thus implicating its role in lantana seed dispersal. The pod fly *Ophiomyia lantanae* significantly reduced the palatability of the lantana fruits to the Philippine turtle dove (Table 1).

Seasonal Survey:

Studies to determine the seasonal fluctuations in abundance and effectiveness of the natural enemies of *lantana camara* on Guam were initiated in June 1988 and are continuing.

The six sites selected for study are in open, sunny locations and are widely separated from one another. They are monitored on a biweekly basis for the following parameters:

1. Leaf damage
 - a) v. light = 10% damage
 - b) light = 10-25% damage
 - c) moderate = 26.50% damage
 - d) severe = 51-100% damage
2. Leaf damaging insects in rank order of effectiveness.
3. Flowers / sq m. and % showing insect damage.
4. Quantitative estimate (%) of species effectiveness (flowers)
5. Fruiting heads/ sq. m.
6. Average # pods/fruitletting head
7. % pod damage
8. Quantitative estimate (%) of species effectiveness (pods)

A summary of the data collected thus far from each site is shown in Table 2.

To date, marked seasonal-and locational-dependant changes in the activities of certain species have been recorded. For example, leaf damage appears to be greatest during the dryer months when populations of the tingid bug, *Teleonemia scrupulosa* are high and plants are heat stressed. As the wet season progresses however, populations of these insects show a dramatic decline with the result that bushes generally show rapid recovery.

In contrast to the seasonal fluctuations observed with *T. scrupulosa*, populations of the leaf mining beetle, *Uroplata girardi*, tended to increase during the wet season. However, they were generally far less effective in stemming the growth of lantana than the former species.

Flower damage was caused predominantly by caterpillars of *Epinotia lantana* and *Lantanophaga pusillidactyla* and was often very extensive (<50% of examined flowers infested). Both species were highly effective in reducing pod

numbers by more than 80% in some cases. Distinct seasonal trends are not immediately apparent for these species.

Likewise, pod damage by the agromyzid fly, *Ophiomyia lantanae*, show no clear seasonal trends at this stage.

A detailed analysis of the date for these and other insects recorded at each site will be presented at the end of the study in the next report.

Biological Control Studies:

The introduction of hitherto absent natural enemies to the lantana infested island of Tinian and Aguijan began with the first shipment of the leaf-mining beetle, *Uroplata girardi*, to the former island in August. Adults were observed at the release site some ten weeks later and studies are continuing to monitor the progress of this species.

Management of Cruciferous Crop Pests

Many experiments were conducted to study the host preference of major cruciferous crop pests on Guam. It was observed that the imported cabbage worm, *Hellula undalis* (F.) preferred radish (*Raphanus sativus* L.) followed by Chinese cabbage (*Brassica pekinensis* cv. Tempest) and mustard (*Brassica juncea*). The cabbage cluster caterpillar, *Crocidolomia binotalis* Zeller preferred Chinese cabbage (*B. pekinensis* cv. Tempest). Radish, mustard and Chinese cabbage (cv. Tempest) were preferred hosts for flea hopper, *Halticus tibialis* (Rwter). Head cabbage was the preferred host for cluster caterpillar, *Spodoptera litura* (F.). Host preference studies on the diamondback moth, *Plutella xylostella* (L.) are being continued.

Table 1: The Palatability of *Ophiomyia lantanae* infested Lantana fruits to the Philippine turtle dove *Streptopelia bitorquata*

Feeding Trial	% lantana fruits* eaten over 6 hour period			
	succulent/non-infested	succulent/infested	dry/non-infested	dry/infested
1	100	72	34	8
2	100	42	10	4
3	100	53	35	0
Average	100	56	26	4

* Between 50-100 berries/catagory used in each trial

Table 2: Summary of Biweekly Observations to Determine Seasonal Changes in the Abundance and Effectiveness of the Natural Enemies of *Lantana camara* from Guam (Data are mean values of 19 biweekly observations)

Site	Severity of leaf attack	Rank order of leaf damaging insects			#/sq.m	Flowers			Fruiting Heads		
		Ur(1)	Te(2)	Hy(3)		% damaged	Insects (%)	#/sq. m.	Av. #. pods	% damaged	Insects(%)
1	Severe	Ur(1)	Te(2)	Hy(3)	5	46.4	La (49.5) Ep (25.1) Te (25.0) Ad (0.4)	5	6	14.4	Op (62.2) Ep (37.8)
2	Moderate	Te(1)	Ur(2)	Hy(3)	6	46.2	Ep (53.5) La (25.1) Te (18.3) Ad (3.1)	3	9	30.5	Ep (54.9) Op (45.1)
3	Moderate	Te(1)	Ur(2)	Hy(3)	13	50.4	La (57.9) Ep (37.1) Te (4.8) Ad (0.2)	10	7	29.7	Ep (57.3) Op(42.7)
4	Light	Ur(1)	Te(2)	Hy(3)	26	48.5	La (63.8) Ep (29.4) Te (6.8) Ad (0.0)	12	6	28.1	Op (63.6) Ep (36.4)
5	Light	Te(1)	Hy(2)	Ur(3)	24	39.9	Ep (43.6) La. (38.3) Te (17.0) Ad (1.1)	36	10	58.4	Op (57.2) Ep (42.8)
6	Light	Ur(1)	Te & Hy (2)		20	43.7	La (47.8) Ep (46.7) Te (3.1) Ad (2.4)	16	10	31.3	Op (57.4) Ep (42.6)

Site Identity: 1 = Yigo; 2 = Tumon; 3 = Dededo; 4 = Piti; 5 = Mangilao; 6 = Inarajan

Species identity: Ad = *Adoxophyes melia*; Ep = *Epinotia lantana*; Hy = *Hypena strigata*; La = *Lantanophaga pusillidactyla*,

Op= *Ophiomyia lantanae*, Te = *Teleonemia scrupulosa*, Ur = *Uroplata girardi*



Biological Control of the Leucaena Psyllid, *Heteropsylla cubana*

Biological control of the leucaena. Studies on the effect of the beetle, *Curinus coeruleus*, released in 1985, were continued. *C. coeruleus* is now established at two sites, Mangilao and Agat. Releases of 150 beetles in three batches of 50 beetles each were made at two additional sites, Asan and Yona. Monitoring populations of psyllids, beetles, and other natural enemies has continued.

Results

In 1988-9, populations of the psyllid remained below 100 nymphs and 50 adults per gram of tip leaves (dry weight). Outbreaks were only noted at two sites (Fig. 1), Asan, and Yona. Levels of general predators have been high. *Olla v-nigrum*, *Orius nibe* (Anthocoridae), *Campylomma lividicornis* (Miridae) and an unidentified mite have also been found associated with psyllid populations. Densities of *Campylomma lividicornis* show a positive correlation with densities of the psyllids, but the mites do not. *O. v-nigrum* is present at only a few sites and did not become abundant until this year, although it has been present on Guam for a long time.

Biological control of leafminers, *Liriomyza* sp.

Ganaspidium utilis was reported established in 1984. Since then it has increased to become the dominant parasitoid on leafminers on a variety of crops. In 1989 work concentrated on determining the

effect of the addition of *G. utilis* to the parasitoid community associated with *Liriomyza* spp.

To estimate the impact of *G. utilis*, populations of parasitoids and leafminers present in untreated beans were compared with those present in untreated beans prior to the introduction of *G. utilis*. Sampling methods are reported in 1982-4 annual reports.

Results

On beans (*Vigna* sp.) numbers of leafminers on untreated beans were low in comparison to populations in the years prior to 1984 release. In 1989 in three trials, peak densities of leafminers remained below seven per leaf compared to populations up to 45 leafminers per leaf in 1982-4 (Fig. 2). Mean seasonal densities dropped from about 11 leafminers per leaflet to less than three.

Primarily two ecto-parasitoids, *Hemiptarsenus semialbiclavus* and *Chrysonotomyia formosa* were attacking the leafminer on yard-long beans. *G. utilis*, an internal parasitoid emerging from the pupa, has complemented those parasitoids and increased the overall level of parasitization on beans.

Estimated gains in yield associated with the introduction of *G. utilis* are about 150 kg per 100 m row, or about 7,000 kg per ha. Some problems with the leafminers are still being experienced by growers, but in most cases it is suspected that these may be pesticide induced outbreaks. *G. utilis* is especially sensitive to insecticides.

Preferences of leafminers and parasitoids for different crops.

Survey reports indicated that both the species of leafminer present and the parasitoids was dependent on the type of crop. To examine this question in more detail, rows of pechay, tomato, long beans (*Vigna* sp.), pole beans (*Phaseolus vulgaris*), and cucumber were planted in a random design. Each crop was planted in 9 m. rows 0.9m apart. Leafminers and parasitoids were sampled every two weeks. The number of miners was counted on 40 leaves in each plot. To sample parasitoids, 15 leaves with mines were collected from each crop and held in the laboratory for emergence of parasitoids and leafminers.

Results

L. trifolii was the only leafminer reared in significant numbers. *L. sativae* was present, but only a few individuals were reared. *L. trifolii* showed distinct host preferences, showing the highest densities on *Vigna* beans and lesser abundances on *Phaseolus*, pechay, tomato and cucumber (Table 1). It was rare on cucumber. On Guam prior to the introduction of *G. utilis*, leafminers were a serious problem on yard-long beans, the most commonly grown bean, and a lesser, but very important problem on pole beans. They were not a problem on tomato, cabbage-related crops or cucurbits.

G. utilis was the dominant parasitoid present (Table 1). *G. utilis* parasitized about half or more of the leafminers in all crops except tomatoes, although it was also the dominant parasitoid that crop.

Distinct preferences for leafminers on certain crops were shown by the different species of parasitoids (Table 1). *Grotonoma micromorpha* and *Disorgama pacifica* preferred cruciferous crops and tomatoes in comparison to beans. *C. formosa*, on the other hand, preferred beans. *G. utilis* was abundant on all crops but was less abundant on tomatoes.

Leafminers attacking yard-long beans were parasitized by several species of parasitoid. In addition to *G. utilis*, the ecto-parasitoids, *Hemiptarsenus semialbiclavus* and *Chrysonotomyia formosa* were abundant. *Disorgama pacifica* was also present, but was much less common (Table 1).

Biological control of the mango shoot caterpillar, *Penicillaria jocosatrix*.

Evaluation of the effectiveness of introduced parasitoids for the control of the mango shoot caterpillar continued in 1989.

Results

Euplectrus sp. and the tachinid *Blepharella lateralis* continued to be abundant and effective in controlling the noctuid caterpillar *P. jocosatrix*. Caterpillar populations were slightly higher in 1989 than in 1988 (Fig. 3), but were not sufficiently abundant to affect yield. Despite rainy weather, which increased the incidence of anthracnose, and a major typhoon which knocked many fruit off the trees, a yield of 1.7 fruit per tip was obtained in 1989. This is a 20 fold

increase over the best pre-introduction base year.

Leaf damage caused by the mango shoot caterpillar was decreased significantly when compared to pre-parasitoid introduction years. About 55% of the new leaf area was consumed in 1983-4 compared to 20% in 1989. Much of the leaf tissue consumed in 1989 was by *Anisodes illepidaria*, rather than by the mango shoot caterpillar.

In 1988-9, mortality of the caterpillars from all parasitoids ranged around 50 percent (Table 2). *Euplectrus* sp. was the dominant parasitoid in the wet season while *B. lateralis* was dominant during the wetter parts of the year. From August through December most of the mortality was due to *B. lateralis*, but April and May, at the height of the dry season, *Euplectrus* sp. was the only parasitoid present in detectable numbers. Thus, there appear to be seasonal conditions which affect the two species, and together they form a complementary system for controlling the mango shoot caterpillar.

Euplectrus sp. parasitizes all instars, but attacks third instars more frequently. Fifth instars are rarely attacked. The number of eggs laid by *Euplectrus* is related to the size of the caterpillar attacked. If the fifth instar is excluded due to the rarity of eggs found on it, there is a linear increase in the number of wasp eggs laid on caterpillars in relation to instar (Fig. 4). First instars invariably received a single egg while fourth instars received an average of about five eggs. Overall,

including both the frequency with which caterpillars are attacked, and the rate of oviposition, about half of the wasp population is produced from the third instar.

In 1989 an outbreak of the geometrid caterpillar *A. illepidaria* took place during the latter part of the dry season. This caterpillar has been picked in earlier surveys of the fauna of mangoes, but was rare. Feeding tests showed it prefers older new leaves than the mango shoot caterpillar. Mango shoot caterpillars survive best and have higher pupal weights on young leaves from 1 - 10 days old. They do very poorly on leaves older than 10 days. *A. illepidaria* does poorly on leaves 1-6 days old. This geometrid has the best survival and highest weight gains on leaves 8-12 days old. I suspect the increase in commonness of the geometrid may be a case of competitive release. In addition to this geometrid, an undescribed species of *Thalassodes* and two other undetermined species were found in the surveys in 1989. None of these species were found in the community surveys done prior to the release of the biocontrol agents.

The flowers were also heavily consumed by the mango shoot caterpillar before the introduction of the natural enemies. Since then, flowers have become more abundant, and several other species have become common on mango. *Thalassodes* sp.n., *A. illepidaria*, *Chloroclystis* sp. n., and two other unidentified species have been found. *A. illepidaria* is abundant enough to cause substantial damage at certain times.

Table 1. Seasonal densities of *Liriomyza* spp. and parasitization rates by different species of parasitoids in intercropped beans, tomatoes, pechay, and cucumber.

Crop	<i>Liriomyza</i> sp.		Species of parasitoid					Total
	Density leafminers	Percent emerging	<i>C.</i> <i>formosa</i>	<i>H.</i> <i>semialbiclavus</i>	<i>G.</i> <i>utilis</i>	<i>D.</i> <i>pacifica</i>	<i>G.</i> <i>micromorpha</i>	
<i>Vigna</i> beans	1.7	31.8	13.7	5.7	48.3	0.5	0.0	68.2
<i>Phaseolus</i> beans	0.8	31.8	2.3	11.4	52.3	2.3	0.0	68.2
Pechay	0.6	8.0	4.0	8.0	68.0	8.0	4.0	92.0
Tomato	0.4	36.4	0.0	18.2	36.4	9.1	0.0	63.6
Cucumber	0.2							

Table 2. Parasitization of mango shoot caterpillars by *Euplectrus* sp. and *B. lateralis* during the wet and dry seasons.

Date	Percentage of larvae parasitized by		Overall % parasitized
	<i>Euplectrus</i>	<i>B. lateralis</i>	
Aug-88	13.3	38.8	52.1
Sep-88	13.6	16.5	30.1
Oct-88	11.9	31.0	42.9
Nov-88	0.0	46.7	46.7
Dec-88	0.0	33.0	33.0
Apr-89	67.7	0.0	67.7
May-89	53.8	0.0	53.8

Table 3. Rate of parasitization and the number of eggs laid by *Euplectrus* sp. on various instars of the mango shoot caterpillar.

Instar	Number of caterpillars	Percent attacked	Average no. eggs laid	Standard deviation	Range	% contribution to wasp pop.
1	49	16.3	1.00	0.00		8
2	125	17.6	2.67	1.08	1, 5	23
3	54	29.6	3.31	1.38	2, 6	48
4	51	7.8	5.00	2.94	1, 8	19
5	79	1.3	3.00			1

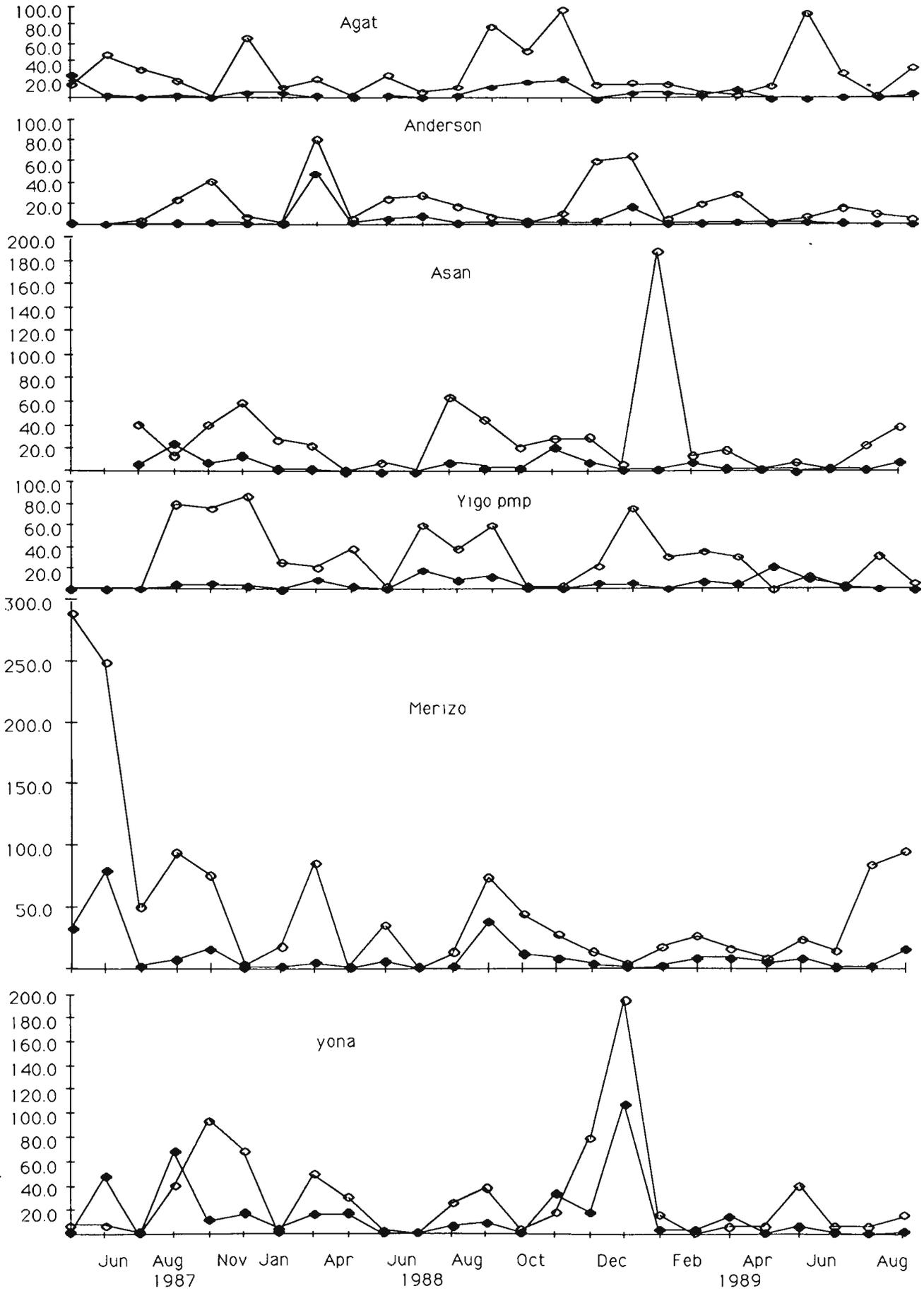


Fig 1. Psyllid populations from 1986 through 1989

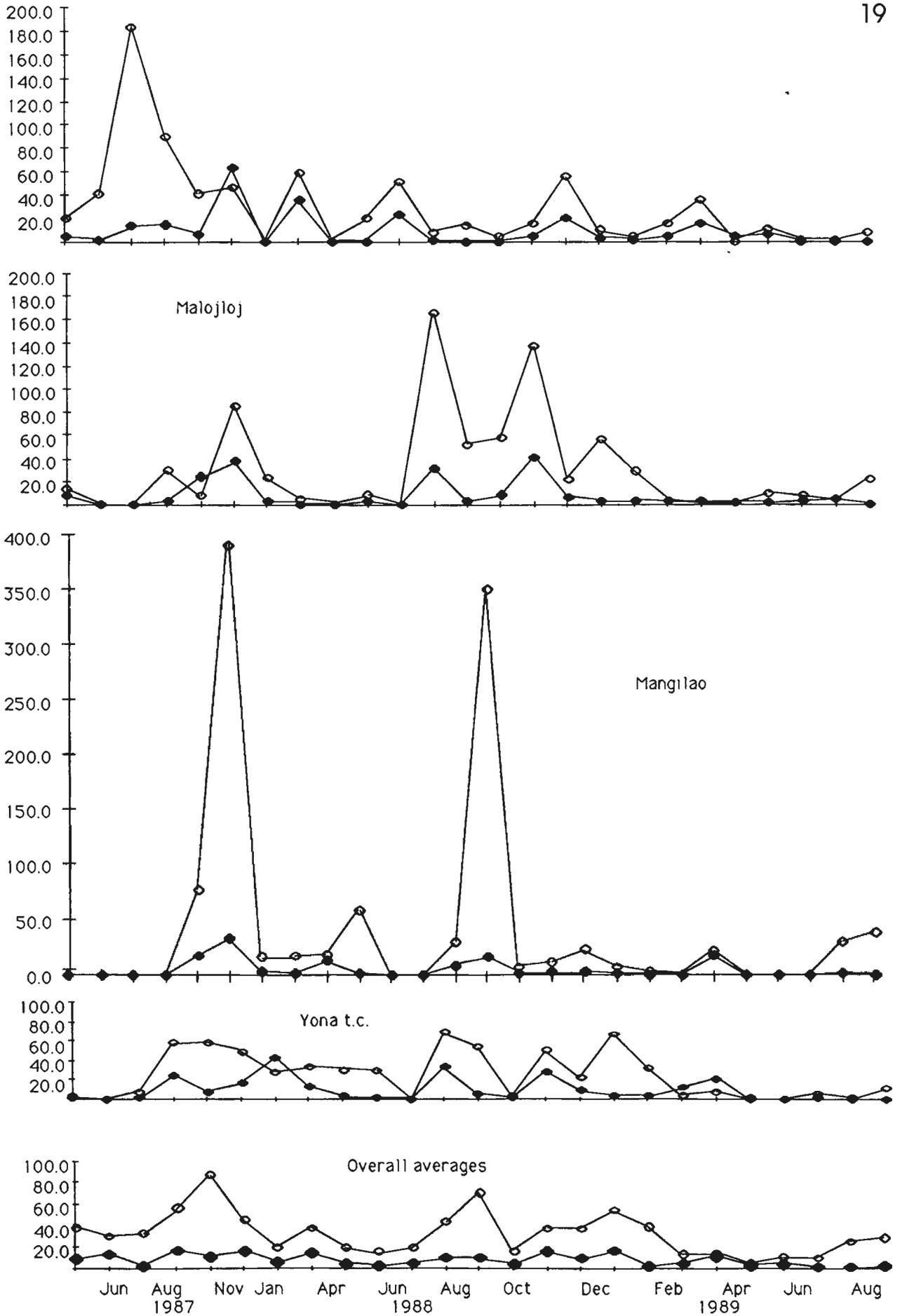


Fig. 1. cont.

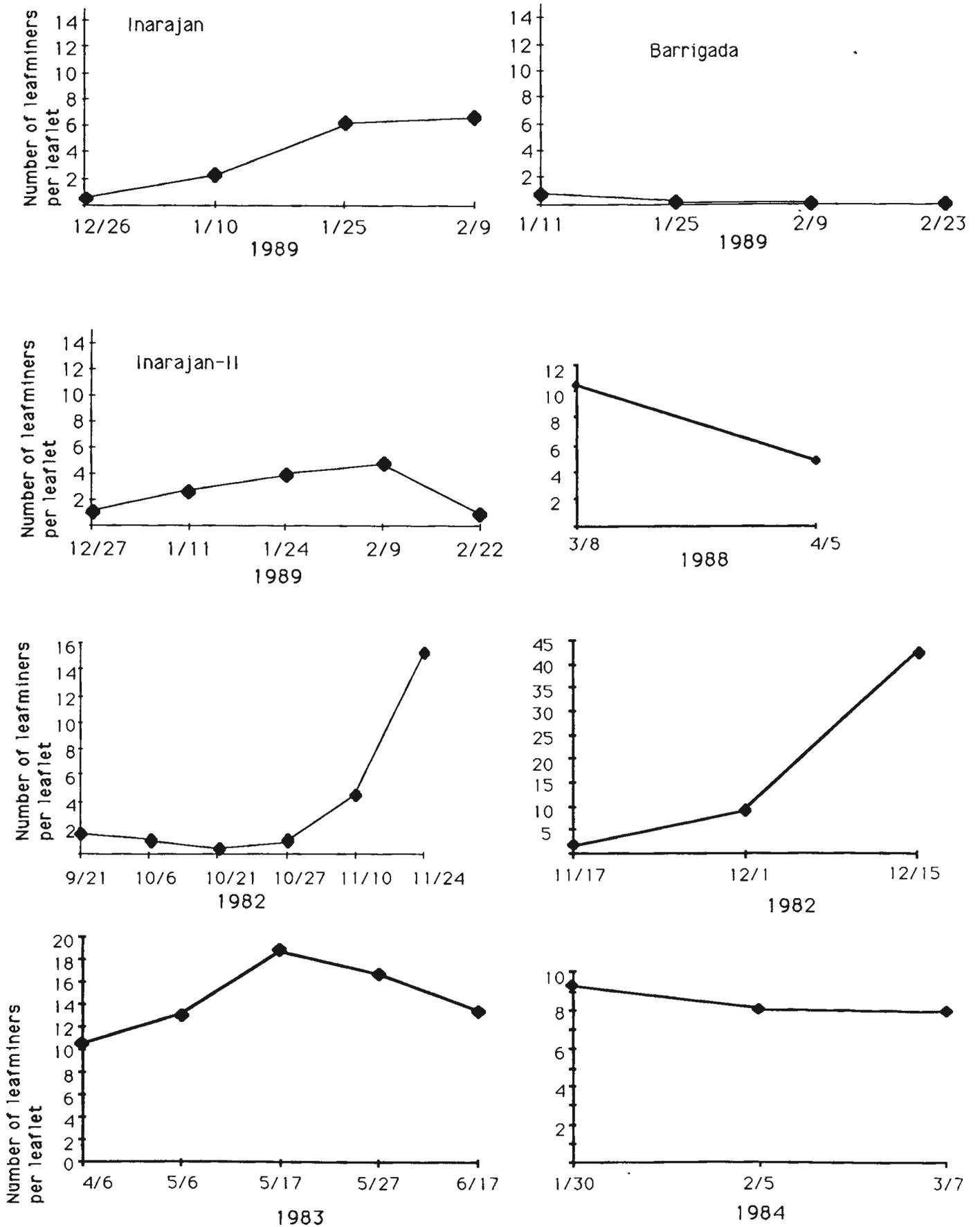


Figure 2. Populations of leafminers prior to and after release of *Ganaspidium utilis*

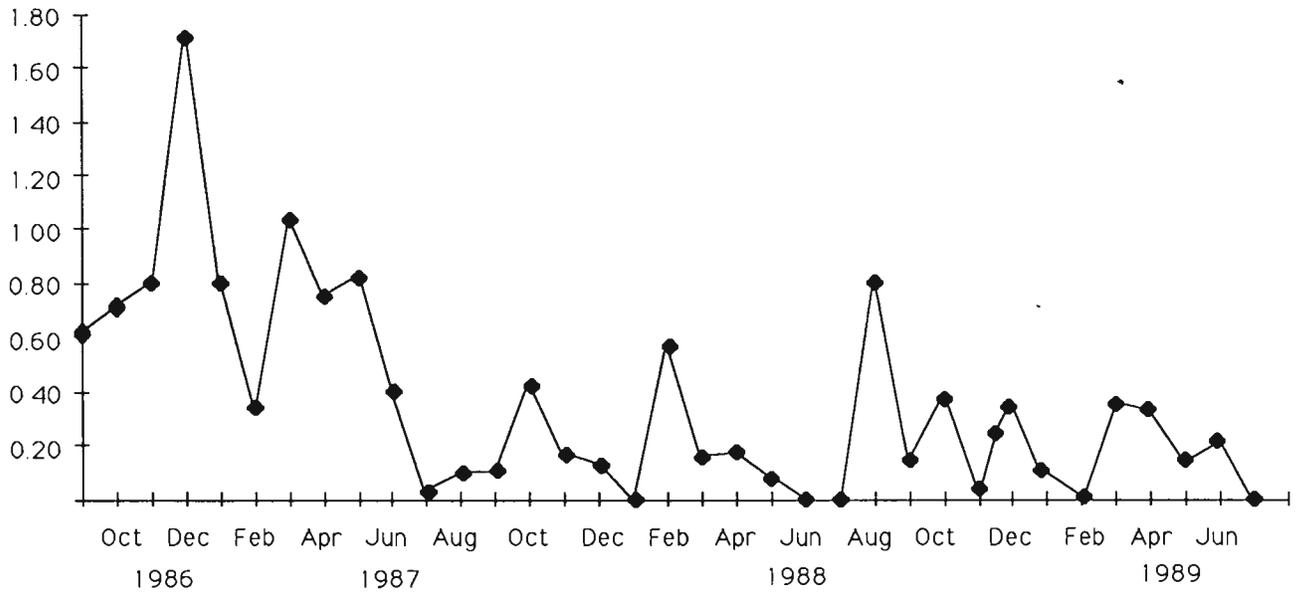


Figure 3. Fluctuation of *P. jocosatrix* populations after the introduction of *E. lateralis* and *Euplectrus* species in late 1986 and early 1987.

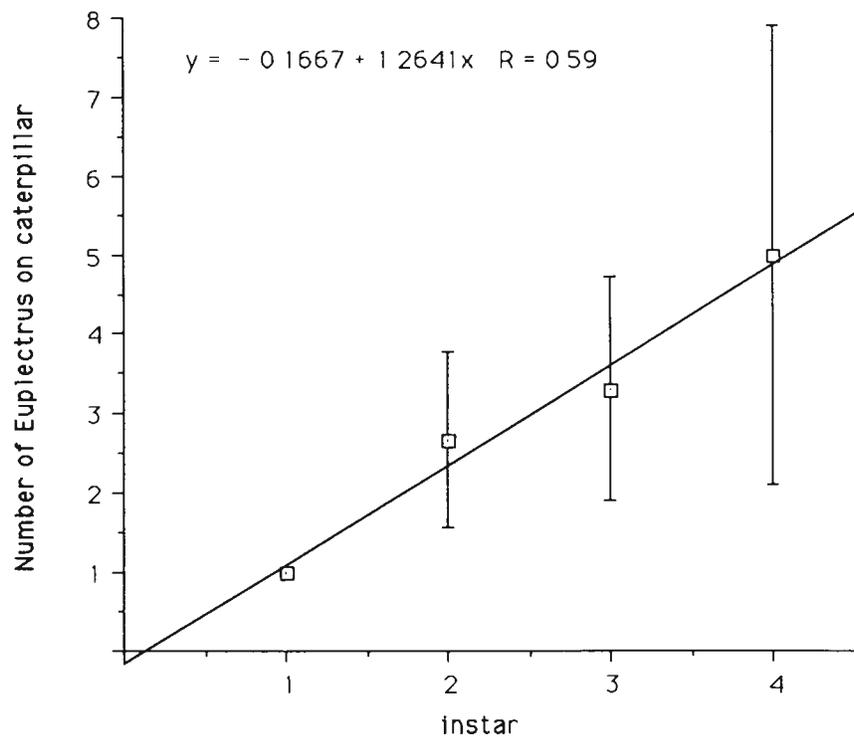


Figure 4. Number of eggs of *Euplectrus* sp. laid on larvae of the mango shoot caterpillar in relation to instar. Values reported are means from field collected caterpillars.



Effects of Trellising on Cucumber Pests

Methods

Cucumber plants were grown in four by four meter plots either horizontally on the ground or vertically trained up nylon nets. Treatments were alternated. The experiment was run in July-Aug. 1989. Plots consisted of four, four meter-long rows one meter apart. In each row, cucumbers were planted in hills one meter apart. After germination, plants were thinned to three plants per hill.

To estimate populations of orange pumpkin beetles and black island fleahoppers, one row in each plot was randomly chosen and sampled using a D-Vac suction apparatus. Populations of melon aphids, melon thrips, predatory mites and anthocorid bugs were estimated by randomly collecting 15 leaves per plot and counting all individuals on the leaf. *Liriomyza* sp. were estimated by counting the number of mines on forty leaves. To select the leaves, a vine tip was randomly selected, and then the number of mines on the 10th leaf back from the tip was counted. Numbers of melon worms, lady beetles and syrphids were also counted on these leaves. Melon fly punctures were counted on each cucumber at harvest.

Results

Responses to the trellising varied depending on the species involved. As in previous experiments, the black island fleahoppers *Halticus tibialis* were much more abundant on the cucumbers growing horizontally. This difference was slight and non-significant during the early season before the cucumbers had climbed very far, but became larger (and statistically significant at the 0.05 level as measured by a *t* test) at each sampling date. In contrast to previous trials, *Aulacophora* beetles were considerably more common on the trellised plants. The difference was statistically significant on the first two sampling dates, but not on the third. *Liriomyza* mines were very scarce, and the influence of plant architecture could not be determined. No clear pattern could be observed in the numbers of melon aphids, *Aphis gossypii*. Sometimes they were more abundant in the horizontal plants and sometimes in the vertical ones, and the differences were never statistically significant. Similar results have been observed in previous experiments. In a previous experiment, their predators, lady beetles and syrphids, appeared to be affected by plant architecture, but this was not observed in the current trial, with one exception. On the last

sampling date, lady beetle larvae were more common in the horizontal plants. This result contrasts with a previous experiment where lady beetles tended to be more common on vertical plants. Syrphid larvae were also more common on the horizontal plants, as they had been in the previous experiment. Melon thrips, *Thrips palmi*, were more common in the vertical plants. The difference was not significant at the first sampling date, approached significance at the second and was highly significant (probability greater than 0.001) at the third sampling date. This is similar to results observed in a previous trial. Their predators were also affected by the trellising. Anthocorid nymphs were more consistently more common on the trellised cucumbers, though the difference was never statistically significant. Predatory mites were more common on the cucumbers on the ground, a difference which was statistically significant on the final sampling date. As in previous trials, melon flies, *Dacus cucurbitae*, did not appear to be affected by height. The amount of damaged fruit was the same in both treatments, 2.3 punctures per fruit in the horizontal cucumbers and 2.4 per fruit in the vertical ones. Other kinds of damage to the fruit also did not differ between treatments. About half the cu-

cucumbers from both plots showed some kind of scarring damage.

To summarize the data from three years experiments, the only insects which were very strongly affected by plant architecture were the black island fleahoppers. These insects preferred horizontal plants. In all three plantings, similar numbers of fleahoppers were present on the cucumbers when the plants were small, but whereas in the horizontal plants the populations continued to increase throughout the season, on the vertical plants they did not, and in some cases even decreased. Thrips and leafminers seemed to show a small preference for vertical cucumbers, but the differences were less clear. Most other insect populations either showed no effect due to plant architecture, or the differences were not consistent from one year to the next.

Control of Orange Pumpkin Beetle on Watermelon

Methods

An experiment was run to test the efficacy of insecticides for the control of *Aulacophora similis* adults and larvae. Watermelons var. Glory were planted 9 Jan. 1989. Plots consisted of eight 9 m rows, 1.2 m apart. Rows were mulched with black plastic, and

the melons were planted in hills 90 cm apart. All plots were treated with a foliar spray of carbaryl (50% WP, 1 lb per 100 gallon) at the seedling stage to prevent the death of young seedlings. The experiment was set up in a split plot-design. Main plots were either treated with or not treated with a foliar spray of carbaryl at the same rate when beetle populations were high. Plots were assigned at random to one of these treatments. Each main plot was subdivided into two subplots. One subplot was chosen at random to be treated preplant with a granular insecticide. The other subplot received no soil insecticide treatment. The granular insecticide used was diazinon (5% G) broadcast at the rate of 4 lbs AI/ acre. Each treatment was replicated four times.

Adult beetles were sampled by using a one meter quadrat. Two plants per row were randomly chosen from the center two rows of each subplot. The wooden quadrat was carefully lowered, centered on the base of the plant. All beetles within the quadrat were counted. Samples were taken once a week, except when a carbaryl treatment was applied, in which case a second sample was taken one day after the spray. The total plot count per subplot (i.e. the sum of the number of beetles sampled in four quadrats)

were analyzed with ANOVA for a split plot design for each sample date.

Beetle larvae were counted by lifting fruits that were at least 10 cm in diameter, and counting how many larvae were visible under the fruits. Twelve fruits were examined per plot. A record was also kept if ants (*Solenopsis geminata*) were observed under the fruits. A further assessment of beetle activity was done by examining all fruits harvested. Each ripe melon was examined, and damage to the tops of the fruits due to adult beetles or to the bottom of the fruits due to beetle larvae was noted.

Results

Effect on numbers of adult beetles

On any given date the ANOVA analysis showed no difference in the number of adult beetles in treated and untreated plots either due to the soil insecticide treatment or due to the foliar treatment (Table 2). This was true for both for sample dates just prior to foliar treatments and those taken one day or one week after the treatment. There was one exception, on March 8, a sample taken just prior to an insecticide spray, the plots to be treated with carbaryl had a significantly higher number of beetles at the 0.03 probability level. This is probably a case of a type 1 error.

The lack of difference among the treated and untreated plots was not because the carbaryl was ineffective. Plots were compared before and after spraying by using a paired t-test (Table 3). There was a highly significant reduction in beetle numbers after the spray which persisted for one week after spraying. No difference could be observed two weeks after spraying. These results imply that adequate control of beetles can be obtained without spraying the whole field. Treatment of only some of the rows would have less impact on predators and parasites of thrips and leafminers and may be desirable in an overall watermelon pest management plan.

Effect on numbers of soil dwelling insects: beetle larvae and ants

The larvae were not randomly distributed in the field and were only found in five of the eight plots in the first sample taken Mar. 10. In the second sample, taken Mar 17, only 1 immature beetle was found. In the Mar. 10 sample immature beetles were only abundant in three of the the plots, one treated with carbaryl and two untreated ones. The distribution of larval beetles appeared to be a function of the early season distribution of adult beetles. For the three plots where beetles were abundant, the average number of larvae in the subplots treated with diazinon was

considerably higher than the number in the subplots with no soil insecticide treatment (15.3 vs 2.7). The difference was not significant when the whole data set was analyzed, because of the high variance between plots (Table 4).

In the Mar. 10 sample, there was a small difference between the number of ants found among treatments. An average of 1.8 out of 12 melons had fire ants under them in the untreated subplots compared to 1.2 melons with ants in the subplots treated with diazinon. The difference became more pronounced by Mar. 17, at which time an average of 4.1 out of 12 melons had ants under them if the subplots were untreated, but only 2.1 did in the diazinon treated plots. The difference was still not significant (Table 4). However there was a significantly more ants in those plots not receiving foliar insecticide sprays.

Effect on beetle damage to watermelons

The number of watermelons damaged by adult or larval beetles was not significantly affected by any of the treatments (Table 4). A large number of melons were stolen from the field before they could be examined so we cannot be certain the data is correct. However, neither the adult or the larval damage penetrated the rind very deeply, so that there was no

spoilage of fruit due to the beetle damage. Damaged melons are readily purchased by consumers in the grocery stores. Probably damaged melons were as likely to be stolen as undamaged ones.

Table 1. Abundance of various insect species on trellised and untrellised cucumbers.

Treatment Sample date	<u>Number /D-vac sample</u>		<u>Number per 40 leaves</u>			<u>Number per leaf</u>			
	<i>Halticus</i>	<i>Aulacophora</i>	Syrphids	Coccinellids	<i>Diaphania</i>	Aphids	Thrips	Predatory Mites	Bugs
Horizontal									
July 15	36	6	0.6	0	11	30	1	0.01	0.00
July 30	99	14	41.3	0.7	20	78	7	0.03	0.03
Aug 15	257	24	0.2	4.3	10	71	4	0.26	0.09
Vertical									
July 15	22	12	0	0	14	8	2	0.01	0.03
July 30	16	37	22.3	1.5	17	53	18	0	0.07
Aug 15	2	27	0.2	0.2	21	98	14	0.03	0.11

Table 2. Mean quadrat count summaries of adult beetles for each sample date. Foliar insecticides were sprayed on Feb 23, Mar 9 and Mar 23.

Treatment	Feb 22	Feb 24	Mar 1	Mar 8	Mar 10	Mar 15	Mar 22	Mar 24
Carbaryl +								
Diazinon preplant	1.5	0.25	0.75	8.0	0.75	0.5	4.75	0.0
Carbaryl	2.25	0.0	0.25	13.0	0.25	0.5	5.25	0.0
Diazinon preplant	3.5	0.25	0.5	4.5	1.5	1.5	11.75	5.25
Check	3.5	0.0	1.0	2.75	1.0	0.5	12.0	6.25
F_{foliar treatment}	0.77	0.00	0.11	13.77	1.59	2.00	1.08	1.90
and probability	0.44	1.00	0.76	0.03	0.30	0.25	0.37	0.26
F_{soil treatment}	0.00	2.00	0.00	0.55	0.35	3.00	0.01	0.06
and probability	0.95	0.21	1.00	0.17	0.57	0.13	0.91	0.81

Table 3. Paired t-test comparison between adult beetle populations pre- and post-foliar insecticide spray.

	<u>Days after Treatment</u>				
	<u>First foliar spray</u>		<u>Second foliar spray</u>		<u>Third foliar spray</u>
Mean difference	2.94	2.25	5.44	6.31	5.56
t	3.319	2.736	4.116	4.014	6.125
Probability	0.005	0.01	0.005	0.005	0.0005

Table 4. Mean number of beetle larvae found under 12 melons on Mar 10 and number of melons with ants under them Mar 10 and Mar 17 and proportion of fruits damaged by adult or larval beetles.

Treatment	Number larvae	Number melons with ants		Percent of fruit damaged by	
		Mar 10	Mar 17	Larvae	Adults
Carbaryl +					
Diazinon preplant	1.25	0.75	1.0	10	16
Carbaryl	2.5	1.0	3.0	13	19
Diazinon preplant	10.75	2.75	3.25	15	9
Check	0.25	1.75	5.5	13	26
F _{foliar treatment}	1.66	1.76	103.08	0.31	0.0
and probability	0.29	0.28	0.002	0.62	0.99
F _{soil treatment}	2.93	1.97	2.59	0.0	1.67
and probability	0.13	0.20	0.17	0.95	0.25



Injury Level of Various Pests of Yardlong Beans

Materials and Methods

Beans were planted 5 Dec 1988. The plots consisted of two 6.85 m rows, 1.5 m apart, separated by sweet corn to minimize drift. The experiment was set up in a randomized complete block design. Half the plots were drenched preplant with oxamyl (Vydate) against root knot nematodes. In the design, one drenched and one non-drenched plot were to have been treated weekly with dimethoate (Cygon), however, through misunderstanding only the undrenched plots were sprayed. Thus there were five replicates of two treatments, and 10 replicates of the plots treated with oxamyl preplant, but with no insecticide.

The number of leafminers per plot was estimated by counting the number of mines on 40 mature leaves in each plot. Samples were taken on Mar. 1 and Apr. 5. Another 40 leaflets per plot were picked and kept in sealed paper bags until parasites and adult flies had emerged and died. Bean flies were sampled by examining 60 seedling per plot on Feb. 12 and determining whether they were infested, and by examining 60 petioles of leaves on Apr. 12. Yield was the total yield for both rows. A subsample of 50 beans per plot was taken from each harvest, and the number that were infested with aphids and with pod borers was counted.

Bean flies were present in very low numbers, less than 5% of petioles or seedling being infested, and so were not counted. However, spider mites (*Tetranychus* sp. primarily *cinnabarinus*) were abundant after flowering. Their numbers were estimated by removing 15 leaves per plot and counting all mites on the underside of the leaves using a compound microscope. Root knot nematodes impact was estimated by carefully digging up two hills of plants per plot (a total of 6 plants) and estimating what percentage of the root hairs were infected by nematodes. Leafminers and their parasites, aphids and bean pod borers were counted as in the previous experiment. In addition, bean bod borers were sampled in the flowers, by picking 40 flowers per plot and counting all larvae in them.

Results

Effectiveness of insecticides

Oxamyl applied preplant had no measurable effect on root knot nematodes (Table 1). However, there was so much variance among samples, that differences may have easily been obscured. As expected, the preplant drenching also did not have any impact on the foliar insects.

Dimethoate significantly reduced leafminer numbers and increased yield. There was a very large reduction in spider mite numbers in the treatments sprayed with dimethoate, though the difference was not quite statistically significant. This was because the spider mites invaded from one side of the experimental plot from an adjacent

planting, and the plots at the far end never became heavily infested. Thus there was large variance. Pod borers and aphids were not significantly reduced by the dimethoate treatments.

Correlations between pest numbers and yield

Replicate was the most important determinant of yield (Table 2). This was due to the slope of the experimental plot. Among the insects, leafminers, and aphids as measured by the number found on the pods, had the most effect on yield. The leafminer populations were low in this trial but even so, their effect was quite large. As the mean number of leafminers increased from 0.8 per leaf to 3.8 per leaf, yield decreased 10 kg per plot, or about 72 kg per 100 m row. Aphids in this trial had a similar effect on yield. As the proportion of beans with aphids on them increased from 1 to 7%, total yield decreased 10 kg per plot. The most abundant arthropods in the bean field were spider mites. Although some plots averaged as many as 312 mites per leaf, this had no detectable effect on yield.

Population Trends for Mango Blotch Miner

Methods

Long term monitoring of *Procon-tarinia* numbers has been taking place since 1986. Two separate samples are done each month. In the first type of sample we sought out four trees in full flush in each of four villages. Twenty young shoots were sampled on each mango tree and the the number of blotch miner

lesions on the bottom most leaf of each shoot was counted. The data was analyzed to determine whether any factors could be used to predict population levels. Factors examined included rainfall in the current and preceding month, proportion of trees flushing in the current and preceding month and the number of leafminers found in the preceding month. Rainfall measurements were obtained from the National Weather Service records. The proportion of trees flushing was estimated as described in the following section.

A set of 16 trees at various locations have been monitored for 3 years and whenever they flushed the number of mines per leaf was estimated in the same manner as above. On each of these trees, twenty shots were tagged, and these were monitored twice a month to determine what proportion of these branches were flushing. Eight of the trees had to be dropped from the final analysis of the data on the proportion of trees flushing because the trees had been repeatedly trimmed by their owners. This may have caused them to flush on a different schedule than untrimmed trees.

In all cases the trees sampled were planted in yards or along roadsides. None were known to be grafted trees, and it is assumed they were all seed grown. The trees were of various sizes, but were more than 15 feet tall and well filled out.

Results.

Overall population trends of the blotch miner

The mean number of blotch miner per leaf varied somewhat from one village to the next, the difference approached significance (Table 3)($F=2.589$, $d.f.=3,488$, $p=0.054$). The mean was lowest in Merizo and highest in Barrigada. The mean population trends are shown in Figure 1. Overall means was never very high, exceeding ten mines per leaf only in 4 months. Only 33 trees during the whole period averaged more than 15 mines per leaf in a given sample. During the sampling period, the median monthly rainfall was 7.95 inches. Eleven trees out of 282 trees sampled in months when the rainfall was below the median had more than 15 mines per leaf. On the other hand 22 trees out of 262 sampled in months when the rainfall was above the median had average mine counts exceeding 15 per leaf (Chi-square corrected for continuity = 3.554, $p<0.10$). The overall mean blotch mine counts were 60% higher in the wet seasons than in the dry seasons, averaging 6.3 mines per leaf during June-November and 3.9 during December-May. This difference was statistically significant ($t=2.241$, $d.f.=33$, $p<0.025$).

Differences among individual mango trees

There were significant differences in the mean number of blotch miners among the 16 trees sampled on a long term basis ($F=3.219$, $d.f.=15,433$, $P<0.001$) (Table 4). Some trees averaged as few as 1.9 mines per leaf during that period,

whereas one tree averaged more than 14 mines per leaf. The peak mean count for a sample was 9 mines per leaf on some of the trees with low overall means, but reached 167 mines per leaf in the most susceptible tree. The tree with the highest peak counts also had the lowest percentage of flushes when no blotch miners were present, but there was only a weak correlation between these two parameters for the data set as a whole. In general there did not seem to much differences among trees, except in one case, Barrigada tree 8 which appeared to be considerably more susceptible to the blotch miners than did other trees. There was a general trend for the trees in Barrigada to have sustained more damage than those in Agat. The mean and peak number of blotch miners was higher ($t=2.144$, $d.f.=14$, $p<0.05$ and $t=1.779$, $d.f.=14$, $p<0.10$, respectively) and the proportion of occasions when the flushing leaves had sustained no damage was lower ($t=2.603$, $d.f.=14$, $p<0.025$) in Barrigada.

Table 1. Effectiveness of pesticide treatments on bean pests, December 1988

Treatment	Spider mites per leaf	Percent of roots with nematodes	% beans with borers	Borers per 40 flowers	% beans with aphids	Mean no. leafminers/leaf	Yield/plot (kg)
Dimethoate	1	4	6	4.2	1	1.0	41
Oxamyl	109	11	5	3.3	3	2.2	33
No treatment	93	13	4	2.6	2	2.6	33
F	3.060	0.962	2.420	0.464	2.669	5.938	5.667
probability	0.073	0.402	0.119	0.636	0.098	0.011	0.013

Table 2. Multiple regression of insect population levels versus yield of beans December 1988

Variable	Parameter estimate	Standard error	t	Probability
Intercept	39.85	6.31	6.313	0.000
Replicate (Position on slope)	3.03	1.08	2.816	0.016
Leafminer mean	-3.30	1.65	-1.997	0.069
Aphids on pods	-3.46	1.74	-1.989	0.070
Bean pod borer (pod)	-1.50	1.58	-0.945	0.363
Bean pod borer (flower)	0.33	0.46	0.720	0.485
Root knot nematode	-0.03	0.14	-0.224	0.826
Spider mite	0.00	0.02	0.111	0.914

Table 3. Mean number of miners in different villages

Village	Number miners	
	Mean	Standard Deviation
Agat	5.4	14.4
Barrigada	7.4	14.4
Merizo	3.7	5.9
Yigo	4.7	5.3

Table 4. Number of miners on 16 mango trees sampled continuously for 3 years

Village	Tree number	Mean number miners per leaf	Peak miner count per leaf	Percent of flushes when 0 miners present
Agat	1	1.5	10.8	58
	2	1.9	13.4	62
	3	2.7	9.3	33
	4	3.4	10.0	28
	5	1.8	8.9	32
	6	2.5	13.6	52
	7	3.1	15.0	34
	8	3.9	21.2	40
Barrigada	1	1.9	11.9	29
	2	2.5	12.0	41
	3	6.1	33.5	21
	4	3.7	27.1	30
	5	5.7	43.5	24
	6	3.9	33.5	41
	7	6.8	29.3	16
	8	14.1	167.4	16

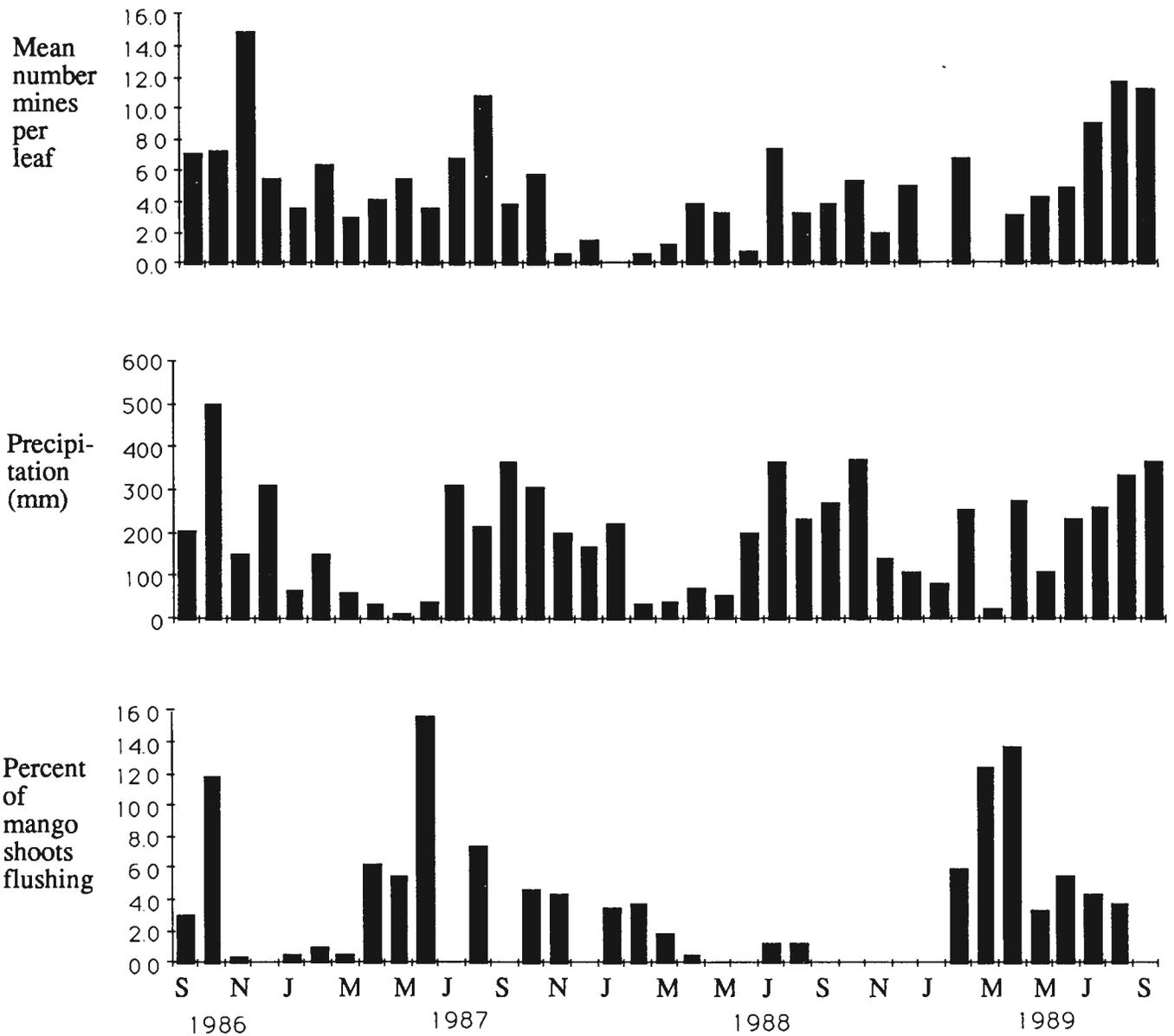


Figure 1. Population levels of mango blotch miners in relations to rainfall and amount of suitable leaves present



Evaluation of Ornamentals for use on Guam

The main purpose of this project is to identify which ornamental crops have potential for commercial production in Guam and to determine optimum conditions for culture. Several crops have been identified for further study. These include several genera of orchids, anthuriums, heliconias and gingers. Currently, cultivars are being evaluated for their flower production. In addition various cultural methods are being evaluated to determine which are suitable for Guam's conditions. Ongoing studies are looking at optimum levels of light, water and fertilizer. Another study is evaluating growing media. It has been found that crushed limestone is suitable for growing vandas, dendrobiums and cattleyas. The coarseness varies with the age of the plants and the genus involved. Overhead irrigation with spinner sprinklers was found to be superior to impact or shrub sprinklers because they give the best distribution of water at low pressures. Spinner sprinklers have also reduce scale populations on the leaves and stems of vandas. This gives the added benefit of needing to spray insecticides less frequently.

Cultivar Release

Aranda Marianas Lila. Parentage: *Aranda* Wendy Scott (4N) X *Vanda* Sanderana 'Alba' (2N). A triploid highly suited for use as a cut flower in Guam. Flowering occurs during much of the year. The flower is purple.

Publications

McConnell, J. and R. Muniappan. 1989. Control of scale by sprinkler irrigation on *Vanda* Miss Joaquim. *HortScience*. 24:86. (Abstr).

McConnell, J. 1990. Crushed limestone aggregate and coconut husk as potting media for *Vanda* Miss Joaquim. Univ. of Guam, Agriculture Experiment Station Publication. 7p.

Environmental Factors Affecting Flowering in Some Vandas and Dendrobiums in the Tropics.

Introduction

Flower production in orchids varies from season to season in the subtropics and tropics. This variation is not consistent year to year as the plants respond to the natural variations in the weather. The primary environmental factors affecting flowering in dendrobiums and vandas have not been clearly identified in relation to how they influence flowering. It has been found that seasonal flower production in *V. Miss Joaquim* was not due to photoperiod. The objective of this study was to compare growth characteristics and flowering in orchids grown in 30% shade and full sun to understand how the plants respond to changes in the environment.

Materials and Methods

Eighty 1-1/2 year-old plants of *Dendrobium* Jaquelyn Thomas 'Uniwai Supreme' were transplanted from 5 cm pots to 3.8l plastic bags in November, 1988. The growing medium was 1/2 inch

crushed limestone. Vandas were planted in 1 gallon plastic bags filled with 3/4 inch crushed limestone. Two cultivars were grown: *Miss Joaquim* and *Miss Joaquim* 'Atherton'. There were 8 plants per bag with 40 plants of each cultivar grown in full sun and 30% shade. Phenological data was recorded for the dendrobiums at different stages of development. Dates of raceme initiation and raceme harvest are recorded every two days'. Dates of shoot initiation and shoot maturation were recorded weekly. The data presented was recorded from December, 1988 through February 15, 1990.

In addition, leaf measurements were made on leaves of five plants growing in full sun, 30% shade. The variable included leaf area, fresh weight, dry weight, and color as measured using a colorimeter.

Results and Discussion

Vandas. The mean number of flowers produced by *V. Miss Joaquim* did not differ between full sun and 30% shade (Table 1). This is a locally grown cultivar. *V. Miss Joaquim* 'Atherton' showed a significant reduction in flowering in 30% shade. These two cultivars also vary greatly in time of flowering.

Dendrobiums. Shading had no effect on the number of growths initiated and number of growths that reach maturity (Table 2). Flowering was found to be significantly affected by reduced light. The number of racemes initiated and harvested per plant was nearly double in full sun compared to 30%

shade. The increased raceme production in full sun also resulted in nearly double the number of individual flowers (Table 2).

In attempting to understand the effect of shading on flowering measurements were taken on several leaf characteristics. All were significantly different between the plants growing in full sun and shade (Table 3). The leaf area had a mean of 48.9 cm² in full sun and 62.5 cm² in the shade. Fresh weight and dry weight were also found to be higher in the plants growing in the shade. Leaf color was measured for plants growing in 70% shade in addition to the other treatments. The leaves were lighter (+L) in full sun and darkest in 70% shade (Table 3). Leaves in full sun had a greener value (-a) and

also had higher yellowness (+b). It has been reported that *V. Miss Joaquim* has Crassulacean Acid Metabolism (CAM). In CAM plants there is an interaction of light and temperature in producing a diurnal fluctuation of titratable acidity and dark CO₂ fixation. CO₂ uptake occurs mostly at night in CAM plants. This behavior was apparently observed in this study in *D. Jaquelyn Thomas 'Uniwai Supreme'*. No CO₂ uptake was observed during daylight hours. Currently measurements are being recorded hourly for a 24 hour periods to identify peak CO₂ uptake. This data will then be compared with seasonal variations in temperature rainfall and solar radiation to determine their effect on photosynthesis and flowering.

Table 1. Mean values of number of flowers produced by *V. Miss Joaquim* and *V. Miss Joaquim' Atherton'* grown under full sun and 30% shade from February, 1989 to December, 1989.

Cultivar Treatment	<i>V. Miss Joaquim</i>	<i>V. Miss Joaquim' Atherton'</i>
30% Shade	19.2±0.1 ^a	14.8±0.2
Full Sun	22.9±0.1	21.9±0.1

^aMeans of 40 plants ± SE.

Table 2. Mean values of various phenological events of *Dendrobium* Jaquelyn Thomas 'Uniwai Supreme' grown under full sun and 30% shade.

<u>Treatment</u>	<u>No. of Growth Initiated</u>	<u>No. of Growths Terminated</u>	<u>No. of Racemes Initiated</u>	<u>No. of Racemes Harvested</u>	<u>No of Flowers per Harvest</u>
30% Shade	4.3±0.3 ^a	3.8±0.3	2.8±0.3	2.7±0.2	27.3±3.2
Full Sun	4.9±0.2	4.4±0.2	4.6±0.3	4.2±0.4	48.4±4.4
Significance Level (t-test)	NS	NS	*	*	*

^aMeans of 40 plants ± SE.
^{NS}. Nonsignificant or significant at P=0.05, respectively.

Table 3. Mean values of various leaf characteristics of *Dendrobium* Jaquelyn Thomas 'Uniwai Supreme' grown under full sun and 30% shade.

<u>Treatment</u>	<u>Leaf Area Index (cm²)</u>	<u>Fresh Weight (g)</u>	<u>Dry Weight (g)</u>	<u>L</u>	<u>Color</u> a b	
30% Shade 29.2±0.9	62.5±2.8 ^a	10.4±0.5	1.1±0.1	46.2±0.9	22.1±0.5	
Full Sun	48.9±2.3	7.6±0.6	0.8±0.1	49.9±0.7	26.6±0.5	36.5±0.6
Significance Level (t-test)	*	*	*	*	*	*

^aMeans of 5 plants + SE.
^{NS}. Nonsignificant or significant at P=0.05, respectively.



Biological Suppression of Soilborne Plant Pathogens

Tests during 1989 focused on bacterial wilt disease of solanaceous crops. The effect of soil texture on bacterial wilt incidence was studied on bell peppers. Two experiments were completed in the greenhouse at Inarajan; The first one included 3 treatments, as follows: 1) infested field soil, 2) a 1:1 mixture of the same soil plus river sand, and 3) sterilized field soil. The infested soil, a heavy clay, was obtained from a field in Mangilao, where bacterial wilt is a chronic problem. Three pots were filled with each of these soil treatments. Bell pepper seedlings were then transplanted, 6 to a pot, and observed for 1 month.

The results are shown in Fig. 1. The addition of sand to the field soil tended to reduce the incidence of bacterial wilt disease. But it wasn't clear if this was due to a change in soil texture, or to a reduced amount of field soil and therefore a reduced amount of bacteria. A second experiment was planted, with more plants per treatment and with an additional treatment, consisting of a 1:1 mixture of infested field soil plus sterilized field soil. After 3 weeks it was clear that soil texture has a significant influence on bacterial wilt of bell peppers. The looser soil with the 1:1 sand mixture resulted in fewer diseased plants. The test indicated that the amount of inoculum in the soil was also important; the infested field soil with a 1:1 mixture of sterilized soil also reduced disease incidence.

Future studies will focus on developing a better understanding of this disease, and on developing practical ways of applying this information to control bacterial wilt.

A Study of the Diseases of Beans and their Control

Previous work has already shown that the two most important diseases of yard-long beans on Guam are powdery mildew, caused by *Oidium sp.*, and a mosaic disease, apparently caused by a virus.

I. A collection of cultivars was started the previous year, including cultivars contributed by local growers and scientists, and where possible, cultivars from seed companies from various countries. Early in 1989, we had enough seed of only four yard-long bean cultivars for testing. These were 1) Takii red-seeded, 2) Ferry Morse Asparagus, 3) a bush variety contributed by Dr. R. Rajendran, and 4) a local red-seeded variety contributed by Mr. G. Pangelinan. Preliminary observations began in a test plot at Inarajan. Little or no disease developed. The test plot was harvested 4 times, then allowed to produce seed. Yields per 5m row are shown in Fig. 2. Number of pods produced is shown in Fig. 3. Highest yields were obtained with the bush variety, which requires no trellis. Pods of this variety, however, are considerably shorter than those of the favorite local varieties.

II. A second test, including 2 cultivars, was planted at Inarajan to study the effect of plastic mulch on yard-long bean culture. An identical test was planted in Yigo, in collaboration with Mr. C. Contreras. The Inarajan test was harvested 5 times and allowed to produce seed, while the Contreras test was harvested 10 times.

Results of these experiments are summarized in Figs. 4 & 5. Again, little or no disease occurred at Inarajan. In Yigo, on the other hand, we had some incidence of mosaic occurring naturally (Table 1). Certain patterns were apparent at both Inarajan and Yigo: the bush variety out-yielded the local red-seeded; for the first 5 harvests, unmulched plots yielded more than plots with plastic mulch. This was mainly due to fewer plants per row in the plastic mulch, as a result of damage during the seedling stage. This was either wind or drought damage, both of which were higher in the plastic mulch plots. In spite of drip irrigation in the whole of the test plots, the plastic mulch plots resulted in fewer established plants. In Yigo, where the plants were harvested for a longer time, the plastic mulch plots finally produced higher overall yields than the unmulched plots. Also, differences in yield between the two varieties became smaller with time.

The bush variety had significantly less mosaic incidence than the local red-seeded one in Yigo (4.5% vrs. 9.4%). This indicates, but does not conclusively prove, that bush has more field resistance; however, this lower disease incidence could be due to a preference of the aphid vectors for the higher-standing local red-seeded. Regression analysis of mosaic incidence on yield suggests that the local red-seeded was less influenced by the disease, although it had more disease than the bush variety. This means that the red-seeded has a certain degree of tolerance to the mosaic disease.

III. Six yard-long bean cultivars were screened for powdery mildew resistance at Radio Barrigada. These were: 1) local red-seeded, 2) bush, 3) Ferry Morse Asparagus, 4) Takii red-seeded, 5) Known-You Kaohsiung, and 6) local black-seeded contributed by Mr. J. Cruz. The test relied on naturally occurring inoculum; spreader rows were planted 3 weeks in advance of the test rows.

The Asparagus and the bush cultivars had significantly less powdery mildew severity than all the others. Differences were only apparent on the upper side of leaves. Although significantly more resistant, these two cultivars still sustained very high levels of powdery mildew severity. Harvests were not possible in this test due to vandalism. Therefore, no yield loss relationships could be explored.

IV. In order to identify the causal agent of the mosaic disease, 53 different genera, species, or cultivars of plants in various families were mechanically inoculated at the Inarajan greenhouse. Previous work had already determined that the disease can be transmitted mechanically.

Thirty-six of these 53 plants tested became infected, either locally or systemically (Table 2). Comparing this host range with that of viruses reported occurring on *Vigna sp.*, the most similar one is Black-eyed Cowpea Mosaic Virus. This is a potyvirus, and is transmitted in a non-persistent manner by a number of aphid vectors.

V. An aphid transmission test from last year showed that *Aphis craccivora* is capable of transmitting this mosaic disease. A similar test was performed again this year.

Disease transmission was successful with this aphid at acquisition periods of 30 seconds and 2 minutes. This indicates that the causal agent of our mosaic disease is a virus of the non-persistent type.

Future work is planned to complete the identification of this mosaic disease, so that we may benefit from what is already known about its control. Resistance screening of cultivars is planned as well. Powdery mildew work will center on fungicide tests.

Identification of Economically Important Diseases on Cucurbit Crops on Guam, and Development of Strategies for their Control

I. A disease of watermelon affecting the fruit was determined to be caused by a bacterium. The organism consistently isolated from diseased fruit samples was used to inoculate healthy fruit and the same disease symptoms were reproduced. These symptoms consisted of watersoaked blotches on the rind, which eventually expanded and rotted the entire fruit. Fruit of cultivar Glory developed fast-growing lesions, while those on Crimson Sweet were smaller and slower growing. This suggests that there may be varying degrees of resistance to this disease in different watermelon varieties. The bacterium corresponds to the description of *Pseudomonas pseudoalcaligenes* subsp. *citrulli* Schaad et al. (Table 3).

II. It was found that all that needs to be done to infect a watermelon seedling is to immerse the seed in a suspension containing the bacteria. Germinating seedlings then develop watersoaked lesions on the cotyledons; later the majority of them die

from the infection, although some survive. Five watermelon varieties were tested in this manner to see if any would resist infection. Those tested were: 1) Glory, 2) Top Yield, 3) Ten Bow, 4) New Crown, and 5) Farmer's Giant. None were found to be resistant to infection by this method.

III. Potted soil from previous experiments with this disease was saved, and re-planted one week later. Watermelon seedlings growing in pots having been previously used to grow infected seedlings developed infection. Seedlings grown in pots previously used with healthy plants were not infected. Therefore, the disease-causing bacteria can survive in soil for at least 1 week (Table 4).

IV. Inoculated watermelon seedlings were grown adjacent to uninoculated seedlings in a tray, which was watered with a watering can. In a separate tray, only uninoculated seedlings were grown, and watered in the same way. The inoculated seedlings developed disease, and so did the uninoculated ones growing next to them, but not the others in the separate tray. This shows that the causal organism can be disseminated either by splashing water or through the soil to immediately adjacent plants.

V. Inoculated seed of watermelon was subjected to various hot water treatments, and it was determined that 50°C for 10 to 20 minutes effectively controlled the disease (Table 5).

VI. Work in conjunction with the ADAP IPM project resulted in the identification of 3 cucurbit viruses. First, the AG-DIA kits based on the ELISA technique for identification of Cucumber Mosaic Virus, Water-

melon Mosaic Virus 1, and Zucchini Yellow Mosaic Virus were used. There were positive results, but cross-reaction was a problem. Subsequent ELISA work was based on antisera developed at Cornell University (D. Gonsalves), and procedures worked out by J. Cho of the University of Hawaii. Four antisera were tested, resulting in the identification of Watermelon Mosaic Virus

1, Zucchini Yellow Mosaic Virus, and Cucumber Mosaic Virus. There were no positive reactions for Watermelon Mosaic Virus II.

Future work will concentrate on looking for resistance to the fruit stage of the fruit blotch disease, and also on looking for resistance to virus diseases in cucurbit crops.

Table 1. Percent mosaic incidence on 2 yard-long bean cultivars, planted with and without black plastic mulch in Yigo.

Cultivar	Treatment		Totals
	Plastic Mulch	No Mulch	
Rajendran Bush	5.75%	3.25%	4.50% *
Pangelinan Red	9.25%	9.50%	9.38%
Totals	7.50%	6.38%	

*Different at .05 level.

Table 2. Plants tested for infection by mosaic virus on yard-long beans.

Genus & Species	Cultivar	Reaction	
Arachis hypogea	Spanish	V	
	Starr	-	
Beta vulgaris	Det.Dk.Red	-	
Chenopodium amaranticolor	UH	+	
	NSSL	+	
C.quinoa	UH	+	
Crotalaria juncea	Local	+	
Cucumis sativus	Sw.Salad	-	
	Chicago Pickling	-	
Cucurbita pepo	Ambassador	-	
Datura stramonium	NSSL	-	
Glycine max	Vinton	V	
	Bragg	V	
Gomphrena globosa	Burpee	+	
	NSSL	+	
Luffa acutangula	UH	-	
Lycopersicon esculentum	Marglobe	-	
Medicago sativa	Du Puis	-	
Nicotiana benthamiana	UH	+	
N. glutinosa	NSSL	-	
N. tabacum	Hicks Broadleaf	-	
	Samsun NN	-	
Ocimum basilicum	Burpee	-	
Petunia hybridum	Plum Pudding	V	
Pisum sativum	Wando	-	
	Perfected Wales	-	
Phaseolus aureus	Berken	+	
P. lunatus	Henderson Bush	+	
P. vulgaris	Greencrop	+	
	1-Black Turtle	+	
Spinacea oleracea	Bloomsdale Lng. Std.	+	
Tetragonia expansa	New Zealand Spinach	+	
Trigonella foenum-graecum	Fenugreek	+	
Triticum aestivum	Minter	-	
Vicia faba	Fava Bush Long Pod	+	
	v. minuta	+	
Vigna unguiculata var. sesquipedalis	Burpee-Asparagus	+	
	Contreras-Asparagus	+	
	Ferry Morse-Asparagus	+	
	Known-You, Green Arrow	+	
	Known-You, Kaohsiung	+	
	Manibusan-Green	+	
	Cruz-Black	+	
	Meno-Red	+	
	Pangelinan-Red	+	
	Manibusan-White	+	
	Rajendran-Bush	+	
	Takii-Red	+	
	Vigna unguiculata	Burpee-Purple Hull	-
		NSSL, Black-eyed Cowpea	+
Miss, Silver		+-	
	Queen Ann	+-	

+ = Positive reaction - = Negative reaction +- = Positive on Inoculated leaf, but not systemic
V = Variable; not all replications positive.

Table 3. Characteristics of the bacterial plant pathogen isolated from watermelon fruit on Guam.

Gram Stain:	Negative
NA Colonies:	Cream
YDC Colonies:	Cream
KB Colonies:	Non-fluorescent, translucent
Anaerobic growth:	Negative
CVP growth:	No pits
41°C growth:	Positive

Table 4. Effect of planting watermelon seed in blotch-contaminated soil.

Treatment	Plants/Pot	Diseased
Contaminated Soil	17.5	7.5
Uncontaminated	18.5	0 *

*Different at .05 level.

Table 5. Effect of seed heat treatment after inoculating watermelon seed with the fruit blotch bacterium.

Date	Treatment	Healthy Plants	Diseased Plant
12/88	Control	19.00 a	0.33 b
	50°C x 10 min	16.00 b	1.00 b
	50°C x 20 min	19.33 a	0 b
	50°C x 30 min	17.67 a	0 b
	Inoculated control	2.67 c	15.3 a
1/89	Control	16.33 a	0 a
	50°C x 10 min	13.33 a	0 a
	50°C x 20 min	14.00 a	0 a
	50°C x 30 min	14.67 a	0 a
	Inoculated control	13.67 b	11.7 b

Different letters (abc) statistically different at .05 level (Fisher PLSD).

Fig. 1. Soil Texture Effect, Bacterial Wilt/Bell Pepper

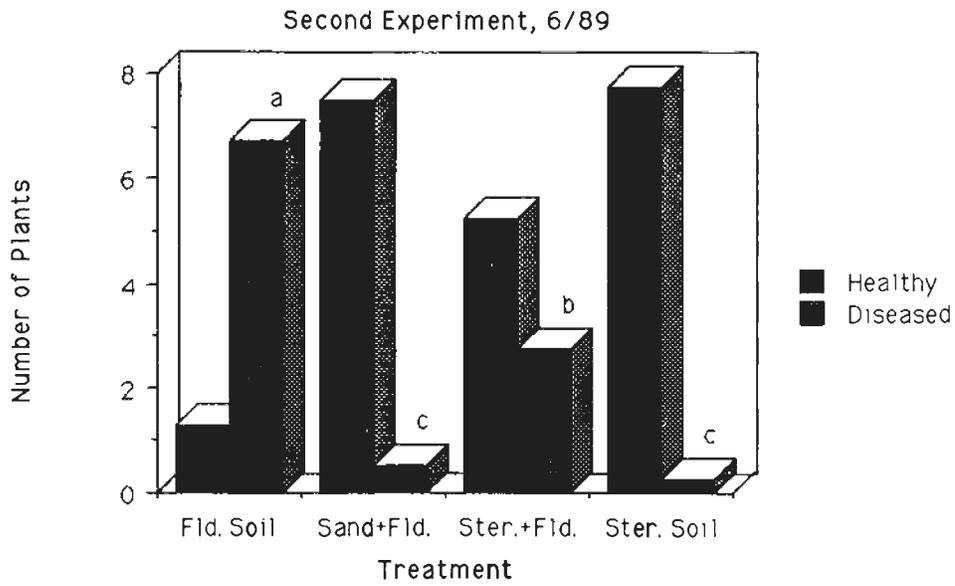
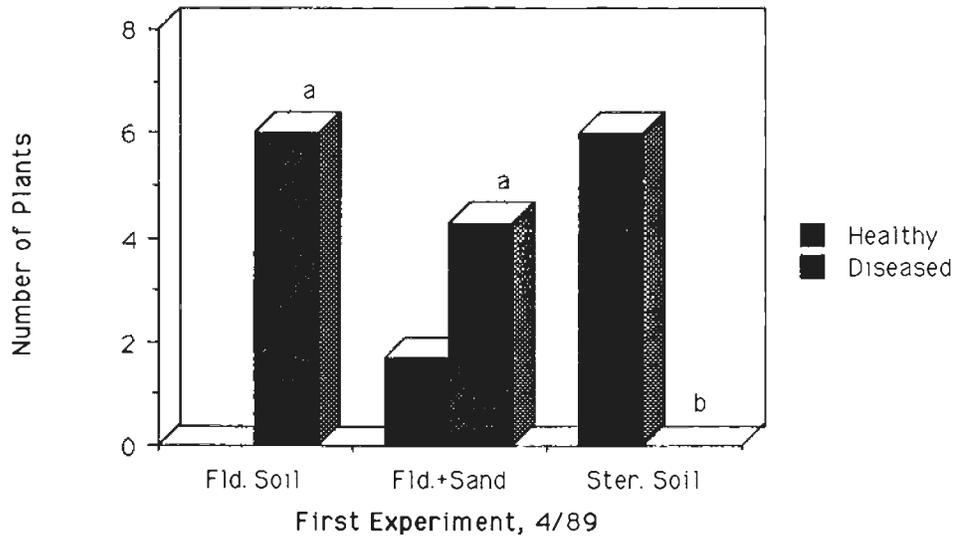


Fig. 2. Yd-Long Bean Varieties, Inarajan 2/89

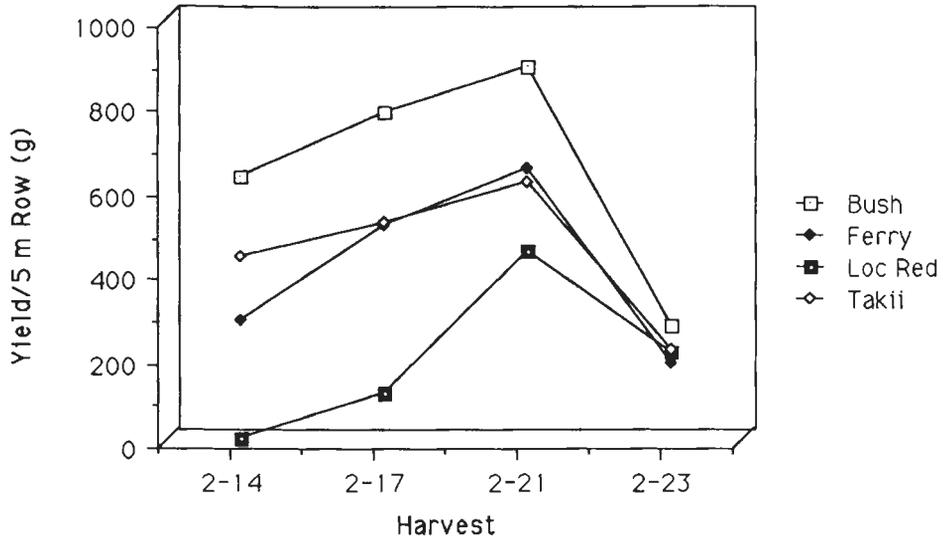


Fig. 3. Yd-Long Bean Varieties, Inarajan 2/89

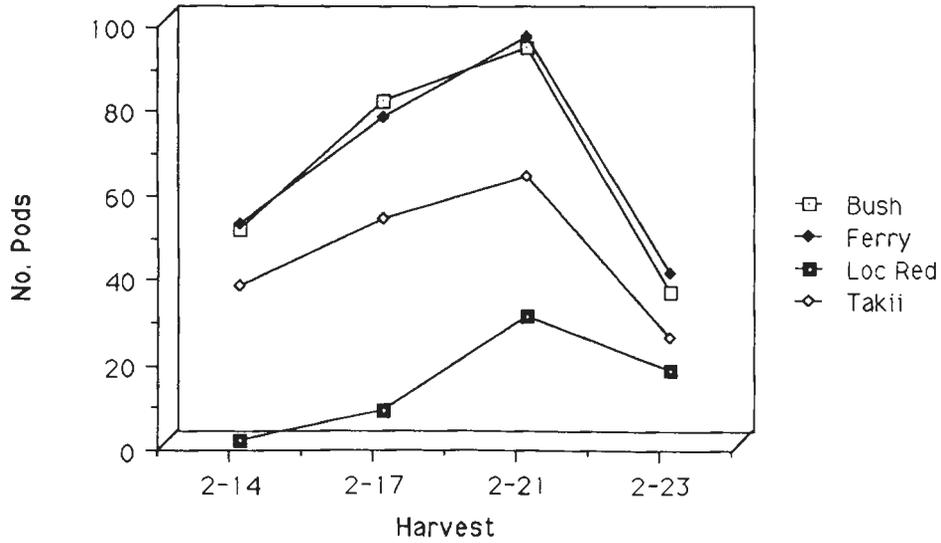
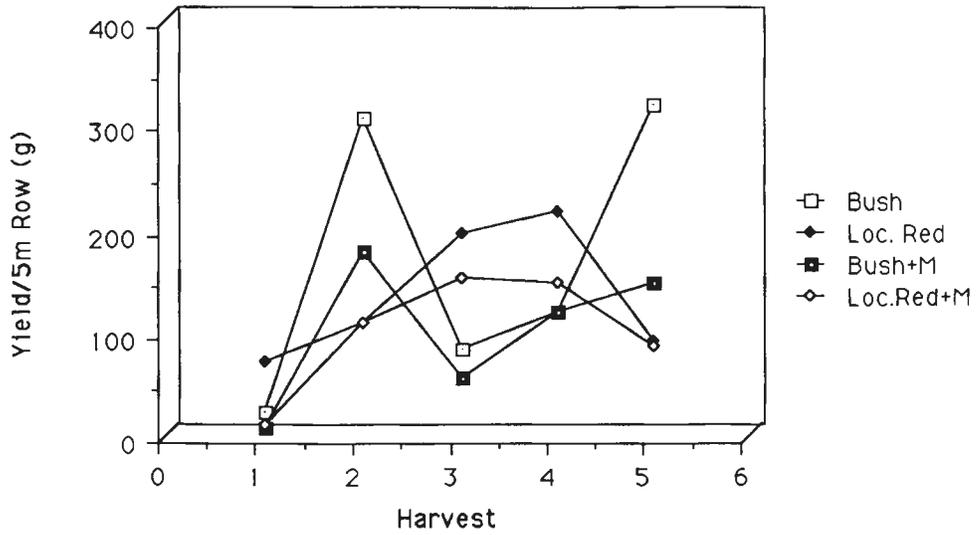


Fig. 4. Pl. Mulch Test, Yd-Long Bean, Inarajan



Pl. Mulch Test, Yd-Long Bean, Yigo

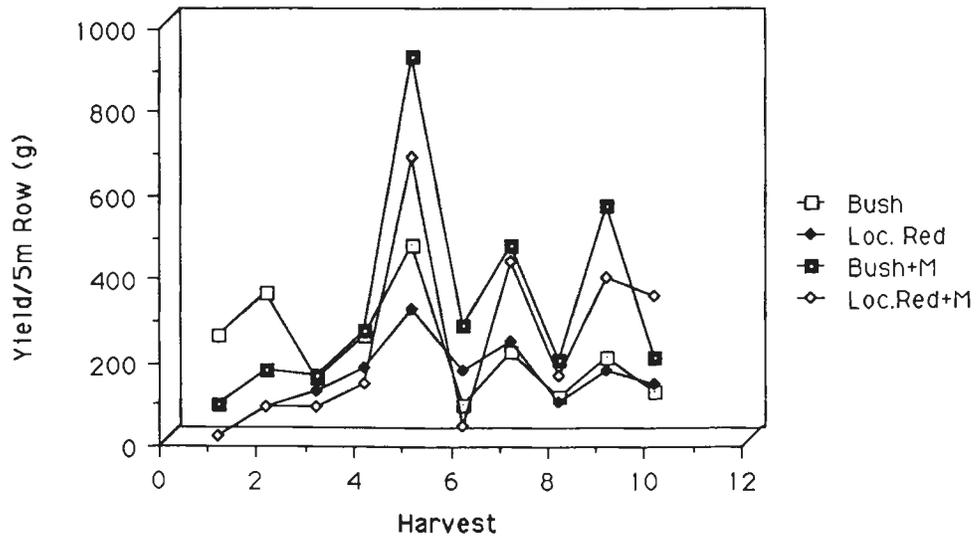
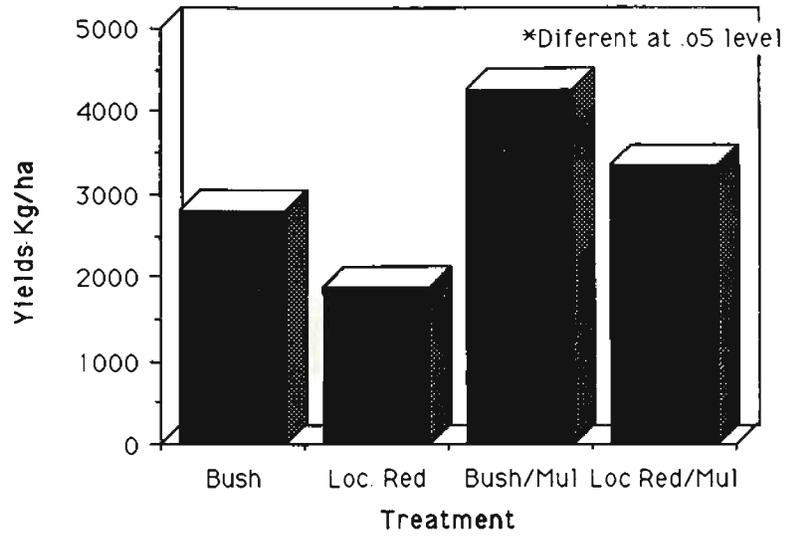


Fig. 5. Total Yields, Yd-Long Bean Test, Yigo





The Reproductive Biology of Three Sea Cucumber Species of Potential Commercial Value

The three species chosen for this study *Holothuria (Microthele) nobilis*, *Actinopyga mauritiana*, and *Thelenota ananas*, were selected for their high commercial value (up to \$12.50 per pound dry weight), and abundance in the coastal habitats of Micronesia.

Sex Ratios

Sex ratios were determined from the animals collected for reproductive studies. Animals were placed in one of three categories, female, male, or unknown. The designation of unknown was for animals without gonads or with gonads in early stages of development that could not be positively identified. The results of these findings are shown in Table 1, below. Those of unknown sex are not included for the determination of the sex ratio, ratios being computed only from individuals of known sex. All of the ratios are very close to 1:1, and no asymmetry in the ratio can be detected that could not be attributed to random variation. Consequently, a 1:1 sex ratio can be assumed to be the case with the species studied.

LIFE HISTORY

Adult Reproduction

Reproduction is a seasonal event in the three species studied. A gonadal index was used to determine the reproductive readiness of the Tumon Bay population. Stages of

gamete development over time were also considered as an indicator for predicting reproductive events. The gonadal index is obtained by dividing the gonadal weight by the whole wet body weight and multiplying by 100. This value is calculated as a percentage and a mean value is generated for each sample period. These values are plotted over time and shown in Figure 1. The peak and postpeak values correspond directly with known periods of spawning activity for *Holothuria nobilis* and *Actinopyga mauritiana*. Results are inconclusive for *Thelenota ananas* at this time.

Gametes from all dissected animals were inspected with a compound microscope. Oocyte diameters were measured with an ocular micrometer, and sperm activity and development were also noted. In both *H. nobilis* and *A. mauritiana* active sperm could be found throughout the year. Also, egg diameters did not change significantly throughout the year (Fig. 2). Gamete development can be used to some degree as a reproductive indicator for *T. ananas*. Oocyte diameter is very sea-

sonal and follows the same trend seen in the gonadal index of this species (Fig. 2). Maturation of testes showed a similar pattern.

Fecundity Index

A fecundity index was determined for *Holothuria nobilis*, *Actinopyga mauritiana*, and *Thelenota ananas* for annual and peak periods by dividing the mean weight of the ovaries by the mean of the oocyte diameter cubed (Conand, 1981). The annual fecundity index for *H. nobilis* is 16,842, reaching peak indices of 29,444 in February and 41,130 in June. *A. mauritiana* exhibits an index of 13,525, reaching a peak value of 30,880 in April. These values are in agreement with those reported by Conand (1989). The mean annual index for *T. ananas* is 22,717 with a peak of 12,167 in April-May. The value reported by Conand for *T. ananas* was much smaller, falling in the range of 2,239-7,861. The large annual fecundity index of *T. ananas* reported from this study is probably not a useful value since oocyte development is highly seasonal.

Table 1. Sample sizes and number of animals within each category, female, male, and unknown. Sex ratio of these samples and the periods of collection are presented.

<u>SPECIES</u>	<u>FEMALE</u>	<u>MALE</u>	<u>UNKNOWN</u>	<u>RATIO</u>	<u>SMPL PERIOD</u>
<i>H. nobilis</i>	122	149	53	1:1.22	Apr.88-Dec.89
<i>T. ananas</i>	80	75	84	1.07:1	Apr.88-Dec.89
<i>A. mauritiana</i>	178	180	63	1:1.01	Apr.88-Dec.89

Size at First Reproduction

The method used to determine the size at which sexual maturity is reached was to plot the percent of sexually mature individuals in specific size classes (whole weight, WW), as described in Conand (1981). The point on the curve at which 50% of the individuals are mature (WW50) is considered to be an approximation of size at first reproduction (Fig. 3). The WW50 for *Holothuria nobilis* is 620g, *Thelenota ananas* 800g, and *Actinopyga mauritiana* 230g.

For *Holothuria nobilis* and *Actinopyga mauritiana*, percentage of sexually mature individuals increases as the size class of the population increases. With *Thelenota ananas* the number of sexually mature individuals decreases after individuals reach a size class of 1800-1900g, where all animals had obtained sexual maturity. This apparent decrease in reproduction with increasing size may be due to the animals becoming sexually senescent, or to an insufficient number of animals sampled within this size class.

Larval Development

1) Fertilization of stock

Successful fertilizations were conducted with gametes obtained from animals that spawned. While sperm that was taken from dissected testes can be used for fertilization, eggs dissected from females did not give good fertilization results. The success of development from embryo to larval stages (auricularia/doliolaria) when using eggs from dis-

sected animals was 0%. All successful larval rearing trials resulted from the use of eggs which were spawned by the females. Spawning of animals is seasonal as indicated by the gonadal indices and by observations of spawning activities (and by egg diameters with *Thelenota ananas*). For *H. nobilis*, spawning from collected animals was observed in 70% of the samples, while males and females of *A. mauritiana* spawned in 50% and 38% of the samples respectively (samples from 1989). In addition, it is the males that almost always initiate spawning, with the females spawning after the males, sometimes by as much as an hour.

Animals can be induced to spawn through temperature stress. This often occurs when the animals are moved from the collection site to the laboratory. Fifteen to twenty animals were placed in 30-40 liter plastic coolers for transportation to the lab. Stress is probably a combination of crowding, rise in water temperature, and lack of water exchange. Animals spawn while in transit and upon release into cement holding tanks. Gametes were collected with Pasteur pipettes during spawning as the gametes were shed from the cephalic gonopore. Gonads were stored separately in 0.45um filtered sea water until mixing for fertilization was conducted. Mixing of gametes was conducted soon after spawning (<2hr). Fertilization was conducted in high egg densities ~10-20 eggs/ml (>500ml containers). Sperm was added only in small quantities to prevent polyspermy (~0.1ml/500ml filtered sea water). All fertilization

was done in 0.45um filtered sea water. Occasional agitation of the fertilization containers or some constant agitation device (not bubbling) has proven to be the best method for gently circulating the zygotes and developing embryos/larvae. Dilution of the fertilization concentrations should be done ~24hr after fertilization. These concentrations should not exceed 2 larvae/ml.

2) Culture of larvae

As noted above, larval rearing is best accomplished by using a slow agitation device other than bubbling, although static cultures (with and without the use of antibiotics) have been used for the successful culture of larvae to a late stage. Larvae were maintained at densities of 1/ml or less after reaching a postgastrula stage. Water changes were conducted weekly with the use of 60um filter screen submerged in filtered sea water. Handling has proven to be a cause of stress and mortality, and should be minimized. Unfortunately, the need for occasional water changes requires some manipulation of the larvae.

Larvae were maintained by several methods. Five-hundred to 3000ml Erlenmeyer flasks were used for static and aerated cultures, with water changes every 3-4d. More successful cultures were conducted with a plexiglass paddle system which maintained slow and steady agitation of the larvae and their food. Two to three different phytoplankton species have been used for feeding the planktotrophic larvae. Tahitian *Isochrysis*, *Rhodomonas lens*, and *Chaetocerus gracilis*, were used

at a combined concentration from between 5000-30,000 cells/ml. Feeding was initiated within 24hr after gastrula formation, or by 24hr after the first larval stage, the auricularia, was reached. Water changes were necessary to maintain the number of phytoplankton in culture. Over-feeding of larvae is to be avoided since food in high densities in the gut appears to be passed through without being properly digested.

Upon reaching the second larval stage, the doliolaria, various treatments were used in an attempt to induce this stage to undergo the next metamorphosis to the pentacula larvae. Treatments have included: exposure to coralline algae, sand, and glass and shell fragments coated with benthic diatoms. These treatments have been carried out in micro conditions, i.e. glass ware, as well as within cement and fiberglass flow-through tanks. Visual inspection of the treated surfaces with the use of dissecting scopes have thus, far, not found pentacula or juvenile sea cucumbers. Due to the small size of the doliolaria larvae, ~200-300um in length, and the lack of pigmentation (the larvae are transparent), observation of the larvae over time has not been easily maintained. Containment vessels in which larvae were introduced are currently being maintained, and periodic searches are being conducted. Further research for the determination of settlement substrates and larval food needs to be continued to overcome such difficulties at this point in larval rearing.

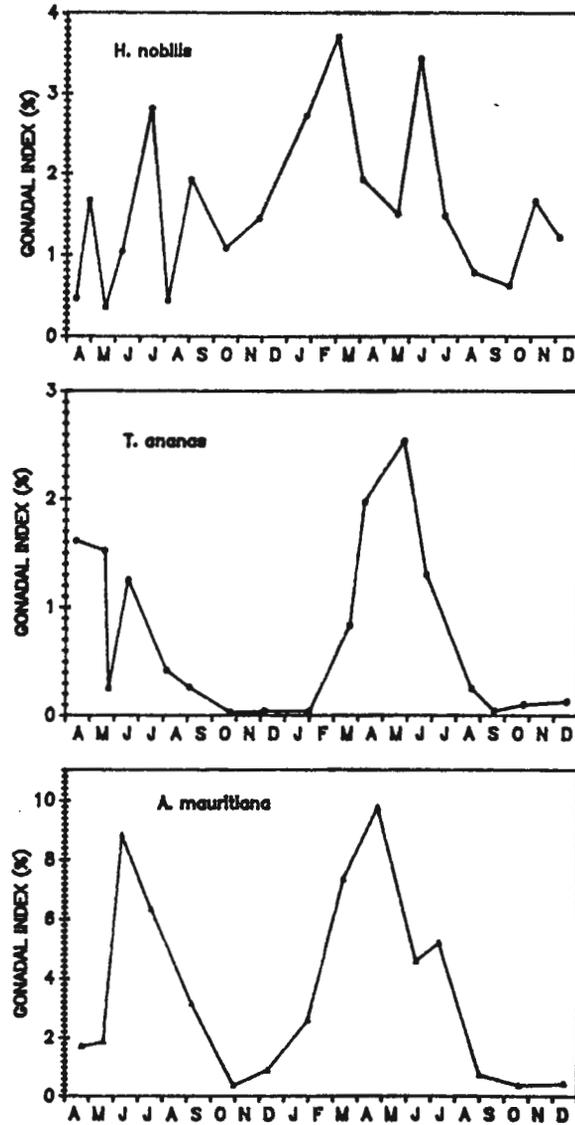


Figure 1. Mean gonadal index over time for each of the 3 study species 1988-1989.

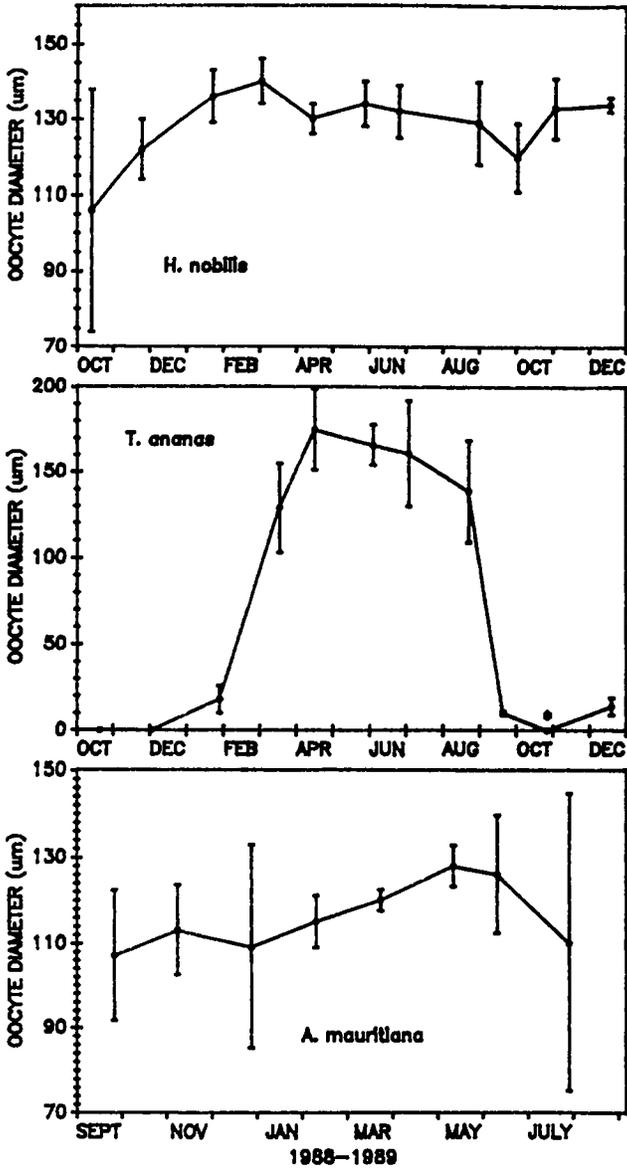


Figure 2. Oocyte diameters of three study species over time. See Tables 1-3 for number of females from each sample. Error bars are sample standard deviations.

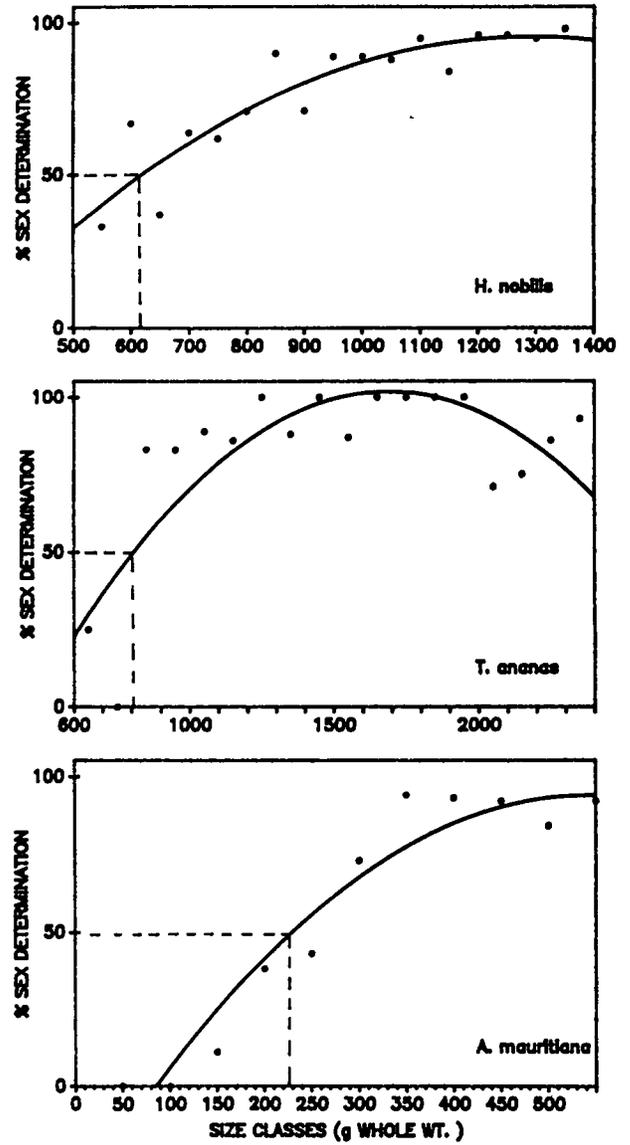


Figure 3. Approximate size class at first reproduction for three species studied, as determined at point in which 50% bear mature gonads (after Conand, 1981).



Development of the Techniques for the Mass Production of Larval Rabbitfishes (*Siganidae*) for Culture in Micronesia

Introduction

In marine fishes, growth and metabolism of early developmental stages are important determinants of survival to metamorphosis. Of particular concern is the transition from endogenous to exogenous nutrition; if the yolk sac and oil globule are depleted before the larvae have developed sufficiently to be able to capture food, survival is adversely affected. The rate of yolk utilization is determined by the metabolic rates, measured as oxygen consumption, of the larvae. For tropical marine species, such information is scant; although there has been work on the metabolic rates of a few sub-tropical species. Information on the rates of respiration is useful in determining the caloric requirements of the larval fishes, an aspect of their biology particularly relevant to the development of mass-rearing procedures.

Our work to date has examined the development, growth, and respiration of larval *Siganus argenteus*, one of the more common siganids of Guam, and *Siganus* sp., a currently undescribed species. Rabbitfishes, or siganids, are found throughout the Indo-West Pacific and are being cultured commercially in several areas. Although there has been other work on the larval growth and development of several species of rabbitfish, to our knowledge there have been no previous studies on the metabolic rates of eggs or larvae of siganids.

Juvenile *S. argenteus* were collected from the Pago Bay reef flat on the eastern coast of Guam and *S.* sp. were collected from the inner portion of Apra Harbor on Guam's western coast. Juveniles of *S. argenteus* are often very abundant during recruitment episodes, so we were able to obtain several hundred fish for broodstock; however, we were able to obtain only 13 specimens of the second species as it is uncommon and has not previously been reported from Guam. The fish were reared to maturity in a cement pond at the Guam Aquaculture Development and Training Center. The broodstock were fed twice daily with commercial pelletized catfish feed at approximately 3 percent of their body weight per day. The broodstock diets were occasionally supplemented with the filamentous green alga *Enteromorpha clathrata*.

When the fish became mature, as evidenced by egg samples taken by cannulation of anesthetized fish, they were induced to spawn by injection with Human Chorionic Gonadotropin (HCG). After from 8 to 12 fish were transferred to 2- or 12-ton larval rearing tanks for spawning. The method of egg collection differed between species. For *Siganus argenteus*, which has neutrally buoyant and non-adhesive eggs, egg collectors consisting of a 300-micron mesh bag suspended in a 120-l tank were placed to receive the outflow from the spawning tanks. The eggs accumulated in the collectors and were then transferred to rearing tanks. The eggs of *Siganus* sp., like those of most other siganids, are demersal and adhesive; to collect these eggs, sheets of corrugated plastic were placed over the bottom of the tank and removed immediately after the fish hatched.

Spawning usually occurred at dawn within 1 to 3 days after the injections and hatching of the eggs occurred from 12 to 15 hours after spawning. Immediately after the eggs had hatched the adults and the plastic sheets, with unhatched eggs and the residuum of the hatched eggs, were removed from the spawning tank. Phytoplankton (*Chlorella* sp.) was introduced to the tanks on the day after hatching at a density of 500,000 cells ml⁻¹. When the larvae developed a functional jaw (on day 2) they were offered a mixture of boiled egg yolk and a commercial diet for larval fishes, both screened through a 60-micron mesh, as a first feed. On the 2nd or 3rd day of culture a small strain (s-type) of marine rotifers *Branchionus plicatilis* were added so that densities reached 20 to 30 per ml by day 3. Later, copepods (*Thisbe holothuridae*) bloomed in the tank providing supplemental feed for the larvae. The larvae were weaned onto artificial feed after approximately 3 weeks when most of the fish had metamorphosed.

Larvae were collected from the rearing tank by gently screening the water through a 120-micron mesh. The rates of oxygen consumption of eggs and larvae were made in a thermostated (28°C) glass microrespirometry chamber. Oxygen consumption was determined with a Radiometer dissolved oxygen probe attached to a Strathkelvin dissolved oxygen meter and a strip-chart recorder. For each set of oxygen consumption determinations, a blank was run which consisted of water from the culture tank without fish. The oxygen consumption of the blanks were used to correct the larval metabolic determinations. The number of individuals used in the chamber varied with size of the larvae.

For older, post yolk-sac, larvae rates of both standard and routine metabolism were determined. For these trials, metabolic rates of each larvae were determined twice: once in the active state and once after the larvae were anaesthetized with MS-222 (trimethane sulphate). The rates of oxygen consumption of the tranquilized larvae were considered standard metabolic rates. We revived all anesthetized larvae by removing them from the anesthetic to insure that the larvae were not moribound.

Results

As has been shown for other warm water marine fishes the oxygen consumption of the eggs of *Siganus* sp. increases with stage of development. We found in siganids that the respiration rate of the developing embryos increases slowly with development during the egg stage and dramatically increases at the time of hatching. A burst of activity upon and immediately following hatching is responsible for the high metabolic rate observed during this period. However, this increased metabolic rate rapidly declines in less than one hour as illustrated in Figure 1. The metabolic rate then remains relatively stable for 2-3 days except for a slight decrease in metabolic rate of larvae undergoing the transition from the yolk-sac stage to

the feeding stage. When the larvae begin to feed the metabolic rate rises again.

Comparisons of anesthetized and unanesthetized larvae showed that the metabolic rates per unit mass of the active larvae were approximately 3 to 4 times greater than standard metabolism (Figure 2). The ratio of the rate of oxygen consumption of routinely active larvae to that of anesthetized larvae estimates the routine factorial metabolic scope of the larvae.

The pattern of oxygen consumption with development of *Siganus* sp. is comprised of several distinct phases: an egg phase, a short post-hatching burst phase, a stable yolk-sac phase, a transition phase where the metabolic rate is slightly depressed, and a growth phase. Although few data are available on the respiration rates of the larvae of tropical marine fishes, similar patterns of oxygen consumption with development have been reported by other investigators.

The difference in metabolic rates between larvae in the yolk-sac stage and those in the transition stage larvae may represent the specific dynamic effect—the calorogenic cost of yolk utilization. Alternatively the slightly lowered meta-

bolic rate at the time of hatching could result from decreased activity. When transition-stage larvae were observed under magnification they appeared more lethargic, often resting on the bottom of the container; the larvae also appear very fragile and mortalities can easily result from handling stress at this stage. The rates of oxygen consumption of the yolk sac larvae of *Siganus* sp. were similar to those reported for the larvae of other warm-water marine fishes.

These data on respiration and growth will be used in the development of a bioenergetic model for larval siganids. The model will be useful in the refinement of hatchery technologies developed for these and other marine fishes. The work to date has been important in establishing the successful production of siganids at the Guam Aquaculture Development and Training Center. These fish are now being cultured on a trial basis by commercial farmers on Guam.

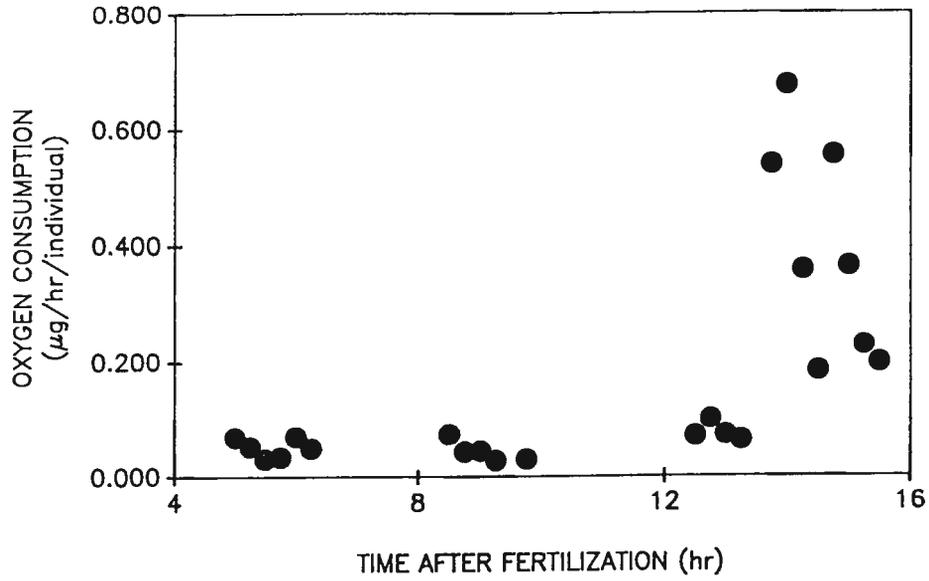


Figure 1. Respiration of eggs and recently hatched larvae of rabbitfish (*Siganus* sp.).

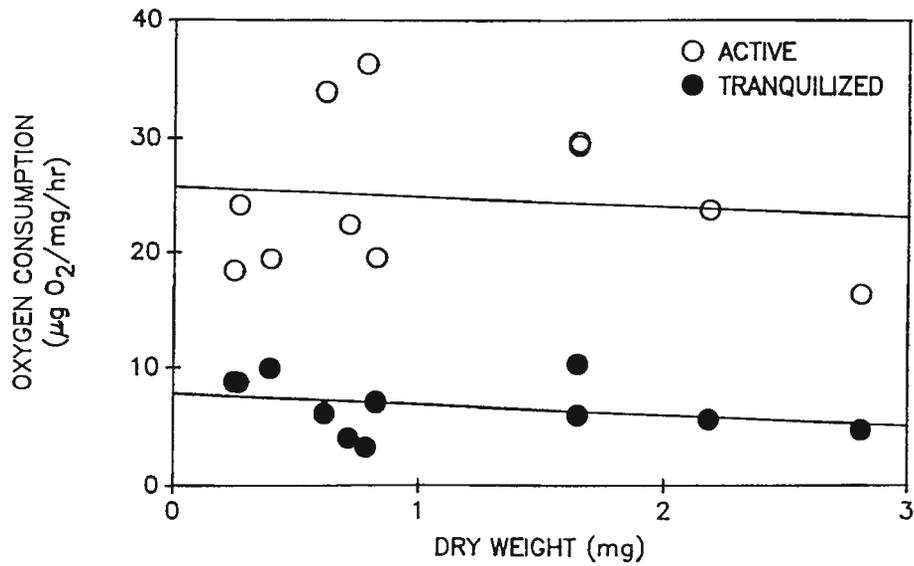


Figure 2. Comparison of respiration rates of active and tranquilized larval rabbitfish (*Siganus* sp.).



Bioenergetics Behavior of Land Rabbitfishes *Siganus* spp

This past year we have refined methods for spawning two species of rabbitfishes found on Guam. Juvenile *Siganus argenteus* and *Siganus* sp. were collected from the nearshore waters of Guam, reared to maturity, and spawned at the Guam Aquaculture Development and Training Center. We examined the development of the larvae, particularly in regard to their use of the yolk sac and to the transition to exogenous nutrition.

Spawning

Broodstock with mature gametes are selected for spawning. Ripe females can often be detected by their bulging sides, and milt can often be easily expressed from ripe males. Fish are selected for spawning on the basis of ripe gametes; gamete samples are collected by aspiration through a cannula attached to a syringe body. Adults with suitable gametes are injected intramuscularly with Human Chorionic Gonadotropin and placed in a 2-ton tank to spawn. Spawning usually occurs within 3 days of the hormone injections. In addition, spontaneous spawnings in broodstock without hormone inducement have not been uncommon. Spawning usually occurs early in the morning.

Different types of egg collectors are needed for *Siganus argenteus* and *Siganus* n. sp, since the two species have different types of eggs. *Siganus argenteus* is unique among siganids in having eggs which are neutrally or slightly negatively bouyant, eggs which are not adhesive; most species have demersal, adhesive eggs.

For *S. argenteus*, the eggs are collected from the overflow of the spawning tank in a 350-micron mesh screen. The eggs in the screen bag are then transferred to a larval rearing tank where they hatch. We found that the mesh bag should have vertical sides; our first egg collectors had sloping sides which resulted in the eggs clumping together at the water line and, subsequently, in a poor hatching success.

Larval Development

The eggs produced ranged from 0.53 to 0.56 mm in diameter. The morphologies of the egg and larval stages for both species were found to be similar to those described for other siganids. The eggs were usually spawned at dawn and hatched from 12 to 15 hours later with the larvae emerging head first from the egg.

Larvae rapidly increase in length during the first 12 hours following hatching (Figure 1); further changes in length were slight until the larvae began feeding. The dry mass of the larvae decreases steadily during the yolk-sac stage. The yolk sac began decreasing in volume immediately after hatching, and by 12 hours the diameter of the oil globule began to decline. Both the yolk sac and oil globule were usually depleted by 36 hours after hatching (Figure 2); however, the time of the disappearance of the oil globule varies between broods and ranges from 24 and 60 hours.

The patterns of depletion of the yolk sac and oil globule for *Siganus* sp. were similar, but somewhat smaller than those reported for other siganids cultured under similar environmental conditions in the Philippines. We found that, as with other tropical marine fishes, siganid larvae must begin feeding early, within 2

to 3 days after hatching. In contrast, larva of subtropical marine fishes first start feeding 70 hours after hatching.

The yolk sac of other tropical marine fishes has been reported to be depleted by 36 hours following hatching, with the yolk sac being depleted more rapidly than the oil globule. However, we found considerable variation in the time of depletion of the oil globule for *Siganus*. This could be related to, among other factors: effects of variation in temperature, egg quality, or feeding success. Earlier success at feeding could result in sparing of the oil globule. Exploration of factors affecting the efficiency and rate of utilization would probably be fruitful. Siganiid larvae which do not feed suffer 100% mortality usually after 5 days. The pattern of growth of the early larvae of *Siganus* sp.— a rapid increase in length during the first 12 hours after hatching after which growth in length slows until the onset of feeding— has been observed in other cultured species.

Transition from Endogenous to Exogenous Nutrition

As other workers have experienced, we found a critical period in the culture of larval siganids to be the transition to exogenous sources of nutrition. At the time that the larvae deplete the yolk sac, they are still too small to feed on rotifers. We tried a number of early diets including boiled egg yolk, commercial feed for larval fish, and the fertilized eggs of sea urchins. We found that the larvae will readily ingest these feeds if they are first screened through a 60-micron mesh screen. So far we have had the best success offering the larvae fertilized eggs of

the sea urchin *Echinometra matheii*. Since it is somewhat difficult to obtain enough sea urchin eggs to provide sufficient densities in the larger (10-ton) rearing tanks, this technique works best in smaller (2-

ton) rearing tanks. Our best results occur when first feeds are offered early, after the jaw has developed but before the oil globule has been depleted.

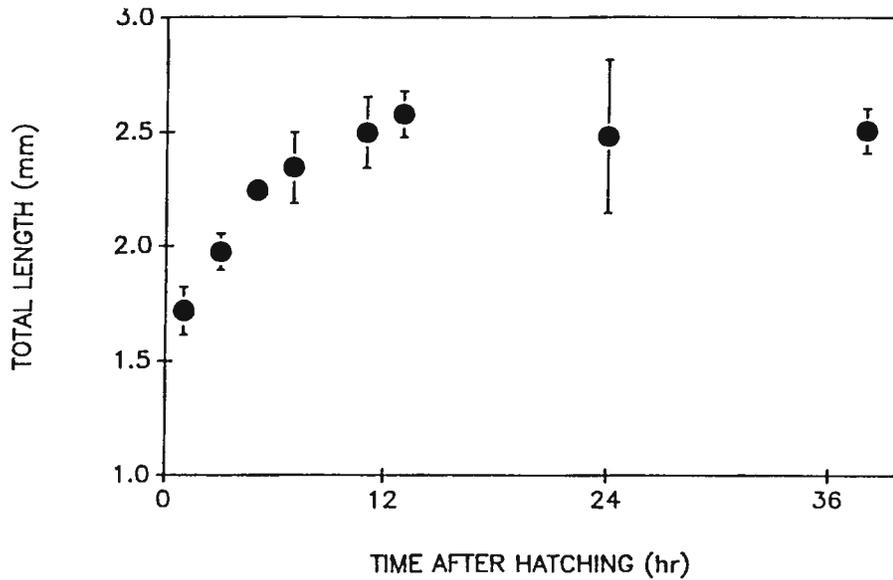


Figure 1. Growth of the larvae of *Siganus* sp. shortly after hatching.

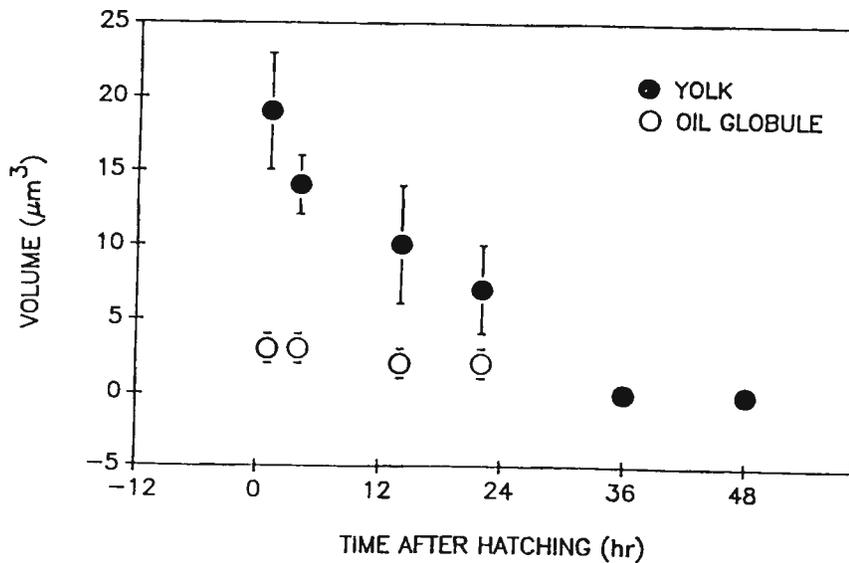


Figure 2. Depletion of the yolk sac and oil globule of *Siganus* sp. larvae.

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