Project Number 82-F9

BIOLOGICAL CONTROL OF THE NAVEL ORANGEWORM

Interim Report III

"Establishment of *Goniozus emigratus* (Rohwer) and *Goniozus legneri* Gordh on Navel Orangeworm, *Amyelois transitella* (Walker) in California and Biological Control Potential"

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S U M M A R Y

The parasites Goniozus emigratus (Rohwer) and Goniozus legneri Gordh found dominant on navel orangeworm, Amyelois transitella (Walker) in south Texas and Uruguay and central Argentina, respectively, were successfully established at experimental sites in California's Central Valley in 1979, following 4 months of releases at rates of ca. 2,500 ♀♀ per ha. Both species were observed to persist into the 1980 and 1981 harvests albeit at lower frequencies than that recorded in 1979. Coexistence with an earlier introduced parasite, Pentalitomastix plethoricus Caltagirone, occurred at all experimental sites. Separate k-value analyses for each parasite indicated significant regulative capabilities by the Goniozus species of their navel orangeworm host during the warm summer season. No such tendency was measured with P. plethoricus during June, August and December samples, which suggests a further examination of this parasite's potential regulative role in different seasons, as perhaps mid spring and autumn.

[End of Summary]

The goal of the present research was to acquire new natural enemies of navel orangeworm, Amyelois transitella (Walker), which following its invasion of California in the 1940's, has become the most important pest of soft shelled almonds. Earlier work resulted in the establishment of an egg-larval parasite from southern Mexico, Pentalitomastix plethoricus Caltagirone (Caltagirone 1966, Caltagirone et al. 1964). At that time the navel orangworm was thought to have originated somewhere in the neotropics from Mexico to 5° south of the equator in Brazil. The transfer of successful parasites from tropical to temperate regions has never met with significant success (DeBach 1964), and trials with other parasites from Mexico (Caltagirone et al. 1964) did not result in further establishments. The success with P. plethoricus may be attributed to the acquisition of temperate strains from moderately high elevations in Oaxaca, but this parasite's ability to regulate navel orangeworm at low densities remains in doubt. Certainly no host density drop was recorded with its establishment on carob moth, Ectomyelois ceratoniae (Zeller) in Israel (Gothilf 1978). Even though P. plethoricus may account for some of the leveling-out of navel orangeworm abundance witnessed in California during the 1970's, economic thresholds set at below 4% damage are lower than that attainable with P. plethoricus alone Unusually warm summers have been followed by higher navel orangeworm damage of double or triple the economic threshold, which could be explain-

ed in part to the inactivation of P. plethoricus, which is adapted to cooler temperatures. Additional natural enemies were desired which could permanently reduce navel orangeworm densities to acceptable levels.

This report traces the acquisition of new parasitic species and measures the potential impact of 3 parasites on navel orangeworm in experimental orchards of 3 major almond producing areas of California's Central Valley.

Procedure

Foreign Exploration.--New species of natural enemies were sought with emphasis on temperate latitudes of both North and South America. Explorations were conducted during 1977-1979 in the natural range of navel orangeworm at high latitudes of Texas, Uruguay and Argentina. Spring, summer and autumn seasonal collections were made in each area. The fruit of host trees surveyed in Texas within 27-32° N. Lat. that produced navel orangeworm were Texas ebony, Pithecellobium flexicaule (Bentham) Coulter; western soapberry, Sapindus drummondii Hooker & Arnott; and Nonpareil almond, Prunus dulcis (Miller) D. A. Webb. In Argentina and Uruguay host trees occurring between 30-35° S. Lat. were Acacia farnesiana (L) Willard; walnut, Juglans regia L.; coral tree, Erythrina crista-galli L.; loquat, Eriobthrya japonica (Thunberg) Lindley; and Prunus dulcis.

Experimental Orchards.--Commercially productive 15-50 year old almond orchards of primarily the Nonpareil soft shell variety were selected in 3 major almond growing areas of California's Central Valley. There were two 6 ha. orchards at Chico, one with 50 and the other with 23 year old trees, and a third 16 ha. orchard with 23 year old trees. At Chowchilla two 3 ha. orchards each with 12 year old trees were selected; and at Wasco a 4 ha. and a 29 ha. orchard were used, both with 12 year old trees.

Experimental intensive study sites of 9 trees arranged in a semicircular pattern were designated, the number varying with orchard size. These sites were separated by at least 5 tree rows. The orchards were unsprayed during the growing season, but received dormant applications of oil and phosphate insecticides for control of European red and brown mites, peach twig borer, Anarsia lineatella Zeller, and San Jose scale, Aspidiotus perniciosus Comstock.

Parasite Releases.--The parasite Goniozus emigratus (Rohwer) from south Texas and Goniozus legneri Gordh from Uruguay and central Argentina were released biweekly from April 17th to August 8th, 1979 at experimental sites for the purpose of establishing the species and measuring its potential impact against the navel orangeworm. The latter was especially important because summer insecticidal spray programs against navel orangeworm were becoming widely adopted, and the 2-3 years time required for firm establishment of most natural enemies could not be guaranteed. Release rates were ca. 2,500 ♀♀ per ha. at each 9-tree experimental site.

Three orchards in Chico with one experimental site each were selected for release of G. emigratus, while G. legneri was released at 5 experimental sites in 2 orchards at Chico, 3 sites in 2 orchards at Chowchilla and 2 sites in 2 orchards at Wasco. The latitude at the Chico sites was 39.6° N., at Chowchilla 37° N. and at Wasco 35.6° N.

Sampling.--Harvest samples of almonds were taken in mid to late August in 1979 and 1980, about 3-5 weeks after hull split. This was sufficient time for the occurrence of a 2nd generation of navel orangeworms (Wade 1961) and the parasitization by the egg-larval parasite Pentalitomastix plethoricus, and the larval parasites G. emigratus and G. legneri. Sample dates at Chico were August 3rd, 1979 and August 22nd, 1980; at Chowchilla August 21st, 1979 and August 9th, 1980; and at Wasco August 21st, 1979 and August 8th, 1980.

A random sample of 100 hull split almonds was taken from each tree. Samples were transported to the laboratory in air conditioned vehicles with temperatures maintained near 25° C. Each almond was examined within 4 days for navel orangeworm damage and the presence of larvae. The larvae were transferred with their respective almond shells and kernels (hulls removed) to separate transparent polystyrene plastic vials with 39 mesh/cm brass screened lids. These vials were separated according to the size of larvae incubating within (small, medium and large) for separate analysis of each group. They were incubated for 5 months at 25.6° ± 1° C., 40-50%

RH, and a photoperiod of 14:10-h L:D supplied by cool white fluorescent lamps, giving a maximum vial level intensity of 25 ft-c, to ensure the full development of all larvae and parasites to the adult stage.

Mummy almonds were sampled during the first week of December 1979, 1980 and in June 1980. Sample dates were December 10th, 1979 and December 12th, 1980 at Chico. At Chowchilla samples were on December 9th, 1979 and December 2nd, 1980; and at Wasco on December 9th, 1979. A June 10th sample was also taken at experimental sites in Chico. A minimum of 10 mummies per tree could be sampled without depleating a tree's entire supply, but usually larger samples were taken.

The mummies were treated similarly to almonds at harvest, each mummy being examined for infestation and the larvae confined with the mummy almond shell and kernel in screened plastic vials where they were incubated for complete development (ca. 5 months). In cases where more than one larva was present, each was isolated separately on almonds gathered from the same orchard to ensure adequate food supply during development.

Statistical Analyses.--The initial larval navel orangeworm density in a tree at the time of field sampling was compared to the final density after parasitization. This was judged directly from the larvae present and by laboratory incubation. This comparison was made in order to measure a parasite species' response to varying host densities in the field, and to determine whether this

response was regulative, where an increasing % of hosts are parasitized at higher host densities. In the first case, the initial density of navel orangeworms per 100 sampled almonds per tree was plotted graphically with the parasitized host density judged after complete incubation. Linear relationships were then tested with a bivariate correlation analysis. In the 2nd case, host regulative response was analyzed by graphing and then correlating the $\log_{10}$ initial density + 1.0 with the difference between $\log_{10}$ initial density + 1.0 and the $\log_{10}$ final density + 1.0 (the "killing power" or "k-value" of Varley et al. 1974). The number of mummy almonds sampled was adjusted to 100 per tree for analysis.

The determination of a parasite's activity from incubation and emergence in the laboratory underestimates the actual activity of the parasite to the extent that hosts which are killed by parasites through their probing and egg laying activity die without giving rise to adult parasites in the manner reported for other species (Legner 1979). Incubation at 25.6° C. and 50% RH is near optimum for development of both the navel orangeworm and the parasites involved in the current investigation (Wade 1961; Legner, unpublished data). Although parasitism by Goniozus spp. that oviposit exteriorly on paralyzed larvae can be judged visually, incubation for these parasites is still desirable to obtain parasite survival and to avoid missing larvae on which parasite eggs might be overlooked. Paralyzed larvae were not readily distinguishable from those that were quiescent or infected with microsporidian

pathogens, even though the frequency of the latter was usually below 1% in field samples. The initial density of P. plethoricus, which attacks host eggs, was inferred from the number of larvae present at sampling. Eggs that were killed during parasitization could not be judged, but such mortality was found to be positively correlated with reproductive capacity in the laboratory as reported for other species (Legner 1979).

Results

Natural Enemy Exploration.--The Texas explorations revealed P. plethoricus parasitizing A. transitella on Nonpareil almonds as far north as Brownwood (32° N. Lat.) and on Texas ebony and western soapberry seeds all along the Gulf of Mexico coast and throughout south Texas. At the latitude of Corpus Christi another parasite, a biparental strain of Goniozus emigratus, was found attacking A. transitella at low densities and in all seasons on western soapberry and Texas ebony. The 1977-1979 South American explorations produced primarily G. legneri at all seasons, parasitizing both A. transitella and the carob moth, E. ceratoniae, on E. crista-galli, A. farnesiana, J. regia and E. japonica, over a broad range of Uruguay and central Argentina to 35° S. Lat. and across the continent to the eastern slopes of the Andes. Another parasite, an ichneumonid Temelucha sp. was collected in large enough numbers to be considered significantly associated with these two hosts; but it represented less than 5% of the total parasiti-

zation, and a culture was not established. Rare parasites reared from these hosts were the ichneumonids Coccygonimus sp. and Venturia canescens (Gravenstein), a braconid Bracon sp. and an encyrtid Copidosoma sp.

Field Establishment.--Both species of Goniozus from south Texas and South America became quickly established at all experimental release sites in California following spring and early summer releases in 1979. Their spread in the orchards was recorded at a maximum distance of 310 m, 4 months after initial liberation at Chico and Wasco. They attacked navel orangeworms in tree mummies after harvest and were observed to persist at the Chico and Chowchilla sites which could be retained until harvest 1981, even though by then surrounding orchards were being sprayed with 2 to 4 spring and summer applications of broad spectrum insecticides for navel orangeworm control, and though orchard mummy almond removal was being practiced within the experimental sites themselves. This interference may have accounted for their lower abundance in the years following liberation.

Parasite Biological Control Potential.--The potential by these parasites for a general lowering of the average density of navel orangeworm was examined in August and September 1979 experimental sites, before the above mentioned interferences intervened. The parasite P. plethoricus was included in this examination as it occurred naturally at all the experimental sites during this

period. Separate analyses of data in which larvae were separated to size in an attempt to measure discrete broods, was similar to the combined analyses, so that only the latter are shown.

The functional response of Goniozus species to host density in a tree is shown in Table 1 for 1979 and for P. plethoricus in both 1979 and 1980 (Table 2) as the greater number of experimental sites available for the latter parasite permitted a longer study period.

Positive functional responses to host density by both G. emigratus and G. legneri were highly significant at all experimental sites in August and at 2 of 3 remaining sites in December (Table 1). On the other hand, P. plethoricus was most highly correlated with host density in December collections (Table 2). Thus, all parasites demonstrated a positive response to higher host densities by increasing parasitization, although this capability was strongest in the Goniozus species. However, it may have resulted merely from the greater chance of finding hosts in trees where they were most abundant and not from the parasite's awareness of these higher numbers.

Correlations with % infested almonds were of lesser significance (Tables 1 & 2).

Whether or not this response of the parasites to host density was indeed potentially regulative, was tested with k-value analyses shown in Tables 3-7 for the 3 parasite species. It was found that G. emigratus demonstrated a significant capacity to recognize

and respond in a regulative fashion at Chico in August by increasing its attack rate on higher host densities (Table 3). Similarly, G. legneri showed a significant capacity to behave in this manner in August at 4 locations in the Central Valley (Table 4). No such tendency was indicated for either of these species in December as shown combined in Table 5.

Data for P. plethoricus in Tables 6 & 7 do not indicate any regulative ability neither in August nor December collections.

Discussion

Goniozus emigratus and G. legneri both appear capable of significantly regulating navel orangeworm density by their ability to increase attack rates in response to rising host densities. This capability may be restricted to the warm summer season as no such tendency was detected in December collections. However, P. plethoricus did not show a regulative response to its host at either sample interval. As this parasite's functional response to the host was strongest in December collections (Table 2), it is possible that earlier collections representing activity in a slightly warmer, but not hot, period such as occurs in October and November in the Central Valley, might have shown a regulative ability with k-value analysis. Because of destructive sampling in restrictive experimental acreage (removal of too great a number of almonds from experimental orchards), this data was not obtainable in the current investigations.

Laboratory data support the temperature hypothesis, where

temperatures above 30° C. are detrimental to the parasitization and development of P. plethoricus, whereas both G. emigratus and G. legneri respond favorably to temperatures in the 35° C. range.

Pentalitomastix plethoricus may have exerted hidden population regulative influences that could not be measured with current techniques. For example, this parasite's destruction of host eggs could have been higher than that discerned from incubation and adult emergence. However, an analysis of host destruction in a manner described for other parasite species (Legner 1979) showed that reproductive capacity and destructive capacity were strongly and significantly correlated. Although the functional response may not have been regulative, a parasite sometimes can defer its regulative capabilities to a later numerical response through the production of proportionately more ♀♀ at higher host densities (Legner 1967). These and other behavioral possibilities would have to be critically evaluated before conclusions about P. plethoricus' impact are final.

The data indicate a potential for G. emigratus and G. legneri in the biological control of navel orangeworm during warmer periods and, thus, their establishment in almond orchards is desirable. The average density of navel orangeworm could drop with both Goniozus spp. and P. plethoricus present in the way that the addition of a warm season adapted parasite, Coccophagoides utilis Doutt resulted in a permanent lowering of olive scale, Parlatoria oleae (Colvee) density (Rosen & DeBach 1977).

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TABLE 1. Functional response of *Goniozus emigratus* and *Goniozus legneri* parasitization combined in Nonpareil almonds and almond mummies at 4 locations in California's Central Valley

AREA	df	Initial den. vs Parasite den. Corr. Coeff(r)	t	Signif. level	% Infest. almonds vs Parasite den.[1/] Corr. Coeff (r)	t	Signif. level
Aug 1979							
Chico-1	32	0.857	9.395	99	0.649	4.828	99
Chico-2	16	0.824	5.621	99	0.736	4.210	99
Chowchilla	21	0.757	5.307	99	0.035	0.159	ns
Wasco	16	0.879	3.697	99	0.452	1.133	ns
Dec 1979							
Chico-1	21	0.855	3.299	99	0.661	1.762	90
Chico-2	15	0.892	3.947	99	0.105	0.212	ns
Chowchilla	18	0.082	0.217	ns	-0.088	-0.234	ns

[1/] Density expressed in No. per 100 sampled almonds per tree.

TABLE 2. Functional response of Pentalitomastix plethoricus parasitization to variable navel orangeworm densities in Nonpareil almonds and almond mummies sampled over 2 years at 4 locations in California's Central Valley.

AREA	df	Initial den. vs Parasite den. Corr. Coeff (r)	t	Signif. level	% Infest. almonds vs Parasite den.[1/] Corr. Coeff (r)	t	Signif. level
Aug 1979							
Chico-1	49	0.623	5.574	99	0.581	5.001	99
Chico-2	30	0.476	2.964	99	0.546	3.569	99
Chowchilla	33	0.145	0.841	ns	0.095	0.541	ns
Wasco	15	0.201	0.741	ns	0.116	0.420	ns
Dec 1979							
Chico-1	44	0.738	7.257	99	0.730	7.159	99
Chico-2	29	0.694	5.196	99	0.698	5.253	99
Chowchilla	36	0.563	4.091	99	0.491	3.377	99
Jun 1980							
Chico-1	10	-0.112	-0.319	ns	-0.109	-0.310	ns
Chico-2	10	0.563	1.928	90	0.445	1.404	ns
Aug 1980							
Chico-1	46	0.469	3.598	99	0.365	2.662	98
Chico-2	32	0.708	5.675	99	0.416	2.591	98
Chowchilla	19	0.238	1.068	ns	-0.169	-0.746	ns
Dec 1980							
Chico-2	29	0.704	5.331	99	0.691	5.142	99
Chowchilla	6	-0.295	-0.617	ns	-0.444	-0.989	ns

1/ Density expressed in No. per 100 sampled almonds per tree.

TABLE 3. Estimates of Goniozus emigratus impact on navel orangeworm in Nonpareil almonds on August 21-30, 1979 at 3 orchards in Chico, California through k-value analysis.

ORCHARD		N.O.W. 1/ Adult hosts emerged per 100 almonds	N.O.W. 1/ % Infested almonds	Goniozus emigrata % 2/ Parasitism	Goniozus emigrata Correlation with N.O.W. 3/ Corr. Coeff (r)	t	Signif. level	df
1	$\bar{x}$	2.78	6.22	3.02	0.658	2.313	90	7
	$s_{\bar{x}}$	0.88	1.13	2.02				
2	$\bar{x}$	2.33	5.56	8.24	0.498	1.520	ns	7
	$s_{\bar{x}}$	0.41	0.50	3.43				
3	$\bar{x}$	2.17	8.33	13.89	0.797	2.640	95	7
	$s_{\bar{x}}$	0.48	1.48	9.04				
Pooled (3 orchards)	$\bar{x}$	2.46	6.50	7.69	0.604	3.556	99	25
	$s_{\bar{x}}$	0.37	0.61	2.70				

1/ navel orangeworm

2/ (No. parasites emerged / initial N.O.W. density) x 100

3/ $(\log_{10}$ Init. Den. + 1.0) vs $[(\log_{10}$ Init. Den. + 1.0) - $(\log_{10}$ Final Den. + 1.0)]

TABLE 4. Estimates of Goniozus legneri impact on navel orangeworm in Nonpareil almonds on August 21-30, 1979 at 4 locations in California's Central Valley through k-value analysis.

AREA		N.O.W. 1/		Goniozus legneri				
		Adult hosts emerged per 100 almonds	% Infested almonds	% Parasitism 2/	Correlation with N.O.W. 3/ Corr. Coeff (r)	t	Signif. level	df
Chico-1	$\bar{x}$	3.91	6.47	9.12	0.655	4.250	99	25
	$s_{\bar{x}}$	0.50	0.59	2.10				
Chico-2	$\bar{x}$	2.82	5.89	14.29	0.686	2.667	98	16
	$s_{\bar{x}}$	0.59	1.30	5.06				
Chowchilla	$\bar{x}$	2.48	2.63	11.38	0.579	1.739	90	21
	$s_{\bar{x}}$	0.39	0.41	4.30				
Wasco	$\bar{x}$	1.43	0.67	17.86	0.735	2.170	95	16
	$s_{\bar{x}}$	0.67	0.27	11.85				

1/ navel orangeworm

2/ (No. parasites emerged / initial N.O.W. density) x 100

3/ $(\log_{10}$ Init. Den. + 1.0) vs $[(\log_{10}$ Init. Den. + 1.0) - $(\log_{10}$ Final Den. + 1.0)]

TABLE 5. Estimates of Goniozus legneri and Goniozus emigratus impact on navel orangeworm in Nonpareil almond mummies on December 10, 1979 at 3 locations in California's Central Valley, through k-value analysis.

AREA		N.O.W.[1]		Goniozus spp.				
		Adult hosts emerged per 100 mummies	% Infested mummies	% Parasitism[2]	Correlation with N.O.W. Corr. Coeff. (r)[3]	t	Signif. level	df
Chico-1	$\bar{x}$	64.18	53.41	10.17	-0.731	-4.909	99	21
	$s_{\bar{x}}$	7.68	5.15	4.87				
Chico-2	$\bar{x}$	38.46	24.10	4.92	-0.034	-0.131	ns	15
	$s_{\bar{x}}$	12.16	4.59	2.49				
Chowchilla	$\bar{x}$	81.70	55.49	3.71	-0.269	-1.185	ns	18
	$s_{\bar{x}}$	7.46	4.39	1.19				

[1] navel orangeworm

[2] (No. parasites emerged/initial N.O.W. density) x 100

[3] $(\log_{10}$ Initial Density + 1.0) vs [$(\log_{10}$ Initial Density + 1.0) - $(\log_{10}$ Final Density + 1.0)]

TABLE 6. Estimates of Pentalitomastix plethoricus impact on navel orangeworm in Nonpareil almonds on August 21-30, 1979 and August 8-22, 1980 at 4 locations in California's Central Valley through k-value analysis.

AREA		N.O.W. 1/		Pentalitomastix plethoricus				
		Adult hosts emerged per 100 almonds	% Infested almonds	% Parasitism	Correlation with N.O.W. 3/ Corr. Coeff (r)	t	Signif. level	df
Aug 1979								
Chico-1	$\bar{x}$	3.69	6.21	26.97	0.045	0.316	ns	49
	$s_{\bar{x}}$	0.42	0.43	2.95				
Chico-2	$\bar{x}$	2.44	4.72	23.32	0.135	0.748	ns	30
	$s_{\bar{x}}$	0.39	0.75	4.91				
Chowchilla	$\bar{x}$	1.40	1.96	6.84	-0.179	-1.043	ns	33
	$s_{\bar{x}}$	0.23	0.28	3.32				
Wasco	$\bar{x}$	0.45	1.45	19.05	-0.018	-0.063	ns	15
	$s_{\bar{x}}$	0.14	0.22	9.86				
Aug 1980								
Chico-1	$\bar{x}$	3.08	7.68	17.73	-0.372	-2.722	99	46
	$s_{\bar{x}}$	0.34	0.54	3.23				
Chico-2	$\bar{x}$	2.19	6.15	10.42	-0.005	-0.027	ns	30
	$s_{\bar{x}}$	0.50	0.69	2.15				
Chowchilla	$\bar{x}$	0.26	3.63	2.54	0.318	1.464	ns	19
	$s_{\bar{x}}$	0.12	0.37	1.51				
Wasco	$\bar{x}$	0.23	1.73	1.85	(orchard sprayed)			
	$s_{\bar{x}}$	0.09	0.27	1.85				

1/ navel orangeworm

2/ (No. parasites emerged / initial N.O.W. density) x 100

3/ $(\log_{10}$ Init. Den + 1.0) vs $[(\log_{10}$ Init. Den. + 1.0) - $(\log_{10}$ Final Den. + 1.0)]

TABLE 7. Estimates of Pentalitomastix plethoricus impact on navel orangeworm in Nonpareil almond mummies on December 10, 1979 and December 12, 1980, at 4 locations in California's Central Valley through k-value analysis.

AREA		N.O.W.[1] Adult hosts emerged per 100 mummies	N.O.W.[1] % Infested mummies	Pentalitomastix plethoricus % Parasitism[2]	Correlation with N.O.W.[3] Corr. Coeff (r)	t	Signif. level	df
Dec 1979								
Chico-1	$\bar{x}$	67.10	55.66	27.62	0.015	0.099	ns	45
	$s_{\bar{x}}$	6.57	4.12	2.63				
Chico-2	$\bar{x}$	37.65	27.49	23.46	-0.501	-3.116	99	29
	$s_{\bar{x}}$	7.63	3.61	3.60				
Chowchilla	$\bar{x}$	76.02	52.02	6.37	0.030	0.182	ns	36
	$s_{\bar{x}}$	5.62	2.98	1.10				
Wasco	$\bar{x}$	46.64	37.48	0	(orchard sprayed)			
	$s_{\bar{x}}$	7.62	4.49	--				
Dec 1980								
Chico-2	$\bar{x}$	41.54	26.59	40.37	-0.693	-5.087	99	28
	$s_{\bar{x}}$	7.87	3.22	4.47				
Chowchilla	$\bar{x}$	81.78	39.65	0.57	-0.114	-0.743	ns	42
	$s_{\bar{x}}$	7.10	2.57	0.57				

1/ navel orangeworm

2/ (No. parasites emerged/initial N.O.W. density) x 100

3/ $(\log_{10}$ Init. Den + 1.0) vs $[(\log_{10}$ Init. Den. + 1.0) - $(\log_{10}$ Final Den. + 1.0)]

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BIOLOGICAL CONTROL OF NAVEL ORANGEWORM

2ND TECHNICAL PROGRESS REPORT

(July 1 - December 31, 1982)

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BIOLOGICAL CONTROL OF NAVEL ORANGEWORM

2nd Technical Progress Report--July 1 - December 31, 1982

Field Experiments

Eight parasitic species have now been mass reared and released in almond orchards since 1979. These are: Goniozus legneri-Uruguay, central Argentina; Goniozus emigratus - south Texas; Chelonus mccombi - south Texas; Diadegma sp. - Loxton, Australia; Chelonus sp.-Ord River, Australia; Bracon sp. - Wasco, CA; Bracon sp. - Rawalpindi, Pakistan; Chelonus sp. - Ethiopia.

Three larval parasites, Diadegma sp., Goniozus legneri and Goniozus emigratus have overwintered in Central Valley almond orchards. Currently, G. legneri and Diadegma sp. continue to be recovered in the 1979 release orchards, with G. legneri spreading to many orchards in the state. This is due undoubtedly to the fact that the latter species has been most widely disseminated.

Statistical analyses run on G. legneri and G. emigratus indicate that these parasitic wasps have a controlling influence on navel orangeworm. This is characterized by an increase in their attack rate in response to rising navel orangeworm densities after hullsplit and into harvest. Statistical analyses run on Pentalitomastix plethorica (almond wasp) at Chico, Chowchilla and Wasco showed that there was no regulative response during June, August and December. This indicates the need for further examination of this parasite's potential role in different seasons, such as mid-spring and autumn. Until now, sampling during these two periods has not been possible due to early abscission of mummied almonds. The 1982 harvest data is still being evaluated, and a December sample will be incubated for 5 months before results are released.

Parasite Establishment.--

Our present goal is to continue to establish new natural enemies as they are obtained, so that they will become firmly associated with the ecology of navel orangeworm. This will permit natural selection for resistance to climatic rigors and to widely used pesticides. We expect to have more parasite numbers and species available for growers in 1983 due to improved mass rearing methods. Rearing technology is being improved with the aim of keeping costs low and easing field release procedure, and to eliminate field ant predation. Individual growers have special needs in establishing the new biological control agents, so that we must often visit a particular orchard to determine the proper seasons and release procedure.

Carob Moth--

Fortunately, the parasitic species we've introduced against N.O.W. also attack carob moth, Ectomyelois ceratoniae, effectively. The two pests are very similar in behavior, but the carob moth appears to attack a wider range of plants. This wider host range can be expected to favor biological control by creating a greater number of survival reservoirs for the natural enemies

outside of almond orchards. One of the reasons for the widespread success of the olive scale biological control effort is thought to be the wide host plant range of the scale.

New Natural Enemy Species.--

A renewed effort to broadly establish Diadegma sp. from South Australia and Goniozus emigratus from south Texas is indicated from the observation that these two parasites have begun to reappear in the 1979-release orchards. We have been stressing Goniozus legneri until now because of its immediate strong response to N.O.W. in the first year of release. Additional parasitic species are known from South America and Australia which were never cultured at the time surveys were being made. These should definitely be acquired. One group of parasites related to the Trichogrammas is now known to occur in Argentina. This group attacks and kills eggs of N.O.W.

The firm establishment of a number of natural enemies on N.O.W. can be expected to directly effect carob moth if this pest becomes widespread. Such natural enemies might even preclude the need for special control practices against carob moth.

Parasite Biology

Studies of the biology of one species, Goniozus emigratus, are completed and presented as follows: Details concerning the immature biology, development, adult longevity, fecundity and behavior of Goniozus emigratus (Rohwer) are provided. Evidence is given to suggest that there are at least two biological forms of this species. Studies show that this parasite attacks Microlepidoptera and is not host specific. Laboratory studies show that inseminated female parasites which are continually provided hosts lived 52.6 ± 7.3 days (n = 20) and mate-deprived males lived 45.2 ± 3.8 days (n = 14) at 25.6 ± 1°C and uncontrolled RH. Under the same conditions, host deprived females lived 37.1 ± 12.4 days (n = 35)while mate-provided males lived 17.2 ± 9.8 days (n = 14). During 1979 females parasitized 9.1 ± 4.2 hosts and laid 118.8 ± 58.3 eggs. During 1980, inseminated female parasites provided hosts with about one day of host deprivation between ovipositional episodes lived 64.1 ± 19.7 days (n = 20), parasitized 15.6 ± 5.6 hosts and laid 232.9 ± 91.5 eggs at 26.7 ± 1°C and 40 ± 5% RH.

Eggs hatch within 24 hours of being deposited on the host; larval development requires about three days; cocoon construction requires about one day; prepupal period requires about 1.5 days and pupation lasts 6-7 days when developing on the navel orange orange worm, Amyelois transitella (Walker).

The sex ratio of the progeny of G. emigratus is strongly skewed towards females, and increases in a female bias with larger clutch size. There is a tendency for broods to become male biased as a female ages and her sperm supply is depleted. Males are protanderous and enter their female sibling cocoons to copulate with pre-emergent females. Alternatively, males will copulate with females that have emerged from their cocoons.

Oviposition sites on the host are not random, and the female strongly prefers the middle segments and dorsal and lateral aspect of the host's body.

Immature Development

EGG: The eggs of G. emigratus are sausage shaped, whitish, partly transparent, and hatch within 24 hours. It is diffiuclt to distinguish between the egg and a newly eclosed lst instar larva because their forms are similar.

LARVA: All bethylid larvae are apodous and it was not possible to determine the number of larval instars, but within 24 hours of eclosion the larva is small, moderately well developed, with slight cephalization. Feeding requires about three days. During this period the parasite larva steadily increases in size. During the first two days following eclosion of the parasite eggs, the host larva is relatively alert, based on its response to a sharp probe and microscopis observation of haemolymph flow and metasynchronous contractions of the circulatory system. During the third day of parasite development the host is more sluggish and the flow of haemolymph in spasmodic. During this time the parasite becomes yellowish and disproportionately larger when compared to the development during the first two days after eclosion. Exsanguination appears rapid during the third day of parasite feeding.

Successful development appears contingent upon the host remaining alive and the circulatory system remaining functional. Twenty-three observations were made in which the hosts were moribund and mummified, and in no instance did the parasites develop to maturity. Failure of the host to remain alive during larval feeding may be a function of the adult female parasite killing the host.

Feeding complete and the host exsanguinated, shrivelled and dead, the parasite larvae detach their falcate mandibles from the host's integument, and begin to spin white cocoons adjacent to the host. The larvae do not migrate. During cocoon formation the parasite larvae each display a dusky spot at the posterior end of the body.

Cocoon formation requires about 24 hours, during which the body shape is essentially that of the feeding larva, with the anterior end enlarged and rather bulbous. The hind gut is not voided during cocoon formation, but occurs within about 24 hours of completion of the cocoon as evidenced by a black spot at the posterior end of the larva.

PUPA: Transformation from the larval to pupal shape occurs 24-36 hours after the hind gut is voided. The perfectly formed pupa is first opaque, and then becomes milky white with the appendages remaining transparent. Coloration in the pupa first occurs in the compound eyes which are initially pink and later become brick red. Concomittantly, the metasome assumes a faint yellow coloration. Within 24 hours of the darkening of the eyes the propodeum becomes dusky posteriomedially. Subsequently, Tergum I becomes dusky. As the mesoscutum and scutellum darken, the posterior margin of the metasomal terga darken and the venter of the metasoma becomes dusky. Within three days of the onset of eye coloration, the body becomes jet black. However, the legs and antennae remain colorless until shortly before emergence. Adults emerge 5-6 days after the eyes begin to become pink.

Adult Behavior

Female attack of the host was slightly different for G. emigratus in that the female parasite more frequently encountered the posterior end of the host, tried to sting this end, and gradually but continuously worked its way anteriad and attached to the first thoracic segment (dorsum) and attempted to sting the venter near the gula. Stinging the host usually requires 5-10 seconds after which the female invariably leaves the host and begins grooming. In instances where the sting session is short the host is immediately immobilized. When the sting session is longer (ca 10 seconds) host paralysis is more gradual, requiring 3-5 minutes although there is an immediate change in overt behavior of the host. Alternatively, the parasite may struggle with the host for several minutes in a repeated attempt to sting the host in what seems to be the ideal "target". The inappropriateness of the attack seems to be a function of the experimental design and not influenced by the host species. Grooming behavior by the adult parasite immediately after host attack is variable, but lasts up to 10 minutes.

The female parasite prefers to lay eggs in a dorsal orientation. In a sample of 848 eggs (55 clutches), 50.1% were laid dorsally, 35.0% were laid laterally, and 14.9% were laid ventrally. However, the preference for the host's dorsum is strong, irrespective of its orientation. Hosts oriented with dorsum up($n = 28$) laid 73.2% of their eggs dorsally, 26.6% laterally, and 0.2% ventrally. Hosts oriented venter-up ($n = 27$) laid 26.1% of their eggs on the relative dorsal position (on the venter), 43.8% laterally, and 30.1% were laid on the relative ventral position (on the dorsum).

The dorsum is the smoothest part of the NOW body, and covering the dorsal aorta, is where haemolymph flow is probably strongest. Conversely, the legs and prolegs make the venter's surface irregular and may interfere with haemolymph flow near the integument. These differences between a host's dorsum and venter can have a significant effect on the survival of the developing parasite. A sample of 97 clutches (1531 eggs) was selected to examine egg-larval survival based on orientation of eggs on the host's body. Eighty-three clutches (1321 eggs) had no eggs on the host's venter, while 12 clutches (199 eggs) had at least 40% of the eggs on the host's venter. Survival until adult emergence in clutches having eggs on the venter was 75.4% vs. 90.2% for clutches with no eggs on venter. This difference was highly significant ($\chi^2 = 37.0$; $P < 0.001$), and although the probability of survival for individual eggs laid on different portions of the host cannot be determined, these results suggest that differential egg mortality accounts for the female parasite's preference for laying eggs on the host's dorsum. There is, however, a trade-off between the preferred ovipositional site and the host's body orientation. When a host is laying upside down and its dorsum is against the substrate, much of its surface is inaccessible to the ovipositing female, and she will lay on the venter and on the lateral portions of the host. This aversion to laying eggs against the substrate, even when this reduces progeny survival, is most likely explained by increased larval mortality as the developing parasites increase in size and press against a rough or abrasive substrate. This was probably not a significant mortality factor in the smooth vials and petri dishes in the laboratory.

Female parasites are able to adjust clutch size to the size of their host. The number of eggs in a clutch and host weight was highly correlated ($r = 0.674$, $P < 0.001$; $n = 175$). This ability maximizes the reproductive potential of the ovipositing female and prevents clutch failure due to over-ex-

ploitation of the host (superparasitism). The behavioral cue that the female uses to evaluate the host is not known.

Adult Longevity

In the 25.6°C test with uncontrolled RH, mated, host-provided females lived 52.6 ± 7.3 days and mate-deprived males lived 45.2 ± 3.9 days. In the 25.6°C test with ambient RH, host-deprived females lived 37.1 ± 12.4 days and mated males lived 17.2 ± 9.8 days. We are unable to explain these differences in longevity other than to ascribe them to the fluctuating environmental conditions. We assume change in photoperiod has no effect on longevity.

In the 1980 part of our study, mated females provided hosts lived 64.1 ± 19.7 days (excluding three females killed when they became entangled in host larva webbing). The last female died on day 96 of the life-table test.

Life History

The life-table data and statistics are given in Table 1. Generation time of 37.1 days under controlled conditions may be shorter than under field conditions. Net reproductive rate (R_0) of 128.037 seems high, but life tables have not been constructed for bethylids and consequently there is no comparison. Similarly, the intrinsic rate of natural increase, (r_m) value of 0.131 cannot be compared with other bethylids now. Studies on another species of Goniozus are currently in progress to determine the comparative aspect of this portion of study.

Immature stage mortality was low, with 90% of the eggs surviving to adult emergence (n = 2746). The major cause of premature adult mortality was female parasites becoming entangled in host webbing. Once entangled, females invariable died within 24 hours. Half of the females survived until presumed sperm depletion, after which they produced all male clutches.

In the first eight clutches (n = 150) of all females (before sperm depletion begins), the sex ratio was 84.6% female. Graph 1 shows the mean preportion of female G. emigratus produced on NOW for 12 female parasties over 15 sequentially offered hosts. As seen from the graph, the proportion of females declines (with the proportion of males increasing gradually) up to host 15. After 15 hosts have been attacked and clutches deposited, virtually all of the progeny emerging are male. There was no differential mortality during development for G. emigratus in 1978 or 1980. The sex ratio of clutches with no mortality in both years was 84.3% female (n = 75).

Depriving females of hosts between ovipositional sessions apparently stimulates egg production. When hosts were continually present, females parasitized 9.1 ± 4.2 hosts and laid 118.8 ± 58.3 eggs. When females were isolated for 24 hours between ovipositional sessions, females parasitized 15.6 ± 5.6 hosts and laid 232.9 ± 91.5 eggs. Both of these differences are statistically significant ($t = 4.1$, $P < 0.001$; $t = 4.7$, $P < 0.001$, respectively). Mean clutch size per host did not differ between the tests, being 13.3 ± 5.2 eggs per host

in 1978 and 15.6 ± 3.1 eggs per host in 1980 ($t = 1.68$; $0.1 < P < 0.2$).

Discussion

The Bethylidae are potentially a group of importance to biological control because all species are fossorial, primary, external parasites of Lepidoptera and Coleoptera larvae, frequently seeking hosts in concealed situations. However, their effectiveness has not been realized because of the comparative weakness of our taxonomic knowledge and biological information regarding bethylids.

Goniozus is a member of the Bethylinae. In North America four genera of this subfamily are known: Bethylus Latreille, Sierola Cameron, Prosierola Kieffer, and Goniozus. Bethylus is Holarctic in distribution, with two species known from North America. Both species appear predominantly boreal in distribution. Sierola is an exceptionally large genus, but only one species is known from North America; it is probably adventive. Sierola is exceptionally well represented in Hawaii and the Marquesas. There have been no comprehensive studies on the biology of this genus, but apparently its species are parasites of Microlepidoptera. Prosierola is poorly represented in North America, but apparently has speciated extensively in the neotropics. Biological studies of this genus are also absent. Goniozus is cosmopolitan in distribution with more than 35 species known from North America. Biologically this genus has been comparatively well studied.

The host spectrum of the Bethylinae appears restricted to Microlepidoptera, but species are not host specific.

Prior, Evans' revision in 1978 there was question regarding the status of Parasierola and Goniozus. Studies on the biologies of species placed in each of these genera suggested a transition in biological attributes which bridged a gap created by an earlier absence of comprehensive biological information. Currently Parasierola is regarded as a subjective junior synonym of Goniozus.

Rohwer (1917) described G. emigratus from material collected on Oahu as a parasite of the pink bollworm. Pectinophora gossypiella (Saunders). Krombein (1979) reports the distribution of G. emigratus as Hawaii and Texas, with the only known host as PBW. Evans (1978) notes that J. C. Bridwell reported it attacking a "variety of Lepidoptera and even beetle larvae in the laboratory". The parasite readily attacks and develops to maturity on NOW, and other Microlepidoptera in the laboratory.

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Pertinent Publications

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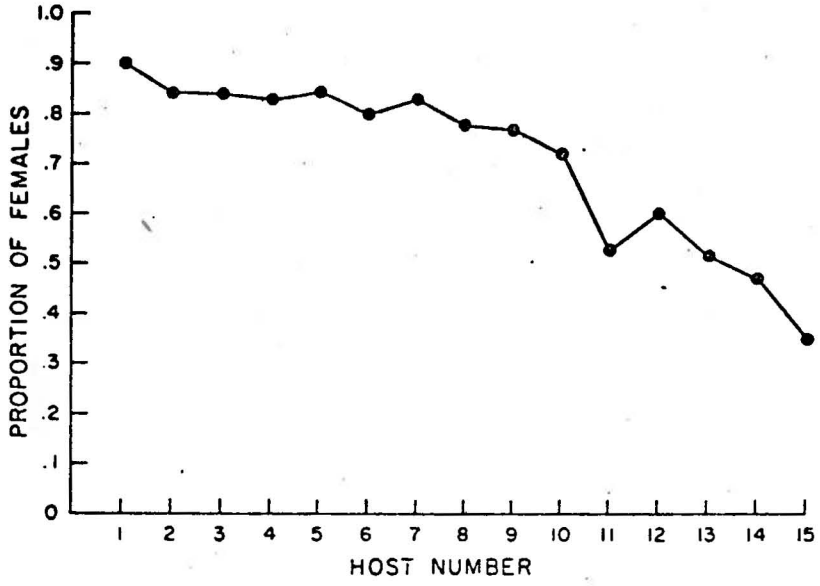
Table 1. *Goniozus emigratus* life table

	Age (x)	Survivorship (l_x)	Fecundity (m_x)
Egg	0	1.000	—
Larva	1–2	.946	—
Prepupa to adult emergence	3–11	.907	—
Pre-oviposition period	12–16	.907	—
	17	.818	1.389
	18	.818	9.122
	19	.818	1.667
	20	.818	1.602
	21	.818	9.451
	22	.818	2.127
	23	.818	1.628
	24	.818	5.639
	25	.818	7.333
	26	.818	0.778
	27	.818	5.561
	28	.818	4.306
	29	.818	4.533
	30	.818	3.256
	31	.818	4.367
	32	.818	3.356
	33	.818	5.194
	34	.818	2.389
	35	.818	4.778
	36	.818	5.433
	37	.818	3.283
	38	.818	2.228
	39	.818	4.467
	40	.818	5.667
	41	.818	1.278
	42	.818	3.506
	43	.818	4.039
	44	.818	3.078
	45	.818	2.389
	46	.818	3.300
	47	.773	2.929
	48	.773	2.541
	49	.773	3.124
	50	.773	3.194
	51	.773	0.600
	52	.773	3.218
	53	.773	1.541
	54	.773	1.194
	55	.773	2.547
	56	.727	1.775
	57	.727	2.250
	58	.727	1.562
	59	.682	2.133
	60	.682	0.667
	61	.682	2.113
	62	.682	2.520
	63	.636	0
	64	.636	2.214
	65	.636	2.286
	66	.591	1.992
	67	.591	0.231
	68	.591	0.769
	69	.591	1.231
	70	.545	0
	71	.545	1.083
	72	.545	0.167
	73	.545	0.250
	74	.545	0.183
	75	.500	1.090
	76	.500	0
	77	.500	0
	78	.455	0.270
	79	.455	0

Generation time = 37.1 days; R_o = 128.037; r_{max} = 0.131.



Graph 1. Proportion of ♀♀ progeny per brood (ordinate) for sequential hosts (abscissa). Based on 12 ♀♀ attacking at least 15 hosts during a lifetime.