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SECOND ANNUAL REPORT

ON

BIOLOGICAL CONTROL OF SCHISTOSOMIASIS TRANSMITTING SNAILS IN SOUTHEAST ASIA

SUBMITTED TO

OFFICE OF THE SCIENCE ADVISOR
PROGRAM IN SCIENCE AND TECHNOLOGY COOPERATION

BY

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THAILAND

GRANT NUMBER 936-5542
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I. BACKGROUND

The award of the project entitled "Biological Control of Schistosomiasis transmitting Snails in Southeast Asia" was presented by Mr. John Gunther Dean, Ambassador of the United States of America to Thailand, to Professor Dr. Natth Bhamarapravati, Rector of Mahidol University, at the Rockefeller Foundation Conference Room, Faculty of Science, Mahidol University, on 5 March 1984. The first payment of the grant was made available to the Faculty of Science in early April 1984. Three reports had been submitted, i.e. the sixth-month report of year one and the annual report of year one, and the sixth month report of year two. This is the annual report of year two covering the period from October 1985 to April 1986.

II. PROJECT OBJECTIVE

The overall objective of this research project is as follows:

1. to study the efficacy of biological control of snails transmitting schistosomiasis in Southeast Asia using biological agents, and

2. to study genetic variability of snails transmitting schistosomiasis in Southeast Asia in relation to degrees of parasite compatibility and susceptibility.

III. EXPERIMENTAL GOALS

1. Laboratory experiments. These studies will include the biological aspects of insects and snail agents and their efficacy against the target snail species. The life

histories of insects and snail agents will be studied and various doses of biological agents and target species will be tested. This phase of study will take approximately 16 months.

2. Simulated field experiments. Biological agents and target species will be tested in simulated field habitats. This phase of study will take approximately 8 months.

3. Genetic and susceptibility studies. Genetic variability of snails transmitting schistosomiasis in relation to degrees of parasite compatibility and susceptibility will be studied under laboratory conditions. This phase of study will take approximately 3 years.

4. Small scale field experiments. Pending the results of "goals" one and two as delineated above, a small scale field trial will be initiated. These experiments will include only local target species. This last stege of study will take approximately one year

RESEARCH FINDINGS

1. Biological control of snails

1.1 Mollusks

1.1.1 Biology of biological agents (mollusks)

The cultivation of the four species of snails employed as biological agents was continued to observe the growth rates and longevity of the snails. The growth tables for the four species of snails, namely, *Melanoides tuberculata*, *Tarebia granifera*, *Brotia costula costula* and *Pila ampullacea* have been completed for the 2 year period.

Among the four species of biological agents, *M. tuberculata* have the shortest life table due to their small sizes. Maturity in *M. tuberculata* is reached within 6-7 months while those in *T. granifera* and *B.c. costula* are 12 months, and 18 months, respectively. All three species of snails have laid their young in the laboratory after the previously mentioned periods of time. For *M tuberculata*, the young were laid after 6-7 months when the snails reached the size of 1.28 cm. in shell length. For *T. granifera*, the young were laid after 12 months when the snails reached the size of 1.89 cm. in shell length. For *B.c. costula*, the young were laid after 18 months when the snails reached the size of 3.82 cm. in shell length. However, the snails reared in the laboratory are considerably smaller than those collected from the field. For *P. ampullacea*, the snails have been reared in the laboratory for 24 months from the eggs laid by field collected snails, and they have reached the size of 4.58 cm. after 18 months and had stopped increasing in shell length. No eggs have been laid in the laboratory-bred snails.

The detail results of the growth tables of the four species of snails are shown in Tables 1-4 and Figures 1-4.

1.1.2 Experimental work on biological control of mollusks in the laboratory

The experimental work on biological control of mollusks in the laboratory has been completed. Since the first annual

report submitted in May 1985, we have completed the rest of the study to test the efficacy of the biological control agents against the target snail species in the laboratory. They were as follows:

Control agent	Target species
Tarebia granifera	*Biomphalaria glabrata*
	Radix rubiginosa
Melanoides tuberculata	*B. glabrata*
	R. rubiginosa
	Bithynia siamensis
	goniomphalos
	Indoplanorbis exustus
Brotia costula costula	*R. rubiginosa*
	Robertsiella kaporensis
Pila ampullacea	*B. glabrata*

The results of the experiment are shown in Tables 5-13 and Figures 5-13.

The biological control studies between *T. granifera*, being the control agent, and *B. glabrata* and *R. rubiginosa* being the target species have shown that *T. granifera* could not control *B. glabrata* (Table 5 and Fig. 5) but did have some effects on the population of another pulmonate, *R. rubiginosa* (Table 6 and Fig. 6). *B. glabrata* have been reared in the laboratory for number of years and they are

certainly well adapted in terms of mortality and numbers of offspring. It usually takes *B. glabrata* 3-4 months to become fully mature, while it takes *T. granifera* 12 months to reach maturity. For *R. rubiginosa*, when put into container, they usually started laying large numbers of eggs, and then the adult snails died. The eggs would hatch into young snails until they reached a certain size, then they started to die off. However, *R. rubiginosa* living together with *T. granifera* did not leave any offspring. It could be that *T. granifera* have exerted some control over *R. rubiginosa*, especially on the reproductive ability of the target snails.

The biological control studies between *M. tuberculata*, being the control agent, and *B. glabrata* (Table 7 and Table 7), *R. rubiginosa* (Table 8 and Fig. 8), *B.s. goniomphalos* (Table 9 and Fig. 9), *I. exustus* (Table 10 and Fig. 10), being the target species could be concluded that *M. tuberculata* could not control the planorbid snails, *B. glabrata* and *I. exustus*. They apparently had some control over *R. rubiginosa*. For *B.s. goniomphalos*, even though the target snails seemed to be decreased in numbers, but the control container of the target snails also behaved in similar fashion. Hence, *M. tuberculata* and *B.s. goniomphalos* seemed to have no reaction with one another.

The biological control studies between *B.c. costula*, being the control agent, and *R. rubiginosa* (Table 11 and Fig. 11) and *R. kaporensis* (Table 12 and Fig. 12), being the target species had revealed that *R. rubiginosa* living

together with the control agent, *B.c. costula* or alone behaved in similar fashion, i.e. the adult snails would start to lay large number of eggs and then died off. The offspring of *B. rubiginosa* would grow to a certain size, then started to die off after 6 weeks. At the end of 16 weeks, there were only a few number of *B. rubiginosa* remained. *B.c. costula* and *B. kaporensis* did not seem to interact with one another.

The biological control study between *P. ampullacea*, being the control agent, and *B. glabrata* being the target species appeared very promising (Table 13 and Fig. 13). Even though *B. glabrata* did not die off completely, but they appeared to be under control of *P. ampullacea*. The effect of *P. ampullacea* on *B. glabrata* could be as an accidental predator on the egg masses of *B. glabrata*. Further study especially using the simulated field conditions has been investigated.

1.1.3 Experimental work on biological control of mollusks under simulated field conditions

For simulated field conditions, the testing was performed outside the laboratory, at a small field station. Habitats simulating standing waters were constructed in such a way that the experimental agents and target species were not able to escape.

The testings were done in cement tanks with the diameter of 80 cm. and the height of 35 cm. The tanks were aerated and furnished with water plants and rocks in order to simulate the snails' habitats. The ratio between biological agent and target species was as follows:

Control agent	Target species
0	100
50	50
100	0

Control agents were *T. granifera* and *M. tuberculata*.

For *B.c. costula* and *P. ampullacea* being control agents, the following ratio has been used.

Control agent	Target species
0	50
25	25
50	0

The target species were:

B. glabrata

I. exustus

R. rubiginosa

T. aperta

O.h. quadrasi

B.s. goniomphalos

The general procedure, observation on mortality rates of both control and target species was done weekly while the counting of the snail offspring was done biweekly. The original snails were marked with finger nail polish so that the offspring could be distinguished from them.

The experiments have been completed for the total period of 8 months. The results of the experiments are shown in Tables 14-37 and Figures 14-37.

Brotia costula costula

Effect on *Biomphalaria glabrata*

The result of the biological control study between *B.c. costula* (control) and *B. glabrata* (target) is shown in Table 14 and Fig. 14. The experimental tank contained equal numbers of *B.c. costula* and *B. glabrata* (25:25). During the first 2-4 months, the target snails started to multiply to a large number, while the control snails did not produce any offspring. After 5 weeks, the number of target species, *B. glabrata* had reduced due to overcrowding, and remained relatively constant until the end of 8 month period. It was further observed, however, that the control agent, *B.c. costula* started to multiply after 10 months, producing a large number of offspring. Hence, it appeared that reproduction of *B.c. costula* could be seasonal under the simulated field condition. No competition interactions were indicated between *B.c. costula* and *B. glabrata*. Both control and target snails maintained separately were mostly alive

with an increase in their numbers. It can be concluded that *B. c. costula* was not an effective biological control agent for *B. glabrata*.

Effect on *Indoplanorbis exustus*

The result of the biological control study between *B.c. costula* (control) and *I. exustus* (target) is shown in Table 15 and Fig. 15. The experimental tank contained 25 *B.c. costula* and 25 *I. exustus*. Two weeks after the initiation of culture, *B.c. costula* started to multiply to a very large number due to their high reproductive rate. After 2 months, the target species, *I. exustus* had grown up to sexual maturity and begun to breed. A month later, adult snails of both species started to die until there were no adult snails left in the experimental tank. There was no sign of competition observed between the control agent and the target species. However, the number of the control offspring had always been greater than that of the target species. By the end of 8 months, the number of *B.c. costula* offspring was almost five times the number of *I. exustus* offspring. Both control and target snails maintained separately were mostly alive with an increase in their numbers. The result has indicated that the control species, *B.c. costula* had more reproductive potential and relatively lower mortality rate than the target species, *I. exustus*

Effect on *Radix rubiginosa*

The result of the biological control study between *B.c. costula* (control) and *R. rubiginosa* (target) is shown in Table 16 and Fig. 16. The experimental tank contained 25 *B.c. costula* and 25 *R. rubiginosa*. Three to four weeks after the initiation of culture, *B.c. costula* began to multiply. Due to overcrowding, the young *B.c. costula* was reduced in number until becoming relatively stable by the end of 5 months. It took *R. rubiginosa* in the experimental tank 2 months to multiply, but the number of offspring produced was not as large as that of *B.c. costula*. At the end of the 7th month, the young snails of *R. rubiginosa* started to die off while those of *B.c. costula* remained relatively stable. By the end of 8 month period, the number of *B.c. costula* snails was almost tenfold the number of *R. rubiginosa*. The control snails, *B.c. costula* maintained alone were mostly alive with an increase in their number. Adult snails of the target species, *R. rubiginosa* mostly died but left a number of young snails behind. The experiment has indicated the superior reproductive ability of *B.c. costula* over *R. rubiginosa*. In addition, *B.c. costula* young snails seemed to have better survival rate than those of *R. rubiginosa* due to their hard shells and opercula and their ability to withstand adversed environmental condition better than *R. rubiginosa*. Owing to the differences in their habitat preferences, i.e. *B.c. costula* usually stayed under water in the sand while *R. rubiginosa* were mostly at the water

surface, there seemed to be no interaction between these two species of snails.

Effect on *Tricula aperta*

The resul. of the biological control study between *B.c. costula* (control) and *T. aperta* (target) is shown in Table 17 and Fig. 17. *B.c. costula* maintained together with *T. aperta* did not produce any young snails. This is due to the fact that when the experimental culture was initiated, *B.c. costula* were not in the breeding season. *T. aperta* in the experimental tank and in the control tank behaved in similar fashion, i.e. they had very high mortality and no reproduction occurred.

In nature, *B.c. costula* and *T. aperta* are found in different habitats. *B.c. costula* are found in mostly slow-running water embedded under the sand. *T. aperta* were found in the river with strong current attached themselves to the underside of the rocks and the water is quite cool most of the year. *B.c. costula* could survive better under the simulated field condition than *T. aperta*. However, the two species of snails did not seem to interact with one another as observed during the whole experimental period.

Effect on *Oncomelania hupensis quadrasi*

The result of the biological control study between *B.c. costula* (control) and *O.h. quadrasi* (target) is shown in Table 18 and Fig. 18. The data presented is much similar to that of *B.c. costula* and *T. aperta*. *B.c. costula* and *O.h.*

quadrasi live in different habitats. *B.c. costula* are aquatic and *O.h. quadrasi* are amphibious in nature. While *B.c. costula* survived and reproduced very well in the cement tank, *O. h. quadrasi* could not and they all died after 4 months. Despite the superior reproductive and survival rates of *B.c. costula* over *O.h. quadrasi*, they did not seem to interact with one another during the experimental period.

Effect on *Bithynia siamensis goniomphalos*

The result of the biological control study between *B.c. costula* (control) and *B.s. goniomphalos* (target) is shown in Table 19 and Fig. 19. Similar to the experimental work on *B.c. costula* and *T. aperta*, the experimental culture of *B.c. costula* and *B.s. goniomphalos* was initiated when *B.c. costula* were not in the breeding season. Neither *B.c. costula* nor *B.s. goniomphalos* reproduced but the former seemed to have a better survival rate than the latter. It is worth noted that if *B.c. costula* culture was initiated during their breeding season, the number of *B.c. costula* would be far greater than that of *B.s. goniomphalos* due to their ovoviviparity and better survival rate.

It can be concluded that *B.c. costula* appear to have some possibility as an effective competitor in certain type of habitat. The major factor favoring *B.c. costula* as bio control agent appear to be superior reproduction and relatively low mortality rate. They seem to be able to control the population of pulmonate snails, *I. exustus* and *R.*

rubiginosa. However, this is not the case for *B. glabrata*. There was no interference observed between *B.c. costula* and *T. aperta* and *O.b. quadrasi* due to the different preferences in habitats.

Melanoides tuberculata

Effect on *Biomphalaria glabrata*

The result of the biological control study between *M. tuberculata* (control) and *B. glabrata* (target) is shown in Table 20 and Fig. 20. In the experimental tank containing 50 *M. tuberculata* snails and 50 *B. glabrata* snails after one month, both species of snails began to multiply. The quantity of young snails of both species fluctuated, but they appeared to have similar reproduction and mortality rates. At the end of 8 months, the numbers of both control and target snails were almost the same. Both control and target snails maintained separately were mostly alive with an increase in their numbers. It can be concluded that *M. tuberculata* might not be a suitable bio control agent for *B. glabrata*.

Effect on *Indoplanorbis exustus*

The result of the biological control study between *M. tuberculata* (control) and *I. exustus* (target) is shown in Table 21 and Fig. 21. In the experimental tank containing both control and target species, *M. tuberculata* started to multiply much later than *I. exustus*. Six weeks after the initiation of culture, *I. exustus* already laid egg masses and

produced offspring, while it took *M. tuberculata* almost 4 months to start producing offspring. In addition, the number of offspring produced by *M. tuberculata* was less than that by *I. exustus*. Both control and target snails maintained separately were mostly alive with an increase in their numbers. It can be concluded that *M. tuberculata* might not be a suitable bio control agent against *I. exustus* due to their lower reproduction and high mortality rates than the target species.

Effect on *Radix rubiginosa*

The result of the biological control study between *M. tuberculata* (control) and *R. rubiginosa* (target) is shown in Table 22 and Fig. 22. After 6 weeks, *M. tuberculata* in the same experimental tank as *R. rubiginosa* started to multiply and the number of young snails remained relatively constant until the end of 8 month period. On the contrary, *R. rubiginosa* produced very few egg masses and they started to die off. At the end of the 8 month period, the number of *R. rubiginosa* was drastically reduced. The control snails maintained alone were mostly alive with an increase in their number. The adult target snails maintained alone all died but left a number of young snails behind. It is apparent that *R. rubiginosa* had lower growth and egg laying rates when in competition with *M. tuberculata* than when maintained alone. Even though no interference was observed between the control and target species due to the different preferences

of habitats, *M. tuberculata* still showed greater adaptability, hardiness and rapid multiplication.

Effect on *Tricula aperta* and *Oncomelania hupensis quadrasi*

The results of the biological control study between *M. tuberculata* (control) and *T. aperta* (target) is shown in Table 23 and Fig. 23 ; and between *M. tuberculata* (control) and *O.h. quadrasi* (target) is shown in Table 24 and Fig. 24. After 8 months, the results were as follows : (1) The target snails, *T. aperta* and *O.h. quadrasi* started to die off after one month. By the end of the 8th month, there were no *T. aperta* snails left while there were only a few *O.h. quadrasi* snails present. (2) The target snails did not reproduce (3) The control snails, *M. tuberculata* both maintained alone or together with target species were present in large numbers.

It was observed during the two experiments that no competition interaction between the control and the two target species was indicated due to the difference in their habitats. The target snails maintained alone behaved in similar fashion as those maintained with the control snails.

Effect on *Bithynia siamensis goniomphalos*

The result of the biological control study between *M. tuberculata* (control) and *B.s. goniomphalos* (target) is shown in Table 25 and Fig. 25. In the experimental tank which contained 50 *M. tuberculata* snails and 50 *B.s. goniomphalos*

snails, six weeks after the initiation of culture, *M. tuberculata* started to multiply in relatively large number while *B.s. goniomphalos* was decreased drastically. At the end of 4 months, no *B.s. goniomphalos* snails present in the experimental tank. There was a complete displacement of *B.s. goniomphalos*, target, by the control snails, *M. tuberculata*. The control snails maintained alone were mostly alive with an increase in their number. The target species maintained alone decreased drastically in number until there were only a few target snails present at the end of the 8 month period. No concrete conclusion could be drawn from this experiment since both *B.s. goniomphalos* maintained alone and with *M. tuberculata* behaved in rather similar fashion. It should be noted, however, that the control agent, *M. tuberculata* are generally very hardy and have a much higher reproductive potential than the target species, *B.s. goniomphalos*.

It can be concluded that *M. tuberculata* appear to have some possibility as an effective competition in certain type of habitat. It is not an effective bio-control agent for *B. glabrata* and *I. exustus*, but they seemed to have some interference with the other pulmonate, *R. rubiginosa* in terms of superior reproduction and relatively low mortality rate. There seemed to be no interference between *M. tuberculata* and *T. aperta* or *O.h. quadrasi* due to the differences in habitats. For *B.s. goniomphalos*, the conclusion could not be drawn but *M. tuberculata* certainly had superior reproductive potential and lower mortality rate than *B.s. goniomphalos*.

Pila ampullacea

Effects on pulmonate snails (*Biomphalaria glabrata*, *Indoplanorbis exustus* and *Radix rubiginosa*).

The results of the biological control studies between *P. ampullacea* (control) and *B. glabrata*, *I. exustus* and *R. rubiginosa* (targets) are shown in Tables 26-28 and Figures 26-28, respectively. The competition between *P. ampullacea* and the three target species of snails has been demonstrated experimentally under the simulated field condition. The three target species, *B.glabrata* (Table 26, Fig. 26), *I. exustus* (Table 27, Fig. 27) and *R. rubiginosa* (Table 28, Fig. 28) behaved in similar fashion when maintained with *P. ampullacea*, the control agent. During the first month, the target species started to lay egg masses. A few weeks later, the first sign of competition had appeared : clutches of eggs of all three target species failed to hatch, some disappeared, and the young of these snails began to die. After two months, it was noticed that all the younger snails had completely disappeared from the experimental tanks, and only a few of the original snails were still alive. In the case of *R. rubiginosa*, all of the adult snails died. By the end of the 8 month period, the numbers of the three target species were drastically reduced to the insignificant numbers for competition. On the contrary, the target species maintained alone were mostly alive with an increase in their numbers.

Based on the results obtained from these experiments,

there is good reason to believe that *P. ampullacea* compete for food and cause interference with the hatching of egg masses, juvenile and adult target snails. They certainly devour egg masses of the target species causing the disappearance of the egg masses present. In addition, due to their large sizes, they may excrete certain waste products which might be lethal to the adult target snails.

Effects on prosobranch snails (*Tricula aperta, Oncomelania hupensis quadrasi* and *Bithynia siamensis goniomphalos*).

The results of the biological control studies between *P. ampullacea* (control) and *T. aperta, O.h. quadrasi* and *B.s. goniomphalos* are shown in Tables 29-31 and Figures 29-31, respectively. In the experimental tanks containing both control and target snails together, all three target snail species had very high mortality, and did not reproduce. *T. aperta* were very sensitive to water and temperature condition and could not tolerate the simulated field condition. Hence, *T. aperta* maintained with *P. ampullacea* or alone both had high mortality rate and all died after 2-4 months (Table 29, Fig. 29). *P. ampullacea* did not seem to have any interaction with *O.h. quadrasi* (Table 30, Fig. 30). *P. ampullacea* could be found in the same natural habitat as *B.s. goniomphalos*, but the result here indicated no interaction since *B.s. goniomphalos* maintained alone did not reproduce and had a very high mortality rate (Table 31, Fig. 31).

The conclusions from the experiments on the competition between *P. ampullacea* and the pulmonate target snail species

of *B. glabrata*, *I. exustus* and *B. rubiginosa* are that the competition results in a reduced growth and reproduction of the target species, and these effects are based on competition for food, physical interference between snails or predation on egg masses of the target species. On the contrary, *P. ampullacea* did not have any interaction with either *T. aperta* or *O.h. quadrasi*, the prosobranch snails. As for the other prosobranch snail, *B.s. goniomphalos*, no conclusion could be drawn. Further work on field trial experiment under natural habitat should yield more conclusive results.

Tarebia granifera

Effect on *Biomphalaria glabrata*

The result of the biological control study between *T. granifera* (control) and *B. glabrata* (target) is shown in Table 32 and Fig. 32. After 2 months, the control and target snails maintained together started to multiply producing a large number of offspring. However, the number of young *T. granifera* was much lower than that of *B. glabrata*, all through the experimental period. The result obtained here was similar to the result of the experiment done under the laboratory condition. So, it is apparent that under either laboratory or simulated field conditions, *T. granifera* could not control the population of *B. glabrata*. *B. glabrata* had been long established and well adapted to the laboratory and simulated field conditions much better than *T. granifera*.

Both control and target snails maintained separately were mostly alive with an increase in their numbers.

Effect on *Indoplanorbis exustus*

The result of the biological control study between *T. granifera* (control) and *I. exustus* (target) is shown in Table 33 and Fig. 33. Six weeks after the initiation of culture, both control and target snail species maintained together or alone started to multiply. On the sixth week, the number of young produced by *I. exustus* exceeded that produced by *T. granifera*. During the next 2-5 months, there were more *T. granifera* snails than *I. exustus* snails. After that, the numbers of the two species became slightly different, with more *T. granifera* than *I. exustus*. At the end of the 8th month, there were more *T. granifera* than *I. exustus*. Both control and target snails maintained separately were mostly alive with an increase in their numbers. The result has indicated that the control snails, *T. granifera* seemed to have more reproductive potential and relatively lower mortality rate than the target species, *I. exustus*.

Effect on *Radix rubiginosa*

The result of the biological control study between *T. granifera* (control) and *R. rubiginosa* (target) is shown in Table 34 and Fig. 34. During the first two months, the target snails, *R. rubiginosa* maintained together with *T. granifera* started to multiply. A few weeks later, all the adult snails of *R. rubiginosa* died, only young snails were

present but the number was decreasing gradually. After 4-5 months, there was no *R. rubiginosa* present in the experimental tank. On the contrary, *T. granifera* maintained together with *R. rubiginosa* started to multiply after 2 months, and they still maintained the relatively constant number of young during the whole experiment. The control species maintained alone were mostly alive with an increase in their number. All adult target snails maintained alone died, only young snails were present. The result indicated the superior reproductive potential and lower mortality rate of *T. granifera* over the target snails, *R. rubiginosa*.

Effects on prosobranch snails (*Tricula aperta*, *Oncomelania hupensis quadrasi*, and *Bithynia siamensis goniomphalos*).

The results of the biological control studies between *T. granifera* (control) and the target species ; *T. aperta*, *O.h. quadrasi* and *B.s. goniomphalos* are shown in Tables 35 37 and Figures 35 37, respectively. All the prosobranch target species behaved in similar fashion. The target snails maintained together with control snails had very high mortality rate and no reproduction occurred. Since the target snail species maintained alone behaved in the same way, it might be concluded that no interaction exist between the control and the three species of target snails, *T. aperta*, *O.h. quadrasi* and *B.s. goniomphalos*. For *T. aperta* and *O.h. quadrasi*, this could be explained on the basis of different habitat preferences.

It can be concluded that *T. granifera* appear to have some possibility as an effective competitor for *L. exustus* and *R. rubiginosa*, the pulmonate snails, i.e. superior reproductive potential and low mortality rate. This is not the case with the other pulmonate, *B. glabrata*. There seemed to be no interference between *T. granifera* and *T. aperta*, *O.h. quadrasi* or *B.s. goniomphalos*.

1.2 Insects

Sciomyzid fly larvae were used as biological control agent against pulmonate and operculate snails in the ratios of 4:0, 3:1, 2:2, 1:3, and 0:4, under simulated field conditions (conditions are same as mollusks), as follows:

Ratios of sciomyzid fly larvae to snails

4:0 3:1 2:2 1:3 0:4

Numbers of sciomyzid fly larvae to snails

400:0 300:100 200:200 100:300 0:400

Lampyrid fly larvae were used as biological control against pulmonate and operculate snails in the ratios of 4:0, 3:1, 2:2, 1:3, and 0:4, under laboratory conditions, as follows:

Ratios of lampyrid fly larvae to snails

4:0 3:1 2:2 1:3 0:4

Numbers of lampyrid fly larvae to snails

40:0 30:10 20:20 10:30 0:40

The results of biological control testings between sciomyzid fly larvae (control agent) and pulmonate and operculate snails (target species) are shown in Tables 38-41.

Tables 38 and 39 show, respectively, the effects of the second and third stage larvae of sciomyzid fly *Sepedon plumbella* on pulmonate snails. The sciomyzid fly larvae caused higher mortality rates in juvenile than in adult snails. The best result of snail control by sciomyuzid fly larvae was obtained at the ratio of 1:3 (snails : sciomyzid fly larvae), i.e. from 88% to 100% for juvenile snails and from 70% to 98% for adult snails.

Tables 40 and 41 show, respectively, the effects of the second and third stage larvae of sciomyzid fly *Sepedon plumbella* on operculate snails. The sciomyzid fly larvae did not have much effect on operculate snails, possibly owing to the fact that the operculate snails possess opercula which can be used to prevent any attack from the fly larvae. In addition, the fly larva can be killed when the snail closes its operculum.

The results of biological control testings between lampyrid fly larvae (control agent) and pulmonate and operculate snails (target species) are shown, respectively, in Tables 42 and 43. The lampyrid fly larvae were more effective in killing snails than the sciomyzid fly larvae. The size of the lampyrid fly larvae was larger, and the larvae were seldom killed while attacking any operculate snails. The lampyrid fly larvae caused high mortality rates at all ratios of pulmonate snails, but were less successful with operculate snails.

2. Genetics and susceptibility studies

2.1 Susceptibility aspect

Susceptibility of *Oncomelania hupensis quadrasi* from different geographic locations to infection with geographic strains of *Schistosoma japonicum.*

Four groups of *O.h. quadrasi* snails from different locations in the Philippines were used, i.e. from Palo, Leyte Island; from Mindoro Island; from Irosin, Luzon Island and from Mindanao island. The snails were collected from the respective areas in the Philippines and were examined for natural schistosome infections prior to use by shedding. The negative snails were isolated and examined for cercarial shedding for another 2 months before use as breeders in the laboratory.

Snail infections were done by exposing individual laboratory bred snails to 4-6 miracidia of *S. japonicum* of different geographic strains. Natural snail hosts were infected at the same time to serve as controls.

Three geographic strains of the parasites were available for use; the Mindoro, Leyte and Mindanao strains. The parasites were maintained in the laboratory by passage through white mice and the natural snail hosts. The mice were usually exposed to infection with 40 cercariae and were killed 6-7 weeks post infection.

The total numbers of snails for each group exposed to infection was variable because of the supply limitation.

Snails from Mindanao Island were limited due to the difficulty of obtaining snails. *S. japonicum* strain from Irosin was lacking due to the scarcity of infected snails in the area. All surviving snails were examined for cercariae 8 weeks after exposure to miracidia by shedding.

Part of the result is shown in Table 44. It can be concluded that strain of *S. japonicum* from Mindoro was able to develop in all geographical strain of snail host, *O.h. quadrasi*. It developed best in *O.h. quadrasi* from Irosin (28.97%), then Mindoro (20%), Mindanao (13.75%) and poorest in Leyte snails (3.5%). The strain of *S. japonicum* from Leyte was able to develop in the snails from Irosin (60.49%), Leyte (12.1%) and Mindoro (7.14%). The strain of *S. japonicum* from Mindanao developed best in its own snails, *O.h. quadrasi* from Mindanao (51.43%). It developed poorly in *O.h. quadrasi* from Irosins (3.06%) and from Leyte (0.90%).

2.2 Genetic aspect

Within the context of the "Biological Control of Schistosomiasis transmitting Snails in Southeast Asia" project, the primary concern of the U.C.S.D. group is to study genetic variability of selected snail populations and species in relation to degrees of parasite compatibility and susceptibility. To achieve this objective, we are studying the genetic characterization of the various species of snails and their parasites.

2.2.1 Genetic variation in *Oncomelania hupensis* in the Philippines and China

Our initial goal was to undertake a pilot study of genetic variation in the medically important snail *Oncomelania hupensis* from the island of Leyte, Philippines. Once the technical aspects of allozyme identification were resolved, we began a geographic survey of variation in this species in other Philippine Islands. The survey is to be extended to other parts of Southeast Asia later in the project. One of the goals of the study is to try to resolve the issue of whether *O. hupensis* is a single widespread species with six recognized subspecies or really a species complex into which several sibling species are presently lumped.

Oncomelania hupensis quadrasi was characterized on the basis of 7 field-collected samples representing four Philippine islands and one laboratory stock of mixed origin (Table 45). *O.h. hupensis* was characterized on the basis of a stock derived from three large samples from Guichi on the Yangtze River about 400 km west of Shanghai. The field-collected snails were maintained in our laboratory at the Center for applied Malacology and Entomology, Faculty of Science, Mahidol University until they were frozen on dry ice, carried to San Diego, and held at -70 ^{o}C until analysis.

Our electrophoretic techniques are described in general terms elsewhere (Woodruff, 1975; Mulvey and

Vrijenhoek, 1981). Individual snails were thawed and quickly homogenized whole in 0.1 ml of grinding solution (0.01 M Tris, 0.001 M EDTA, 0.05 mM NADP, pH 7.0) with a glass rod. The homogenate was centrifuged at 10,000 g for 2 minutes, and the supernatant was absorbed onto Whatman no. 3 chromatography paper wicks (3x9 mm) and inserted into 12% horizontal gels made of Sigma starch (Sigma Chemical Co., St. Louis, Missouri). Electrophoresis was carried out for 15 hrs. by which time a bromophenol blue marker dye had migrated 100-120 mm anodally. Four to five slices were then cut from each gel, and each slice was stained for a specific enzyme following standard methods (Shaw and Prasad, 1970; Harris and Hopkinson, 1977). Electrophoretic conditions for the resolution of the 21 allozymes reported here are described in Table 46. The esterase substrate was alpha naphthyl acetate, and the peptidase substrate was leucyl alanine. Snail from different samples were run on each gel to facilitate comparisons: isozymes were numbered, and allozymes were assigned superscript letters a,b....., in order of decreasing anodal mobility of the primary electromorph. Commonly used enzyme abbreviations are typeset in capital letters to indicate the protein and in italics to indicate the presumed allele.

The mean number of alleles per locus, the proportion of loci polymorphic, P (a locus was considered polymorphic if more than one allele was detected), and the mean individual heterozygosity, $\overline{H}$ (by direct count) were

calculated for each sample. Allozyme frequencies for the polymorphic loci were tested for their agreement with Hardy Weinberg expectations for a panmictic population by X^2 test where possible and by the Fisher exact test. Population genetic structuring was studied with Wright's hierarchical F statistics (F_{IS}, F_{ST} and F_{IT}) calculated for each locus and sample. Genetic distance coefficients (*D*) of Nei (1978) and the genetic similarity coefficient (*S*) of Rogers (1972) were calculated and clustered by the UPGMA algorithm. The above analyses were performed with the BIOSYS 1 computer program (Swofford and Selander, 1981).

Consistent and genetically interpretable results were obtained for 20-21 loci per sample. Allele frequencies are shown for all polymorphic loci in Table 47.

In the snails from the Philippines, the mean number of alleles per locus ($\bar{A}$) was 1.2-1.4; Chinese snails were more variable, $\bar{A}$ = 2.0-2.4. The proportion of loci polymorphic (*P*) was 0.14-0.29 in the field collected samples from the Philippines and slightly higher (*P* = 0.38) in the mixed origin laboratory stock. In contrast, the Chinese snails were three times as polymorphic (*P* = 0.52-0.67) and varied at 7 loci which were fixed or almost fixed in the Philippines. Mean individual heterozygosity also differed significantly between the Philippines and China ($\bar{H}$ = 0.02-0.10 vs 0.20-0.21).

No significant departure from panmixia was detected in the samples studied. Genotype frequencies were in close agreement with Hardy Weinberg expectations in 35 of 38 tests (polymorphic loci/sample) involving Philippine snails. No special significance is attached to the three cases involving slight heterozygote deficiencies (*Pep 4*) in Luzon A, *Gpi* in Luzon B and *Lap* in Lub stock) as all the remaining variable loci (3-7) in each of these samples were in equilibrium. Similarly, in the case of the more variable Chinese samples, no significant departure from Hardy Weinberg expectations was detected in 24 of 25 tests. (The remaining case, *Sordh* in China A, also involved a deficiency of heterozygotes; Fisher exact tests P = 0.048, fixation index 0.43). With only these four minor exceptions among 63 tests, we conclude that in nature these snails are outbreeding at random. This conclusion is supported by the finding that Wright's multilocus F_{IS} statistic was 0.092, a value not significantly different from zero.

Study of Table 47 shows that the five samples from Leyte and Luzon are practically indistinguishable at the 20 loci examined. Mindoro snails differ from those on Leyte-Luzon in having different allele frequencies at 6 of 19 loci; the greatest differences involving *Lap* and *Pgm-2*. Snails from Mindanao were characterized by slower *Acp* and *Idh-1* alleles but were otherwise similar to the other Philippine snails. In contrast, the Chinese snails had unique alleles at 13 of the 21 loci examined. Philippine and Chinese snails

can be distinguished from one another on the basis of variation at *Aat*, *Es-1*, *Es-3*, *Gpi* and *Pgm-1*. The major differences between Chinese snails and the others is underscored by the calculated value of Wright's F_{ST} (0.64), a value significantly different from zero.

We estimated overall multilocus genetic differentiation between these samples using Nei's (1978) unbiased coefficient of genetic distance (Table 48; Roger's *S* values are shown for comparative purposes). Within the Philippines intersample *D* values were in the range 0.001 0.118 and *D* 0.032. *D* between the two Chinese samples was a very similar 0.035. In marked contrast, the China-Philippines comparison yielded *D* 0.628.

The first goal of the present study was to establish whether *Oncomelania* were variable enough to permit the preparation of a molecular or genetic phylogeny. The samples studied were found to be well suited to this type of analysis based on allozyme variation. *O. hupensis* and *O. quadrasi* differ in their levels of variability (P 0.52-0.67 in the former, P 0.14 0.29 in the latter, $\bar{H}$ 0.20 0.21 vs 0.02-0.10) but neither taxon is unusual, approximate mean levels of variation in amphimictic molluscs are $\bar{P}$ = 0.47 (range 0 1.00) and $\bar{H}$ = 0.14 (range 0 0.30) (Selander and Ochman, 1983; Nevo *et al.*, 1984). Our preliminary study has revealed up to 14 polymorphic loci per sample--well in excess of the number needed to develop robust molecular phylogenies.

In a subsequent report, we will characterized the various allelic variants quantitatively so as to facilitate comparative studies in other laboratories.

Our second goal was to explore the extent of genetic differentiation within *Oncomelania quadrasi* in the Philippines. Not surprisingly we found a strong relationship between the degree of physical isolation and the genetic distance between any two samples. Physical isolation in the case of *Oncomelania* is related to both geographic distance and saltwater barriers. Thus, the three samples collected within a few km of one another on Leyte are genetically indistinguishable. The Luzon samples, collected 140 km and three islands to the north, are very weakly differentiated from those on Leyte; map distance belies the fact that the snails of Luzon and the almost continuously distributed populations of Leyte-Samar are only separated by the shallow (135 m), narrow (<10 km) San Bernardino Strait. Mindoro snails are more differentiated from those of Luzon Leyte having higher frequencies of certain alleles at *Lap* and *Pgm 2*. The Mindoro sample was collected 250 km from Leyte and 160 km from southern Luzon, and although Mindoro is only 20 km from central Luzon the two islands are separated by a deep and permanent saltwater strait. Mindanao is the most distinctive of the samples (with a unique *Acp*); the sample was taken 300 km south of Leyte. Today, Leyte and Mindanao are separated by a 30 km wide strait with water 80 m deep. Additional samples of snails from throughout the archipelago

are to fully document the apparent pattern of isolation related differentiation. In addition, future studies will need to relate the alleles found in various Philippine populations to those occurring in and possibly introduced by migratory birds from China, Taiwan and Japan. We, ourselves, plan to test the hypothesis that the Sulawesi *Oncomelania* are derived from Mindanaoan stock.

Our final goal was to initiate a study of host-parasite coevolution between the snails of the *Oncomelania hupensis* superspecies and the trematodes referred to as *Schistosoma japonicum*. As *O. hupensis* is the sole or obligate intermediate host of *S. japonicum* the schistosome could not have established itself in an area until after the snails were introduced. If parasite and host evolve at similar rates, we would predict that the genetic distance between source (China) and derived (Philippines) populations would be less for the parasite than for the host. This is, in fact, the case: *D* 0.45 between *S. japonicum* from China and Leyte (based on 11 loci; Fletcher *et al.*, 1980; Woodruff *et al.*, in press) and *D* 0.63 for *O. hupensis* *O. quadrasi*. The relatively large *D* value between Chinese and Philippine strains of the parasite suggests that the schistosomes were introduced to the archipelago in bird-borne infected snails long before man arrived. This would explain why *S. japonicum* has an unusually wide range of mammalian hosts (Mao and Shao, 1982) compared with most other schistosomes. Clearly, the genetic and evolutionary interactions of *Oncomelania* and

Schistosoma merit closer attention both at the level of the genetic regulation of compatibility (Yuan, 1958; Davis and Ruff, 1973; Chiu *et al.*, 1981) and at the population genetic level (Rollinson and Southgate, 1985; Woodruff, 1985). The present preliminary study indicates that some more taxonomic surprises may be forthcoming.

2.2.2 Genetic varintion in the Triculini

We are now applying the insights gained in our successful pilot study of *Oncomelania* to the smaller snails of the genus *Tricula*. Despite obvious limitations related to their size, we have already resolved isozyme patterns for more than 12 genes. Preliminary results indicate that *Tricula* and *Oncomelania* have no common alleles. Such a genetic finding would be in agreement with present hypotheses regarding the great antiquity of the divergence of these two subfamilies.

Samples representative of the following taxa are currently being examined: *Tricula aperta* (alpha, beta and gamma races), *T. bollingi*, Malaysian *Robertsiella kaporensis*, and unknown triculines from Pitsanulok and Kanchanaburi, Thailand.

2.2.3 Genetic variation in Asian *Schistosoma*

Four strains of *S. japonicum* from the Philippine islands of Leyte, Luzon and Mindoro were examined. All were cycled through *Oncomelania hupensis quadrasi* snails and Swiss albino outbred mice. LEYTE LAB was founded in 1980

with 20-30 infected snails collected from several sites near Palo, Leyte, and supplemented with schistosomes from about 20 additional infected snails collected in the same area in 1982. LEYTE FIELD was founded in 1984 with about 10 infected snails and passed through mice once. Again, several sites close to Palo were represented among the founders. MINDORO was founded in 1984 with about 50 infected snails collected in a churchyard in Victoria, Mindoro, and passaged through mice once. LUZON was founded in 1984 with schistosomes from 3-4 infected snails collected at Irosin, near Sorsogon, in southern Luzon island and passed through mice once.

One strain of *S. mekongi* was examined: MEKONGI. Originally collected in *Tricula aperta* snails at Khong Island, Laos, in 1971 and maintained initially at the Department of Tropical Medicine, Faculty of Tropical Medicine, Mahidol University. This strain has been cycled in Dr. Upatham's laboratory since 1979.

One strain of the Malaysian schistosome was examined: MALAYSIA. Founded in 1982 with about 10 infected *Robertsiella kaporensis* snails collected near Kuala Koyan, Pahang State, northern Peninsular Malaysia and passaged through Swiss albino mice.

All schistosomes were cultured at the Center for Applied Malacology and Entomology (Department of Biology, Faculty of Science, Mahidol University, Bangkok), and the older strains were cycled at a rate of 2-3 passages a year.

Adult worms were recovered by perfusion and dissection of anesthetized infected mice and were briefly washed in distilled water before being frozen at -30 ^{o}C. They were then flown to San Diego and stored at 70 ^{o}C for up to 3 months before electrophoresis.

Horizontal starch gel electrophoresis was performed to separate each animals structural gene products and histochemical staining techniques resolved specific allozyme patterns. Techniques were very similar to those described elsewhere (Fletcher *et al.*, 1981) except that we used 12.5% gels made of Sigma strach (Sigma Chemical Co., St. Louis, Missouri). Prior to electrophoresis, the adult worms were thawed, quickly separated by sex, and homogenized individually in 7 *ul* (females) or 10 *ul* (males) of chilled distilled water. The homogenate was immediately absorbed onto a single 10 x 3 mm tab of chromatography paper and inserted in the gel. The electrophoretic buffer and stain recipes are given elsewhere except that superoxidase dehydrogenase (SOD) was resolved on gel slices stained for malate dehydrogenase (MDH), diaphorase (DIA) was resolved using the technique of Harris and Hopkinson (1977) and the peptidase (PEP) appenred with a leucylglycylglycine substrate. We employed a discontinuous Poulik buffer (pH 8.2/8.7) to resolve aldolase (ALD, E.C. 4.1.2.13), DIA (1.6.2.2), glucose 6-phosphate dehydrogenase (G6PD, 1.1.1.49), hexokinase (HK, 2.7.1.1) and phosphoglucomutase (PGM, 2.7.5.1), a discontinuous citrate with aminopropylmor-

pholine buffer (pH 6.4/7.4) to resolve adenylate kinase (AK, 2.7.4.3), mannose phosphate isomerase (MPI, 5.3.1.9) and PEP (3.4.1.3), and a continuous citrate with aminopropylmorpholine buffer (pH 7.0) for glucose phosphate isomerase (GPI, 5.3.1.9, formerly called phosphoglucose isomerase), MDH (1.1.1.37) and SOD (1.15.1.1).

The number of individuals electrophoresed and the mean and range of the number of individuals scored per locus were: LEYTE LAB 397, 90 (32-151), LEYTE FIELD 69, 32 (18-69), MINDORO 360, 85 (13-155), LUZON 4, 1.5 (1-2), MEKONGI 29, 16 (11-24) MALAYSIA 211, 48 (7-121). Schistosomes of different strains were run on the same gel to facilitate comparison of electromorphs. Allozymes were designated a,b,c in order of decreasing electrophoretic mobility and by the use of the same abbreviations employed to represent the enzymes, set in lower case and italicized. Unbiased estimates of genetic distance between the various strains were calculated using Nei's revised formula (Nei, 1978) and the BIOSYS computer program (Swofford and Selander, 1981).

Consistent and interpretable electromorph patterns were obtained for 11 enzymes representing 11 structural gene loci. Patterns for *Dia* and *Pep* in Asian schistosomes are described here for the first time: DIA resolved as a single sharply defined band of activity migrating anodally, PEP resolved as series of 2-3 intense

evenly spaced bands migrating anodally. The presumption is that each of these enzymes is coded as a single monomorphic locus. All other enzymes gave patterns similar to those reported previously except as noted. MDH 1 resolved as a single band of activity in all strains; this band varied in relative mobility between taxa. When MDH 1 is denatured, it breaks down to give a 3 banded pattern sometimes indistinguishable from the patterns of heterozygotes for dimeric allozymes. We are convinced, however, that these *Mdh 1* pseudo heterozygotes are actually denatured homozygotes as affected individual schistosomes also showed blurred patterns for PEP, MPI and GPI and generally no activity for AK.

Our genetic results, pooled where appropriate with those previously published, are summarized in Tables 49 and 50. The proportion of loci polymorphic (*P*) and the mean individual heterozygosity per locus (*H*) were both zero in all four strains of *S. japonicum*, in *S. mekongi* and in the Malaysian schistosome.

Four strains of *S. japonicum* from the Philippines were examined. The LEYTE LAB and LEYTE FIELD strains were identical at all 11 loci. The LUZON strain is not well characterized (based on number of loci and number of worms), but was identical to the LEYTE strains at all 8 loci examined. In contrast, the strain from MINDORO was weakly differentiated from the others being fixed for a different allele at *G6pd and at Pep*.

Schistosoma mekongi apparently shares a *Gpi* allele with Philippine *S. japonicum*, but otherwise the two taxa differ at 11 out of 12 (92%) loci. The Malaysian schistosome also differs from *S. japonicum* at the same 11 loci; it shares 5 of these 11 allozymes with *S. mekongi*.

Electrophoretically detected allozyme variation is described in strains of *S. japonicum* (4 Philippine strains), *S. mekongi* (Laos), and an undescribed anthropophilic *S. japonicum* like schistosome from Peninsular Malaysia. Results, together with those reported previously for 8 other strains (*S. japonicum* China, Formosa, Japan, Philippines; *S. mekongi* - 2 substrains; Malaysian schistosome 2 strains) permit a composite genetic characterization of 15 strains of Asian schistosomes at 9 18 presumptive loci. The proportion of polymorphic loci (P) and the mean heterozygosity per locus ($\overline{H}$) were zero in all strains. Although this was expected for strains that had been in laboratory culture for 4 50 years, we expected to detect variation in strains based on 10 50 recently field-collected infected snails. We expected *S. japonicum* to be as variable as *S. mansoni* ($\overline{P}$ = 0.13 (0 0.33), $\overline{H}$ 0.04, 18 loci, 22 strains) as it reproduces sexually, has an evolutionary history of several million years, inhabits a wide geographic range, coevolved with a genetically variable intermediate host snail, and has a diversity of mammalian hosts.

No differences were detected between the 5 *S. japonicum* strains from Leyte and Luzon (Philippines), the 3

S. mekongi strains, or the 3 Malaysian schistosome strains; these groups and the remaining *S. japonicum* strains representing Mindoro (Philippines), China, Formosa and Japan all have distinctive multilocus electromorphic patterns. Nei's genetic distances (D) were calculated to estimate interstrain and interspecific divergence. Interstrain genetic distances in *S. japonicum* averaged > 0.3; much higher than those reported previously for *S. mansoni* ($\bar{D}$ 0.06, D (max) 0.24). *S. japonicum* (Mindoro) was moderately differentiated from the Luzon strains (D = 0.29, 12 loci). Estimates of the *S. japonicum* China Philippine distance (D > 0.4, 11 loci) are high for conspecific populations, and further studies of the still poorly characterized Chinese parasite may reveal that it is, in fact, a separate species. *S. japonicum* is shown to be only distantly related to *S. mekongi* and the Malaysian schistosome (D > 1); the latter is closely related to, but genetically quite distinct from, *S. mekongi* (D = 0.61 ± 0.14, 11 loci) and warrants recognition as a new species.

The report has the following medical and public health consequences. First, the possible medical correlates of the increasingly complex taxonomic situation merit more attention than they have received. It is reasonable to expect the different species and species groups of schistosomes to differ in their epidemiology, pathology and response to various treatments. Second, our continued failure to find intrastrain variation in the Asian

schistosomes has important ramifications. although isogenic strains are ideal for some investigations (immunological experiments and sources for monoclonal antibodies), single isogenic strains may be inappropriate models for wide ranging species. Development of broadly applicable disease diagnosis, treatment and control techniques involving vaccines and drugs may be more difficult in view of this pattern of marked geographic variation (Woodruff, 1985). There is, after all, apparently much more regional differentiation within *S. japonicum*, as presently defined, than within *Homo sapiens*.

REFERENCES

CHIU, J.K., ONG, S.J., YU, J.C., KAO, C.Y. & IJIMA, T. 1981. Susceptibility of *Oncomelania hupensis formosana* recombinants and hybrids with *Oncomelania hupensis nosophora* to infection with *Schistosoma japonicum*. *Intl. J. Parasitol.*, *11* : 391-397.

DAVIS, G.M. & RUFF, M. 1973. *Oncomelania hupensis* (Gastropoda : Hydrobiidae) : hybridization, genetics, and transmission of *Schistosoma japonicum*. *Malacol. Rev.*, *6* : 181-197.

FLETCHER, M., LOVERDE, P.T., & WOODRUFF, D.S., 1981. Genetic variation in *S. mansoni*: enzyme polymorphisms in populations from Africa, Southwest Asia, South America, and the West Indies. *Amer. J. Trop. Med. Hyg.*, *30* : 406-421.

HARRIS, H. & HOPKINSON, D.A. 1977. *Handbook of Enzyme Electrophoresis in Human Genetics*. North-Holland, Amsterdam.

MAO, S. & SHAO, B. 1982. Schistosomiasis control in the People's Republic of China. *Amer. J. Trop. Med. Hyg.*, *31* : 92-99.

MULVEY, M. & VRIJENHOEK, R.C. 1981. Genetic variation among laboratory strains of the planorbid snail *Biomphalaria glabrata*. *Biochem. Genet.*, *19* : 1169-1182.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, *89* : 583-590.

ROGERS, J.A. 1972. Measures of genetic similarity and genetic distance. *Univ. Tex. Stud. Genet.*, *7* : 145-153.

ROLLINSON, D. & SOUTHGATE, V.R. 1985. Schistosome and snail populations: genetic variability and parasite transmission. In *Ecology and Genetics of Host Parasite Interactions*, Rollinson, D. and R. Anderson, eds. Linn. Soc. London. Pp 91-109.

SHAW, C.R. & R. PRASAD. 1970. Starch gel electrophoresis of enzymes. A compilation of recipes. *Biochemical Genetics*, *4* : 297-320.

SWOFFORD, D.L., & SELANDER, R.B. 1981. BIOSYS I: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered., 72 : 281-283.

WOODRUFF, P.S. 1975. Genetics and morphometrics of *Cerion* at Pongo Carpet: a new systematic approach to the enigmatic land snails. *Systematic Zoology*, *23* : 518-535.

WOODRUFF, D.S. 1985. Genetic control of schistosomiasis: a technique based on the manipulation of intermediate host snail populations, pp. 41-68. In T. Cheng, ed. *Parasitic and Related Diseases: Basic Mechanisms, Manifestations and Control.* Comparative Pathobiology, Vol. 8, Plenum, New York.

YUAN, H.C. 1958. A preliminary study of the susceptibility of geographic strains of *Oncomelania hupensis* to geographic strains of *Schistosoma japonicum* from different provinces in China. *Chinese Med. J.*, *77* : 575.

TABLE 1

Growth table for *Melanoides tuberculata*.

Week	Ave. shell length per week (mm.)	Ave. shell growth per week (mm.)
1	0.8	0.4
2	1.2	0.3
3	1.5	0.3
4	1.8	0.4
5	2.2	0.3
6	2.5	0.5
7	3.0	0.6
8	3.6	0.4
9	4.0	0.5
10	4.5	0.6
11	5.1	0.8
12	5.9	0.6
13	6.5	0.5
14	7.0	0.5
15	7.5	0.5
16	8.0	0.6
17	8.5	0.5
18	9.0	0.5
19	9.5	0.4
20	10.0	0.4
21	10.3	0.7
22	11.0	0.6
23	11.5	0.5
24	12.0	0.8
25	12.8	

Mean shell growth per week = 0.5 mm.

TABLE 2

Growth table for *Turbia granifera*.

Month	Ave. shell length per week (cm.)	Ave. shell growth per week (cm.)
1	0.30	0.31
2	0.61	0.14
3	0.75	0.07
4	0.82	0.14
5	0.96	0.02
6	1.08	0.05
7	1.13	0.28
8	1.41	0.15
9	1.56	0.05
10	1.61	0.09
11	1.70	0.19
12	1.89	

Mean shell growth per month = 0.14 cm.

TABEL 3

Growth table for *Brotia costula costula*.

Month	Ave. shell length per week (cm.)	Ave. shell growth per week (cm.)
1	0.29	0.16
2	0.45	0.18
3	0.63	0.28
4	0.91	0.21
5	1.20	0.38
6	1.58	0.03
7	1.61	0.30
8	1.91	0.19
9	2.10	0.16
10	2.26	0.07
11	2.33	0.25
12	2.58	0.19
13	2.77	0.25
14	3.02	0.18
15	3.20	0.22
16	3.42	0.18
17	3.60	0.22
18	3.82	

Mean shell growth per month = 0.20 cm.

TABLE 4

Growth table for *Pila ampullacea*.

Month	Ave. shell length per month (cm.)	Ave. shell growth per week (cm.)
1	0.42	0.33
2	0.75	0.36
3	1.11	0.31
4	1.42	0.44
5	1.86	0.45
6	2.31	0.73
7	3.04	0.30
8	3.34	0.14
9	3.48	0.34
10	3.82	0.34
11	4.16	0.23
12	4.39	0.02
13	4.41	0.08
14	4.49	0.05
15	4.54	0.04
16	4.58	

Mean shell growth per month = 0.28 cm.

TABLE 5

Biological control study between *Tarebia granifera* (control) and *Biomphalaria glabrata* (target) under laboratory condition.

Week	Number of snails									
	1		2		3		4		5	
	Tg	Bg	Tg	Bg	Tg	Bg	Tg	Bg	Tg	Bg
0	40	0	30	10	20	20	10	30	0	40
2	40	0	30	10	20	20	10	30	0	40
4	90	0	29	40	20	25	2	38	0	52
6	643	0	0	94	0	106	0	82	0	76
8	324	0	0	151	0	306	0	255	0	134
10	77	0	0	70	0	114	0	77	0	108
12	89	0	0	65	0	102	0	86	0	96
14	95	0	0	63	0	96	0	93	0	87
16	102	0	0	58	0	94	0	101	0	75

Tg = *Tarebia granifera*

Bg = *Biomphalaria glabrata*

Ratio of *T. granifera* : *B. glabrata*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 6

Biological control study between *Tarebia granifera* (control) and *Radix rubiginosa* (target) under laboratory condition.

Week	Number of snails									
	1		2		3		4		5	
	Tg	Rr	Tg	Rr	Tg	Rr	Tg	Rr	Tg	Rr
0	40	0	30	10	20	20	10	30	0	40
2	40	0	30	3	17	14	9	12	0	6
4	90	0	55	0	13	4	6	0	0	180
6	450	0	38	0	20	1	63	0	0	182
8	324	0	81	0	62	1	24	1	0	112
10	77	0	137	0	84	0	31	0	0	100
12	76	0	168	0	95	0	16	0	0	94
14	84	0	97	0	88	0	11	0	0	72
16	81	0	53	0	84	0	15	0	0	70

Tg = *Tarebia granifera*

Rr = *Radix rubiginosa*

Ratio of *T. granifera* : *R. rubiginosa*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 7

Biological control study between *Melanoides tuberculata* (control) and *Biomphalaria glabrata* (target) under laboratory condition.

Week	Number of snails									
	1		2		3		4		5	
	Mt	Bg	Mt	Bg	Mt	Bg	Mt	Bg	Mt	Bg
0	40	0	30	10	20	20	10	30	0	40
2	50	0	30	10	20	20	10	30	0	40
4	53	0	30	90	19	50	10	32	0	52
6	79	0	24	108	15	64	8	45	0	76
8	102	0	15	112	15	76	5	80	0	134
10	65	0	8	125	10	87	3	112	0	108
12	37	0	2	139	5	95	2	135	0	96
14	46	0	2	135	5	144	2	107	0	87
16	44	0	1	111	1	115	2	78	0	75

Mt = *Melanoides tuberculata*

Bg = *Biomphalaria glabrata*

Ratio of *M. tuberculata* : *B. glabrata*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 8

Biological control study between *Melanoides tuberculata* (control) and *Radix rubiginosa* (target) under laboratory condition.

Week	Number of snails									
	1		2		3		4		5	
	Mt	Rr	Mt	Rr	Mt	Rr	Mt	Rr	Mt	Rr
0	40	0	30	10	20	20	10	30	0	40
2	42	0	30	6	20	12	10	19	0	6
4	53	0	41	0	20	0	10	0	0	140
6	79	0	57	0	52	0	24	3	0	142
8	102	0	69	0	51	0	50	3	0	112
10	65	0	56	0	56	0	43	3	0	100
12	37	0	40	0	55	0	40	2	0	94
14	46	0	50	0	47	0	16	7	0	72
16	44	0	47	0	47	0	18	2	0	70

Mt = *Melanoides tuberculata*

Rr = *Radix rubiginosa*

Raito of *M. tuberculata* : *R. rubiginosa*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 9

Biological control study between *Melanoides tuberculata* (control) and *Bithynia siamensis goniomphalos* (target) under laboratory condition.

Week	Number of snails									
	1		2		3		4		5	
	Mt	Bsg	Mt	Bsg	Mt	Bsg	Mt	Bsg	Mt	Bsg
0	40	0	30	10	20	20	10	30	0	40
2	42	0	30	10	20	20	10	30	0	40
4	53	0	39	6	23	16	17	22	0	29
6	79	0	63	6	39	11	65	17	0	25
8	102	0	55	5	32	10	60	15	0	22
10	65	0	54	5	36	10	65	15	0	20
12	37	0	53	5	38	10	72	13	0	20
14	46	0	30	4	42	8	37	13	0	11
16	44	0	36	4	52	8	54	13	0	20

Mt = *Melanoides tuberculata*

Bsg = *Bithynia siamensis goniomphalos*

Ratio of *M. tuberculata* : *B.s. goniomphalos*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 10

Biological control study between *Melanoides tuberculata* (control) and *Indoplanorbis exustus* (target) under laboratory condition.

Week	Number of snails									
	1		2		3		4		5	
	Mt	Te	Mt	Te	Mt	Te	Mt	Te	Mt	Te
0	40	0	30	10	20	20	10	30	0	40
2	42	0	30	10	12	50	10	30	0	40
4	53	0	41	77	13	50	10	64	0	131
6	79	0	30	26	4	46	2	150	0	43
8	102	0	37	92	3	84	2	81	0	60
10	65	0	35	45	3	55	2	56	0	60
12	37	0	32	58	2	50	2	18	0	27
14	46	0	31	26	2	130	2	103	0	63
16	44	0	35	253	2	363	2	230	0	123

Mt = *Malanoides tuberculata*

Te = *Indoplanorbis exustus*

Ratio of *M. tuberculata* : *I. exustus*

1	=	40	:	0
2	=	30	:	10
3	=	20	:	20
4	=	10	:	30
5	=	0	:	40

TABLE 11

Biological control study between *Brotia costula costula* (control) and *Radix rubiginosa* (target) under laboratory condition.

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Rr	Bcc	Rr	Bcc	Rr	Bcc	Rr	Bcc	Rr
0	20	0	15	5	10	10	5	15	0	20
2	18	0	10	3	7	5	2	6	0	3
4	18	0	6	150	6	80	1	160	0	90
6	17	0	6	76	5	90	1	57	0	96
8	15	0	1	11	5	31	1	15	0	56
10	15	0	6	18	5	14	1	7	0	42
12	14	0	5	2	5	0	1	4	0	39
14	14	0	5	2	4	0	1	3	0	32
16	24	0	5	2	4	0	1	2	0	30

Bcc = *Brotia costula costula*

Rr = *adix rubiginosa*

Ratio of *B.c. costula* : *R. rubiginosa*

1	=	20	:	0
2	=	15	:	5
3	=	10	:	10
4	=	5	:	15
5	=	0	:	20

TABLE 12

Biological control study between *Brotia costula costula* (control) and *Robertsiella kaporensis* (target) under laboratory condition.

Week	Number of snails									
	1		2		3		4		5	
	Bcc	Rk	Bcc	Rk	Bcc	Rk	Bcc	Rk	Bcc	Rk
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	10	10	5	15	0	20
4	20	0	15	5	10	10	5	15	0	20
6	20	0	16	5	10	9	5	14	0	19
8	20	0	24	4	10	9	5	14	0	17
10	20	0	14	4	10	9	5	12	0	17
12	20	0	14	4	10	9	5	12	0	17
14	20	0	14	4	10	9	5	11	0	15
16	20	0	14	4	10	9	5	11	0	15

Bcc = *Brotia costula costula*

Rk = *Robertsiella kaporensis*

Ratio of *B.c. costula* : *R. kaporensis*

1	=	20	:	0
2	=	15	:	5
3	=	10	:	10
4	=	5	:	15
5	=	0	:	20

TABLE 13

Biological control study between *Pila ampullacea* (control) and *Biomphalaria glabrata* (target) under laboratory condition.

Week	Number of snails									
	1		2		3		4		5	
	Pa	Bg	Pa	Bg	Pa	Bg	Pa	Bg	Pa	Bg
0	20	0	15	5	10	10	5	15	0	20
2	20	0	15	5	10	10	5	14	0	20
4	20	0	15	5	10	10	5	14	0	99
6	18	0	14	5	10	10	5	14	0	108
8	18	0	14	5	6	24	5	32	0	151
10	17	0	13	5	6	19	5	14	0	74
12	17	0	10	4	5	19	4	15	0	67
14	16	0	10	3	4	20	4	12	0	66
16	15	0	10	3	4	15	4	12	0	56

Pa = *Pila ampullacea*

Bg = *Biomphalaria glabrata*

Ratio of *P. ampullacea* : *B. glabrata*

1	=	20	:	0
2	=	15	:	5
3	=	10	:	10
4	=	5	:	15
5	=	0	:	20

TABLE 14

Biological control study between *Brotia costula costula* (control) and *Biomphalaria glabrata* (target) under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Bg	Bcc	Bg	Bcc	Bg
0	50	0	25	25	0	50
2	1278	0	25	25	0	50
4	1680	0	24	23	0	47
6	1483	0	23	76	0	495
8	985	0	23	242	0	176
10	975	0	23	276	0	95
12	743	0	22	302	0	180
14	723	0	22	336	0	137
16	748	0	20	394	0	173
18	748	0	20	461	0	65
20	736	0	20	223	0	125
22	706	0	20	256	0	367
24	732	0	19	206	0	507
26	543	0	19	277	0	468
28	529	0	19	275	0	392
30	638	0	18	130	0	368
32	321	0	17	155	0	342

Bcc = *Brotia costula costula*
Bg = *Biomphalaria glabrata*

Ratio of *B.c. costula*	:	*B. glabrata*
1 = 50	:	0
2 = 25	:	25
3 = 0	:	50

TABLE 15

Biological control study between *Brotia costula costula* (control) and *Indoplanorbis exustus* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Ie	Bcc	Ie	Bcc	Ie
0	50	0	25	25	0	50
2	1278	0	875	21	0	50
4	1680	0	645	20	0	48
6	1483	0	804	20	0	152
8	985	0	642	22	0	115
10	975	0	429	116	0	128
12	743	0	522	215	0	166
14	723	0	387	142	0	190
16	748	0	378	169	0	149
18	748	0	383	142	0	114
20	736	0	372	124	0	166
22	706	0	364	118	0	128
24	732	0	360	105	0	142
26	543	0	349	98	0	160
28	529	0	338	79	0	112
30	638	0	337	51	0	142
32	321	0	297	69	0	151

Bcc = *Brotia costula costula*
Ie = *Indoplanorbis exustus*

Ratio of *B.c. costula* : *I. exustus*				
1	=	50	:	0
2	=	25	:	25
3	=	0	:	50

TABLE 16

Biological control study between *Brotia costula costula* (control) and *Radix rubiginosa* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Rr	Bcc	Rr	Bcc	Rr
0	50	0	25	25	0	50
2	1278	0	25	25	0	45
4	1680	0	707	23	0	37
6	1483	0	980	21	0	37
8	985	0	648	21	0	37
10	975	0	411	78	0	123
12	743	0	476	230	0	199
14	723	0	285	69	0	147
16	748	0	371	128	0	188
18	748	0	341	226	0	262
20	736	0	341	226	0	197
22	706	0	212	155	0	130
24	732	0	238	153	0	95
26	543	0	219	101	0	68
28	529	0	138	63	0	148
30	638	0	133	32	0	81
32	321	0	122	18	0	90

Bcc = *Brotia costula costula*
Rr = *Radix rubiginosa*

Ratio of		*B.c. costula*	:	*R. rubiginosa*
1	=	50	:	0
2	=	25	:	25
3	=	0	:	50

TABLE 17

Biological control study between *Brotia costula costula* (control) and *Tricula aperta* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Ta	Bcc	Ta	Bcc	Ta
0	50	0	25	25	0	50
2	1278	0	25	25	0	50
4	1680	0	25	18	0	40
6	1483	0	24	17	0	36
8	985	0	24	14	0	28
10	975	0	23	10	0	24
12	743	0	22	8	0	19
14	723	0	22	6	0	16
16	748	0	20	3	0	9
18	748	0	20	0	0	5
20	736	0	20	0	0	0
22	706	0	20	0	0	0
24	732	0	19	0	0	0
26	543	0	19	0	0	0
28	529	0	19	0	0	0
30	638	0	18	0	0	0
32	321	0	18	0	0	0

Bcc = *Brotia costula costula*
Ta = *Tricula aperta*

Ratio of	*B.c. costula*	:	*T. aperta*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

TABLE 18

Biological control study between *Brotia costula costula* (control) and *Oncomelania hupensis quadrasi* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Oq	Bcc	Oq	Bcc	Oq
0	50	0	25	25	0	50
2	1278	0	25	25	0	40
4	1680	0	25	18	0	32
6	1483	0	113	10	0	30
8	985	0	106	6	0	29
10	975	0	163	6	0	19
12	743	0	181	2	0	16
14	723	0	137	2	0	15
16	748	0	141	2	0	12
18	748	0	121	1	0	11
20	736	0	112	0	0	11
22	706	0	119	0	0	11
24	732	0	127	0	0	11
26	543	0	141	0	0	11
28	529	0	148	0	0	11
30	638	0	145	0	0	10
32	321	0	137	0	0	10

Bcc = *Brotia costula costula*
Oq = *Oncomelania hupensis quadrasi*

Ratio of *B.c. costula* : *O.h. quadrasi*				
1	=	50	:	0
2	=	25	:	25
3	=	0	:	50

TABLE 19

Biological control study between *Brotia costula costula* (control) and *Bithynia siamensis goniomphalos* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Bcc	Bsg	Bcc	Bsg	Bcc	Bsg
0	50	0	25	25	0	50
2	1278	0	25	25	0	50
4	1680	0	25	25	0	48
6	1483	0	25	25	0	48
8	985	0	22	20	0	44
10	975	0	20	20	0	38
12	743	0	20	17	0	26
14	723	0	20	14	0	24
16	748	0	20	12	0	24
18	748	0	20	8	0	21
20	736	0	19	4	0	17
22	706	0	19	2	0	13
24	732	0	19	2	0	12
26	543	0	18	2	0	12
28	529	0	18	1	0	11
30	638	0	18	1	0	10
32	321	0	18	0	0	10

Bcc = *Brotia costula costula*
Bsg = *Bithynia siamensis goniompalos*

Ratio of	*B.c. costula*	:	*B.s. goniomphalos*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

TABLE 20

Biological control study between *Melanoides tuberculata* (control) and *Biomphalaria glabrata* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Bg	Mt	Bg	Mt	Bg
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	50	0	95
6	236	0	76	125	0	990
8	286	0	131	48	0	353
10	223	0	68	140	0	189
12	312	0	165	324	0	361
14	384	0	162	288	0	273
16	514	0	590	568	0	345
18	656	0	775	433	0	131
20	504	0	675	186	0	251
22	264	0	606	497	0	734
24	246	0	511	321	0	1014
26	267	0	199	227	0	936
28	300	0	154	226	0	784
30	662	0	210	193	0	736
32	643	0	184	162	0	684

Mt = *Melanoides tuberculata*
Bg = *Biomphalaria glabrata*

Ratio of *M. tuberculata* : *B. glabrata*				
1	=	100	:	0
2	=	50	:	50
3	=	0	:	100

TABLE 21

Biological control study between *Melanoides tuberculata* (control) and *Indoplanorbis exustus* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Ie	Mt	Ie	Mt	Ie
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	190	0	43	49	0	95
6	236	0	40	333	0	303
8	286	0	40	460	0	229
10	223	0	38	262	0	256
12	312	0	25	297	0	332
14	384	0	23	368	0	380
16	514	0	167	97	0	297
18	656	0	93	127	0	227
20	504	0	154	202	0	312
22	264	0	96	161	0	259
24	246	0	73	188	0	286
26	267	0	78	162	0	230
28	300	0	85	146	0	224
30	662	0	92	215	0	284
32	643	0	105	226	0	301

Mt = *Melanoides tuberculata*
Ie = *Indoplanorbis exustus*

Ratio of M. tuberculata : I. exustus				
1	=	100	:	0
2	=	50	:	50
3	=	0	:	100

TABLE 22

Biological control study between *Melanoides tuberculata* (control) and *Radix rubiginosa* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Rr	Mt	Rr	Mt	Rr
0	100	0	50	50	0	100
2	100	0	48	43	0	100
4	100	0	48	43	0	100
6	236	0	136	35	0	73
8	286	0	190	23	0	73
10	223	0	223	12	0	180
12	312	0	298	8	0	194
14	384	0	286	8	0	196
16	514	0	202	12	0	523
18	656	0	195	12	0	394
20	504	0	184	19	0	334
22	264	0	192	14	0	261
24	246	0	188	12	0	191
26	267	0	176	12	0	136
28	300	0	183	10	0	296
30	662	0	175	10	0	162
32	643	0	187	10	0	181

Mt = *Melanoides tuberculata*
Rr = *Radix rubiginosa*

Ratio of *M. tuberculata*	:	*R. rubiginosa*
1 = 100	:	0
2 = 50	:	50
3 = 0	:	100

TABLE 23

Biological control study between *Melanoides tuberculata* (control) and *Tricula aperta* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Ta	Mt	Ta	Mt	Ta
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	49	42	0	80
6	236	0	49	30	0	72
8	286	0	131	18	0	56
10	223	0	145	10	0	48
12	312	0	194	5	0	38
14	384	0	258	0	0	32
16	514	0	334	0	0	18
18	656	0	308	0	0	10
20	504	0	288	0	0	0
22	264	0	205	0	0	0
24	246	0	200	0	0	0
26	267	0	174	0	0	0
28	300	0	185	0	0	0
30	662	0	181	0	0	0
32	643	0	193	0	0	0

Mt = *Melanoides tuberculata*
Ta = *Tricula aperta*

Ratio	of	*M. tuberculata*	:	*T. aperta*
1	=	100	:	0
2	=	50	:	50
3	=	0	:	100

TABLE 24

Biological control study between *Melanoides tuberculata* (control) and *Oncomelania hupensis quadrasi* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Oq	Mt	Oq	Mt	Oq
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	38	0	63
6	236	0	277	26	0	60
8	286	0	378	26	0	41
10	223	0	627	23	0	38
12	312	0	801	19	0	32
14	384	0	1428	15	0	30
16	514	0	825	15	0	24
18	656	0	748	15	0	20
20	504	0	636	15	0	20
22	264	0	498	15	0	14
24	246	0	580	14	0	14
26	267	0	677	14	0	14
28	300	0	722	13	0	14
30	662	0	537	10	0	14
32	643	0	526	10	0	14

Mt = *Melanoides tuberculata*
Oq = *Oncomelania hupensis quadrasi*

Ratio of *M. tuberculata*	:	*O.h. quadrasi*
1 = 100	:	0
2 = 50	:	50
3 = 0	:	100

TABLE 25

Biological control study between *Melanoides tuberculata* (control) and *Bithynia siamensis goniomphalos* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Mt	Bsg	Mt	Bsg	Mt	Bsg
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	50	0	95
6	236	0	165	40	0	95
8	286	0	221	16	0	87
10	223	0	208	8	0	56
12	312	0	225	2	0	51
14	384	0	238	2	0	48
16	514	0	238	0	0	48
18	656	0	215	0	0	42
20	504	0	204	0	0	34
22	264	0	186	0	0	26
24	246	0	178	0	0	24
26	267	0	189	0	0	24
28	300	0	192	0	0	22
30	662	0	174	0	0	22
32	643	0	179	0	0	20

Mt = *Melanoides tuberculata*
Bsg = *Bithynia siamensis goniomphalos*

Ratio of	*M. tuberculata*	:	*B.s. goniomphalos*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

TABLE 26

Biological control study between *Pila ampullacea* (control) and *Biomphalaria glabrata* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Bg	Pa	Bg	Pa	Bg
0	50	0	25	25	0	100
2	50	0	26	20	0	100
4	48	0	25	20	0	95
6	48	0	25	20	0	990
8	48	0	25	18	0	353
10	44	0	25	9	0	189
12	40	0	25	9	0	361
14	40	0	23	9	0	273
16	38	0	23	9	0	345
18	38	0	22	9	0	131
20	38	0	22	9	0	251
22	38	0	22	9	0	734
24	38	0	22	9	0	1014
26	36	0	22	8	0	936
28	36	0	22	8	0	784
30	36	0	21	7	0	736
32	36	0	21	7	0	684

Pa = *Pila ampullacea*
Bg = *Biomphalaria glabrata*

Ratio of	*P. ampullacea*	:	*B. glabrata*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

TABLE 27

Biological control study between *Pila ampullacea* (control) and *Indoplanorbis exustus* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Ie	Pa	Ie	Pa	Ie
0	50	0	25	25	0	50
2	50	0	25	25	0	50
4	48	0	25	12	0	48
6	48	0	25	7	0	152
8	48	0	25	7	0	115
10	44	0	25	7	0	128
12	40	0	25	6	0	166
14	40	0	25	6	0	190
16	38	0	23	6	0	149
18	38	0	23	2	0	114
20	38	0	23	2	0	166
22	38	0	23	2	0	128
24	38	0	20	2	0	142
26	36	0	20	2	0	160
28	36	0	20	2	0	112
30	36	0	20	2	0	142
32	36	0	20	2	0	151

Pa = *Pila ampullacea*
Ie = *Indoplanorbis exustus*

Ratio of	*P. ampullacea*	:	*I. exustus*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

TABLE 28

Biological control study between *Pila ampullacea* (control) and *Radix rubiginosa* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Rr	Pa	Rr	Pa	Rr
0	50	0	25	25	0	50
2	50	0	25	25	0	45
4	48	0	24	17	0	37
6	48	0	24	11	0	37
8	48	0	24	6	0	37
10	44	0	24	0	0	123
12	40	0	24	0	0	199
14	40	0	23	0	0	147
16	38	0	23	0	0	188
18	38	0	23	0	0	262
20	38	0	22	0	0	197
22	38	0	22	0	0	130
24	38	0	22	0	0	95
26	36	0	21	0	0	68
28	36	0	20	0	0	148
30	36	0	18	0	0	81
32	36	0	18	0	0	90

Pa = *Pila ampullacea*
Rr = *Radix rubiginosa*

Ratio of	*P. ampullacea*	:	*R. rubiginosa*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

TABLE 29

Biological control study between *Pila ampullacea* (control) and *Tricula aperta* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Ta	Pa	Ta	Pa	Ta
0	50	0	25	25	0	50
2	50	0	25	25	0	50
4	48	0	22	16	0	40
6	48	0	22	10	0	36
8	48	0	22	3	0	28
10	44	0	20	0	0	24
12	40	0	20	0	0	19
14	40	0	19	0	0	16
16	38	0	19	0	0	9
18	38	0	19	0	0	5
20	38	0	19	0	0	0
22	38	0	18	0	0	0
24	38	0	18	0	0	0
26	36	0	18	0	0	0
28	36	0	18	0	0	0
30	36	0	18	0	0	0
32	36	0	18	0	0	0

Pa = *Pila ampullacea*
Ta = *Tricula aperta*

Ratio of	*P. ampullacea*	:	*T. aperta*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

TABLE 30

Biological control study between *Pila ampullacea* (control) and *Oncomelania hupensis quadrasi* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Oq	Pa	Oq	Pa	Oq
0	50	0	25	25	0	50
2	50	0	25	25	0	40
4	48	0	25	18	0	32
6	48	0	24	10	0	30
8	48	0	22	4	0	29
10	44	0	20	0	0	19
12	40	0	20	0	0	16
14	40	0	19	0	0	15
16	38	0	19	0	0	12
18	38	0	19	0	0	11
20	38	0	19	0	0	11
22	38	0	19	0	0	11
24	38	0	19	0	0	11
26	36	0	18	0	0	11
28	36	0	18	0	0	11
30	36	0	18	0	6	10
32	35	0	18	0	0	10

Pa = *Pila ampullacea*
Oq = *Oncomelania hupensis quadrasi*

Ratio of	*P. ampullacea*	:	*O.h. quadrasi*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

TABLE 31

Biological control study between *Pila ampullacea* (control) and *Bithynia siamensis goniomphalos* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Pa	Bsg	Pa	Bsg	Pa	Bsg
0	50	0	25	25	0	50
2	50	0	25	25	0	50
4	48	0	25	25	0	48
6	48	0	25	18	0	48
8	48	0	20	12	0	44
10	44	0	20	7	0	38
12	40	0	20	0	0	26
14	40	0	20	0	0	24
16	38	0	19	0	0	24
18	38	0	19	0	0	21
20	38	0	19	0	0	17
22	38	0	18	0	0	13
24	38	0	18	0	0	12
26	36	0	18	0	0	12
28	36	0	18	0	0	11
30	36	0	18	0	0	10
32	36	0	18	0	0	10

Pa = *Pila ampullacea*
Bsg = *Bithynia siamensis goniomphalos*

Ratio of	*P. ampullacea*	:	*B.s. goniomphalos*
1 =	50	:	0
2 =	25	:	25
3 =	0	:	50

TABLE 32

Biological control study between *Tarebia granifera* (control) and *Biomphalaria glabrata* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Bg	Tg	Bg	Tg	Bg
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	50	0	95
6	92	0	48	50	0	990
8	390	0	43	48	0	353
10	650	0	279	567	0	189
12	667	0	128	769	0	361
14	327	0	614	1510	0	273
16	444	0	471	987	0	345
18	390	0	317	832	0	131
20	396	0	205	448	0	251
22	219	0	186	430	0	734
24	216	0	163	344	0	1014
26	246	0	122	350	0	936
28	189	0	96	322	0	784
30	190	0	75	342	0	736
32	259	0	52	350	0	684

Tg = *Tarebia granifera*
Bg = *Biomphalaria glabrata*

Ratio of		*T. granifera*	:	*B. glabrata*
1	=	100	:	0
2	=	50	:	50
3	=	0	:	100

TABLE 33

Biological control study between *Tarebia granifera* (control) and *Indoplanorbis exustus* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Ie	Tg	Ie	Tg	Ie
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	50	0	95
6	92	0	196	386	0	303
8	390	0	502	257	0	229
10	650	0	150	361	0	256
12	667	0	465	374	0	332
14	327	0	315	221	0	380
16	444	0	520	202	0	297
18	390	0	676	191	0	227
20	396	0	664	127	0	312
22	219	0	223	201	0	259
24	216	0	277	149	0	286
26	246	0	230	191	0	230
28	189	0	238	168	0	224
30	190	0	278	159	0	284
32	259	0	229	159	0	301

Tg = *Tarebia granifera*
Ie = *Indoplanorbis exustus*

Ratio of	*T. granifera*	:	*I. exustus*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

TABLE 34

Biological control study between *Tarebia granifera* (control) and *Radix rubiginosa* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Rr	Tg	Rr	Tg	Rr
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	42	14	0	100
6	92	0	42	36	0	73
8	390	0	161	151	0	73
10	650	0	142	93	0	180
12	667	0	152	52	0	194
14	327	0	155	28	0	196
16	444	0	177	12	0	523
18	390	0	184	0	0	394
20	396	0	210	0	0	334
22	219	0	191	0	0	261
24	216	0	185	0	0	191
26	246	0	169	0	0	136
28	189	0	162	0	0	296
30	190	0	166	0	0	162
32	259	0	174	0	0	181

Tg = *Tarebia granifera*
Rr - *Radix rubiginosa*

Ratio of	*T. granifera*	:	*R. rubiginosa*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

TABLE 35

Biological control study between *Turebia granifera* (control) and *Tricula aperta* (target). under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Ta	Tg	Ta	Tg	Ta
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	48	41	0	80
6	92	0	48	32	0	72
8	390	0	77	18	0	56
10	650	0	102	6	0	48
12	667	0	102	0	0	38
14	327	0	94	0	0	32
16	444	0	112	0	0	18
18	390	0	105	0	0	10
20	396	0	99	0	0	0
22	219	0	95	0	0	0
24	216	0	81	0	0	0
26	246	0	84	0	0	0
28	189	0	95	0	0	0
30	190	0	100	0	0	0
32	259	0	109	0	0	0

Tg = *Turebia granifera*
Ta = *Tricula aperta*

Ratio of	*T. granifera*	:	*T. aperta*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

TABLE 36

Biological control study between *Tarebia granifera* (control) and *Oncomelania hupensis quadrasi* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Oq	Tg	Oq	Tg	Oq
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	49	48	0	63
6	92	0	47	42	0	60
8	390	0	45	34	0	41
10	650	0	186	21	0	38
12	667	0	187	21	0	32
14	327	0	168	12	0	30
16	444	0	205	11	0	24
18	390	0	149	11	0	20
20	396	0	172	11	0	20
22	219	0	128	10	0	14
24	216	0	101	9	0	14
26	246	0	170	9	0	14
28	189	0	188	6	0	14
30	190	0	181	6	0	14
32	259	0	179	6	0	14

Tg = *Tarebia granifera*
Oq = *Oncomelania hupensis quadrasi*

Ratio	of	*T. granifera*	:	*O.h. quadrasi*
1	=	100	:	0
2	=	50	:	50
3	=	0	:	100

TABLE 37

Biological control study between *Tarebia granifera* (control) and *Bithynia siamensis goniomphalos* (target), under simulated field condition.

Week	Number of snails					
	1		2		3	
	Tg	Bsg	Tg	Bsg	Tg	Bsg
0	100	0	50	50	0	100
2	100	0	50	50	0	100
4	100	0	50	50	0	95
6	92	0	62	50	0	87
8	390	0	351	43	0	56
10	650	0	447	45	0	51
12	667	0	339	33	0	48
14	327	0	146	28	0	48
16	444	0	193	16	0	42
18	390	0	154	10	0	34
20	396	0	136	5	0	26
22	219	0	171	5	0	24
24	216	0	179	5	0	24
26	246	0	478	3	0	22
28	189	0	363	3	0	22
30	190	0	468	2	0	20
32	259	0	629	0	0	20

Tg = *Tarebia granifera*
Bsg *Bithynia siamensis goniomphalos*

Ratio of	*T. granifera*	:	*B.s. goniomphalos*
1 =	100	:	0
2 =	50	:	50
3 =	0	:	100

TABLE 38

Results of biological control testings between second instar larvae (L2) of sciomyzid fly (control agent) and pulmonate snails (target species), under simulated field conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to sciomyzid fly larvae (L2)									
	Death (days)	Sn:L2 4:0	Death (days)	Sn:L2 3:1	Death (days)	Sn:L2 2:2	Death (days)	Sn:L2 1:3	Death (days)	Sn:L2 0:4
B. glabrata										
juvenile	28	7.5:0.0	28	47.5:2.0	28	70.0:10.0	28	90.0:15.8	28	0.0:5.0
adult	28	6.0:0.0	28	42.2:2.0	28	51.0:13.0	28	70.0:17.3	28	0.0:8.5
B. (P.) abyssinicus										
juvenile	12	6.5:0.0	9	43.6:14.0	4	83.0:14.0	4	94.0:16.5	14	0.0:3.5
adult	14	5.0:0.0	10	80.9:18.0	8	72.0:16.0	5	88.0:17.8	16	0.0:4.5
I. exustus										
juvenile	21	7.5:0.0	21	46.9:14.0	21	73.5:15.0	21	90.0:9.6	21	0.0:7.25
adult	28	7.0:0.0	28	39.6:16.0	28	59:5:17.5	28	72.0:8.6	28	0.0:9.5
B. rubiginosa										
juvenile	14	9.5:0.0	14	57.1:8.0	14	81.5:0.0	14	93.0:6.9	14	0.0:5.5
adult	21	7.0:0.0	21	50.5:6.0	21	72.0:0.0	21	81.0:5.9	21	0.0:7.0

TABLE 38

Results of biological control testings between third instar larvae (L3) of sciomyzid fly (control agent) and pulmonate snails (target species), under simulated field conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to sciomyzid fly larvae (L3)									
	Death (days)	Sn:L3 4:0	Death (days)	Sn:L3 3:1	Death (days)	Sn:L3 2:2	Death (days)	Sn:L3 1:3	Death (days)	Sn:L3 0:4
B. glabrata										
juvenile	28	8.0:0.0	28	69.5:15.0	28	75.0:8.0	28	90.0:15.0	28	0.0:10.5
adult	28	6.0:0.0	28	55.0:12.0	28	70.0:10.0	28	88.0:12.0	28	0.0:10.0
B. (P.) abyssinicus										
juvenile	12	4.5:0.0	9	69.3:12.0	7	87.5:10.2	4	100:5.4	14	0.0:10.0
adult	14	6.3:0.0	10	72.5:9.0	8	89.3:12.2	5	98.5:5.6	16	0.0:9.5
I. exustus										
juvenile	21	6.3:0.0	21	65.5:0.0	21	68.0:12.0	21	88.0:15.0	21	0.0:10.3
adult	28	6.3:0.0	28	60.5:2.0	28	65.0:14.0	28	92.0:18.0	28	0.0:10.8
R. rubiginosa										
juvenile	14	9.8:0.0	14	68.2:15.0	14	90.5:14.2	10	97.0:10.2	14	0.0:8.7
adult	21	9.3:0.0	21	62.8:11.0	21	87.0:34.0	14	96.0:15.1	21	0.0:10.3

TABLE 40

Results of biological control testings between second instar larvae (L2) of sciomyzid fly (control agent) and operculate snails (target species), under simulated field conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to sciomyzid fly larvae (L2)									
	Death (days)	Sn:L2 4:0	Death (days)	Sn:L2 3:1	Death (days)	Sn:L2 2:2	Death (days)	Sn:L2 1:3	Death (days)	Sn:L2 0:4
B.s. goniomphalos										
juvenile	28	10.0:0.0	28	23.5:15.0	21	28.0:12.0	28	30.0:6.7	28	0.0:10.5
adult	30	11.0:0.0	30	18.0:17.0	21	15.0:15.0	30	20.0:18.6	30	0.0:10.5
O.h. quadrasi										
juvenile	18	9.0:0.0	15	5.0:25.0	8	8.0:8.5	10	15.0:20.0	18	0.0:12.0
adult	21	10.0:0.0	17	4.0:30.0	9	8.0:7.5	12	10.0:12.5	21	0.0:11.0
R. kaporensis										
juvenile	18	10.0:0.0	18	6.7:2.5	18	10.0:5.0	18	30.0:16.7	18	0.0:11.0
adult	21	10.0:0.0	21	13.3:2.5	21	15.0:10.0	21	20.0:16.7	21	0.0:9.0
T. aperta										
juvenile	27	5.0:0.0	30	6.7:10.0	27	14.0:5.0	27	21.0:6.7	27	0.0:10.0
adult	35	5.0:0.0	37	6.7:20.0	35	10.0:5.0	35	15.0:13.3	35	0.0:11.0

TABLE 41

Results of biological control testings between third instar larvae (L3) of sciomyzid fly (control agent) and operculate snails (target species), under simulated field conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to sciomyzid fly larvae (L3)									
	Death (days)	Sn:L3 4:0	Death (days)	Sn:L3 3:1	Death (days)	Sn:L3 2:2	Death (days)	Sn:L3 1:3	Death (days)	Sn:L3 0:4
B.s. goniomphalos										
juvenile	28	12.0:0.0	28	26.7:8.0	21	27.0:10.0	14	30.0:6.7	28	0.0:10.5
adult	30	14.0:0.0	30	23.3:12.0	21	24.0:12.0	14	26.0:8.6	30	0.0:8.5
O.h. quadrasi										
juvenile	18	15.0:0.0	15	16.7:30.0	8	20.0:8.5	10	20.0:23.3	18	0.0:10.5
adult	21	12.5:0.0	17	16.7:27.0	9	18.0:15.5	12	19.0:18.6	21	0.0:10.0
R. kaporensis										
juvenile	18	15.0:0.0	18	16.7:5.0	18	18.0:10.0	18	30.0:13.3	18	0.0:12.5
adult	21	12.5:0.0	21	13.3:5.0	21	14.0:5.0	21	20.0:6.7	21	0.0:12.5
T. aperta										
juvenile	27	7.4:0.0	30	10.0:36.0	27	12.0:10.0	27	18.0:15.6	27	0.0:12.0
adult	35	5.0:0.0	37	6.7:40.0	35	10.0:10.0	35	10.0:17.5	35	0.0:10.0

TABLE 42

Results of biological control testings between lampyrid larvae (control agent) and pulmonate snails (target species), under laboratory conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to lampyrid fly larvae (La)									
	Death (days)	Sn:La 4:0	Death (days)	Sn:La 3:1	Death (days)	Sn:La 2:2	Death (days)	Sn:La 1:3	Death (days)	Sn:La 0:4
B. glabrata										
juvenile	14	5.0:0.0	17	86.6:0.0	14	90.0:5.0	9	100:0.0	14	0.0:10.0
adult	18	5.0:0.0	20	83.3:0.0	18	80.0:5.0	12	90.0:3.3	18	0.0:10.0
B. (P.) abyssinicus										
juvenile	14	7.5:0.0	14	83.3:0.0	12	85.0:0.0	9	90.0:3.3	14	0.0:5.0
adult	18	5.0:0.0	21	76.6:0.0	16	80.0:0.0	12	90.0:3.3	18	0.0:5.0
I. exustus										
juvenile	14	2.5:0.0	14	96.6:0.0	10	85.0:0.0	9	90.0:3.3	14	0:0.7.5
adult	18	2.5:0.0	18	93.2:0.0	15	80.0:0.0	12	80.0:6.6	18	0.0:5.0
R. rubiginosa										
juvenile	7	10.5:0.0	14	96.6:0.0	7	100:0.0	7	0.0:0.0		
adult	9	10.0:0.0	21	86.6:0.0	9	95.0:0.0	9	100:6.7	9	0.0:0.0

TABLE 43

Results of biological control testings between lampyrid larvae (control agent) and operculate snails (target species), under laboratory conditions (4 trials).

Species of snails	Mortality rates (%)									
	Ratios of snails (Sn) to lampyrid fly larvae (La)									
	Death (days)	Sn:La 4:0	Death (days)	Sn:La 3:1	Death (days)	Sn:La 2:2	Death (days)	Sn:La 1:3	Death (days)	Sn:La 0:4
B.s. goniomphalos										
juvenile	28	10.0:0.0	28	23.3:0.0	28	36.7:0.0	28	50.0:0.0	28	0.0:0.0
adult	35	7.5:0.0	34	19.9:10.0	35	23.3:0.0	35	40.0:13.3	35	0.0:0.0
O.h. quadrasi										
juvenile	28	9.5:0.0	28	29.9:0.0	28	35.0:0.0	28	40.0:0.0	28	0.0:0.0
adult	35	10.0:0.0	35	16.3:0.0	35	30.0:0.0	35	30.0:0.0	35	0.0:2.5
R. kaporensis										
juvenile	28	10.5:0.0	28	23.3:0.0	28	30.0:0.0	28	40.0:3.3	28	0.0:0.0
adult	35	7.5:0.0	35	16.7:10.0	35	30.0:0.0	35	30.0:3.3	35	0.0:5.0
T. aperta										
juvenile	28	7.5:0.0	28	26.6:10.0	28	30.0:0.0	28	40.0:6.6	28	0.0:0.0
adult	35	10.0:0.0	35	13.3:10.0	35	25.0:0.0	35	30.0:0.0	35	0.0:0.0

TABLE 44

Development of geographic strains of *Schistosoma japonicum* in various strains of *Oncomelania hupensis quadrasi*.

Oncomelania hupensis quadrasi (location)	Geographic strains of *Schistosoma japonicum*			
	Mindoro	Leyte	Irosin	Mindanao
Mindoro				
No. exposed	235	50	-	In progress
No. survived	185	42	-	
No. positive (%)	37 (20)	3 (7.14)	-	
Leyte				
No. exposed	96	100	-	204
No. survived	57	90	-	110
No. positive (%)	2 (3.5)	11 (12.10)	-	1 (0.90)
Irosin				
No. exposed	163	100	-	200
No. survived	107	81	-	98
No. positive (%)	31 (28.97)	49 (60.49)	-	3 (3.06)
Mindanao				
No. exposed	100	In progress	-	220
No. survived	80			140
No. positive (%)	11 (13.75)			72 (51.43)

TABLE 45.

Samples of *Oncomelania hupensis* studied.

Sample name	Locality	Collection date	N	Voucher*
O. hupensis quadrasi				
Leyte A	Vicob Ck., Palo	6.83	>1000	102
Leyte B	Mandaraq Ck., Palo	"	"	101
Leyte C	South Main Canal, Palo	"	"	103
Luzon A	San Isidro, Sorsogon	6.86	200	105
Luzon B	San Augustin, Sorsogon	"	50	106
Mindoro	Victoria	6.83	"	100
Mindoro	Mawab, Davao	8.84	500	107
Lab Stock	Palo and Victoria	'79 & '82	>1000	-
O. hupensis hupensis				
China A	Guichi, Anhui	4.82	> 500	110
China B	Guichi, Anhui	2.84	> 400	-

* Voucher specimens were placed in the museum at the Center for Applied Malacology and Entomology, Mahidol University and were catalogued with the prefix MUFS PHOO-# for Philippine samples and MUFS CHOO # for Chinese.

TABLE 46

Electrophoretic buffers giving optimal resolution of proteins in *Oncomelania hupensis*.

Enzyme (E.C. #)	Abbreviation	Buffer*
Acid phosphatase (3.1.3.2)	ACP	TC-2
Aspartate aminotransferase (2.6.1.1)	AAT	TBE, LiOH
Catalase (1.11.1.6)	CAT	AP
Esterase (3.1.1.1)	ES-1	TBE
	ES-3	TBE, LiOH
Glucose-6-phosphate dehydrogenase (1.1.1.49)	G6PDH	TBE, LiOH
Glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12)	GAP	AP
α-Glycerophosphate dehydrogenase (1.1.1.8)	GPDH	TC-2, TC-1
Glucose phosphate isomerase (5.3.1.9)	GPI	TC-1 AP
Isocitrate dehydrogenase (1.1.1.42)	IDH-1	TC-2
	IDH-2	TC-2
Leucine aminopeptidase (3.4.11)	LAP (PEP-2)	TC-1, LiOH
Malate dehydrogenase (1.1.1.37)	MDH	TC-1
Malic enzyme (1.1.1.40)	ME	TC-2, TC-1
Mannose phosphate isomerase (5.2.1.8)	MPI	TC-2
Peptidase (3.4.11)	PEP-1	TBE, LiOH
	PEP-4	TBE
6-Phosphogluconate dehydrogenase (1.1.1.44)	PGD	AP
Phosphoglucomutase (2.7.5.1)	PGM-1	TC-1
	PGM-2	AP
Sorbitol dehydrogenase (1.1.1.14)	SorDH	TBE

* AP. 0.04 M citrate adjusted with N-(3-aminopropyl)-morpholine to pH 6.0; diluted 1:19 for gels and undiluted for electrodes (15 hr. 80 V). LiOH. Solution A: 0.03 M LiOH, 0.19 M borate, pH 8.1; Solution B: 0.008 M citrate, 0.05 M Tris, pH 8.4; 10% A plus 90% B for gel, A for electrode (15 hr. 180 V). TBE. 0.087 M Tris, 0.086 M borate, 0.001 M EDTA, pH 9.0; diluted 1:3 for gels. 0.5 M Tris, 0.065 M borate, 0.02 M EDTA, pH 8.0; undiluted for electrodes (15 hr., 80 V). TC-1. 0.378 M Tris, 0.165 M citrate, pH 6.0; 13.5 ml diluted to 400 ml for gel and undiluted for electrodes (15 hr. 60 V). TC-2. 0.188 M Tris, 0.065 M citrate, pH 6.8; diluted 1:9 for gels and 1:5 for electrodes (15 hr. 150 V).

TABLE 47

Allele frequencies for 16 polymorphic loci in 10 samples of *Oncomelania hupensis* with summary statistics of genetic variability.*

Locus/allele	Leyte			Luzon		Mindoro	Mindanao	China		
	A	B	C	A	B			Lab	A	B
Aat *a*									0.92	1.00
b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.08		
Acp *a*	1.00	1.00	1.00	1.00	1.00	1.00	0.08	1.00		
b									0.08	0.75
c							0.92		0.92	0.10
d										0.15
Es-1 *a*									0.30	0.55
b									0.70	0.45
c	0.82	0.90	0.83	0.92	0.90	1.00	1.00	0.96		
d	0.18	0.10	0.17	0.08	0.10			0.04		
Es-3 *a*									0.02	
b									0.96	1.00
c	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.02	
Gap *a*	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
b									1.00	1.00
Gpi *a*		0.01		0.20	0.05	0.30		0.16		
b									0.08	0.10
c	1.00	0.99	1.00	0.80	0.95	0.70	1.00	0.84		
d									0.32	0.45
e									0.59	0.40
f									0.01	0.05

Locus/allele	Leyte			Luzon		Mindoro	Mindanao	China		
	A	B	C	A	B			Lab	A	B
Idh-1a	1.00	1.00	1.00	1.00	1.00	1.00	0.67	0.99	0.02	0.10
b							0.33	0.01	0.98	0.90
Idh-2a									0.02	
b	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.98	1.00
Lap-a									0.05	0.10
b									0.55	0.40
c					0.02	0.41		0.14	0.39	0.35
d	0.99	0.97	0.96	0.99	0.98	0.40	1.00	0.73	0.09	0.15
e									0.02	
f	0.01	0.03	0.04	0.01		0.19		0.13		
Me-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		
b									1.00	1.00
Mpi-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.56	0.70
b									0.44	0.30
Pep-4a	0.57	0.76	0.41	0.03	0.25	0.02	0.98	0.11	0.36	0.40
b	0.31	0.23	0.45	0.93	0.75	0.27	0.02	0.55	0.62	0.60
c	0.12	0.01	0.14	0.04		0.71		0.34	0.02	
Pgd-a									0.17	0.20
b	0.99	1.00	1.00	1.00	1.00	0.98	1.00	0.96	0.77	0.80
c	0.01					0.02			0.01	
d								0.04	0.02	
e									0.03	

Locus/allele	Leyte			Luzon		Mindoro	Mindanao	China		
	A	B	C	A	B			Lab	A	B
Pgm-1a	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00		
b									0.68	0.60
c									0.30	0.35
d						0.01			0.02	0.05
Pgm-2a						0.31	0.06	0.25	0.04	0.10
b	0.98	1.00	1.00	1.00	1.00	0.56	0.94	0.69	0.61	0.55
c									0.03	0.05
d	0.02					0.13		0.06	0.18	0.20
e									0.08	0.10
f									0.06	
Sordh-a	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	0.88	0.85
b								0.03	0.12	0.15
$\overline{N}$	75.9	6.1	62.2	56.2	17.4	52.5	77.5	62.2	32.4	9.6
(S.E.)	6.8	5.6	6.8	6.2	1.3	2.2	5.1	4.2	0.4	0.4
$\overline{A}$	1.3	1.2	1.2	1.2	1.2	1.4	1.2	1.5	2.4	2.0
P	2[illegible]	19.0	14.3	19.0	19.0	28.6	19.0	38.1	66.7	52.4
$\overline{H}$	0.04	0.03	0.04	0.03	0.02	0.10	0.03	0.08	0.20	0.21

* Samples are described in Table 1; $\overline{N}$ = mean sample size (and standard error) per locus, $\overline{A}$ = mean no. alleles per locus, *P* = proportion of loci polymorphic (monomorphic loci: *Cat*, *Glpdh*, *α-Gpdh*, *Mdh 1*, *Pep 1*, excluded from this table), $\overline{H}$ = mean individual heterozygosity.

TABLE 48

Matrix of genetic similarity and distance coefficients*

Sample	1	2	3	4	5	6	7	8	9	10
1 Leyte A	*****	0.986	0.990	0.957	0.973	0.905	0.911	0.939	0.500	0.514
2 Leyte B	0.002	*****	0.980	0.955	0.972	0.902	0.921	0.936	0.492	0.506
3 Leyte C	0.001	0.005	*****	0.964	0.980	0.909	0.903	0.947	0.505	0.518
4 Luzon A	0.019	0.028	0.012	*****	0.981	0.914	0.879	0.952	0.499	0.510
5 Luzon B	0.008	0.013	0.003	0.003	*****	0.909	0.894	0.950	0.506	0.518
6 Mindoro	0.045	0.053	0.040	0.045	0.045	*****	0.842	0.951	0.506	0.523
7 Mindana	0.057	0.051	0.064	0.077	0.077	0.118	*****	0.870	0.538	0.515
8 Labstock	0.016	0.023	0.011	0.012	0.011	0.012	0.088	*****	0.515	0.529
9 China A	0.657	0.663	0.652	0.644	0.545	0.668	0.550	0.652	*****	0.910
10 China B	0.620	0.625	0.617	0.611	0.610	0.630	0.591	0.615	0.035	*****

* Below diagonal: Nei (1978) unbiased genetic distance; above diagonal: Rogers (1972) genetic similarity.

TABLE 49

Variation at 18 loci in four Asian *Schistosoma* species. The total number of schistosomes found to have each particular locus/allele is shown.

Locus/ allele		*Schistosoma japonicum* Leyte	Luzon	Mindoro	China	Formosa	Japan	*S. mekongi* Laos	*S.* sp. Malaysia
Acp	*a*	79			22	40	59	-	
	b	-			-	-	-	26	
Ak	*a*	62	2	58				-	-
	b	-	-	-				-	34
	c	-	-	-				34	-
Ald	*a*	128		13	-	-	59	-	
	b	-		-	22	40	-	-	
	c	-		-	-	-	-	26	
Dia	*a*	104	1	79					23
Ga3pd	*a*	79			22	40	59	-	
	b	-			-	-	-	26	
Gdh	*a*	79			22	40	59	26	
G6pd	*a*		-	45	-	-	-	-	-
	b	159	1	-	-	-	-	-	-
	c	-	-	-	22	40	59	45	23
Gpi	*a*	-	-	-	22	-	-	-	-
	b	249	4	155	-	40	59	-	-
	c	-	-	-	-	-	-	37	21
Hk	*a*	200	1	91	22	40	59	-	-
	b	-	-	-	-	-	-	65	-
	c	-	-	-	-	-	-	-	85

Locus/ allele		*Schistosoma japonicum* Leyte	Luzon	Mindoro	China	Formosa	Japan	*S. mekongi* Laos	*S.* sp. Malaysia
Ldh	*a*	79			22	40	59	-	6
	b					-		33	-
Mdh-1	*a*	262	4	136	22	40	59	-	-
	b	-	-	-	-	-	-	41	56
Mdh-2	*a*	62		96	22	40	59		-
	b	-		-	-	-	-		26
Me	*a*	3						-	-
	b	-						-	4
	c	-						2	-
Mpi	*a*	93		96				-	-
	b	-		-				16	-
	c	-		-				-	76
Pep	a	-	-	-					74
	b	-	-	138					-
	c	141	2	-					-
Pgd	*a*	7						-	-
	b	-						12	12
Pgm	*a*	-	-	-	-	-	-	70	78
	b	-	-	-	22	-	-	-	-
	c	229	1	105	-	40	59	-	-
Sod	*a*	34		30				-	-
	b	-		-				1	2

TABLE 50

Genetic distance (D), standard error of D and the number of loci examined between various pairs of strains of Asian schistosomes.

Strain	LEYTE	MINDORO	CHINA	FORMOSA	JAPAN	MEKONGI	MALYSIA
S. japonicum							
LEYTE		0.288	0.452	0.201	0.182	2.708	1.946
		0.08	0.11	0.07	0.07	0.48	0.33
		12	11	11	11	15	14
MINDORO			1.099	0.405	0.182	∞	1.705
			0.27	0.13	0.08	N.A.	0.32
			7	7	7	9	11
CHINA				0.201	0.405	1.705	1.099
				0.07	0.11	0.34	0.27
				11	11	10	7
FORMOSA				-	0.182	1.705	1.099
					0.07	0.34	1.27
					11	10	7
JAPAN					-	1.792	1.099
						0.35	0.27
						10	7
S. mekongi						-	0.606
							0.14
							11
Malaysian schistosome							

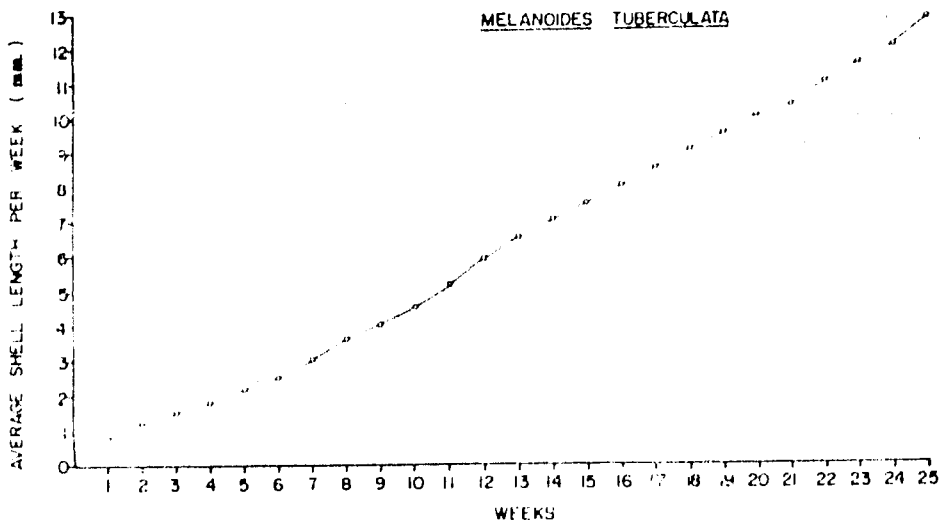


Fig. 1. Growth table for *Melanoides tuberculata*

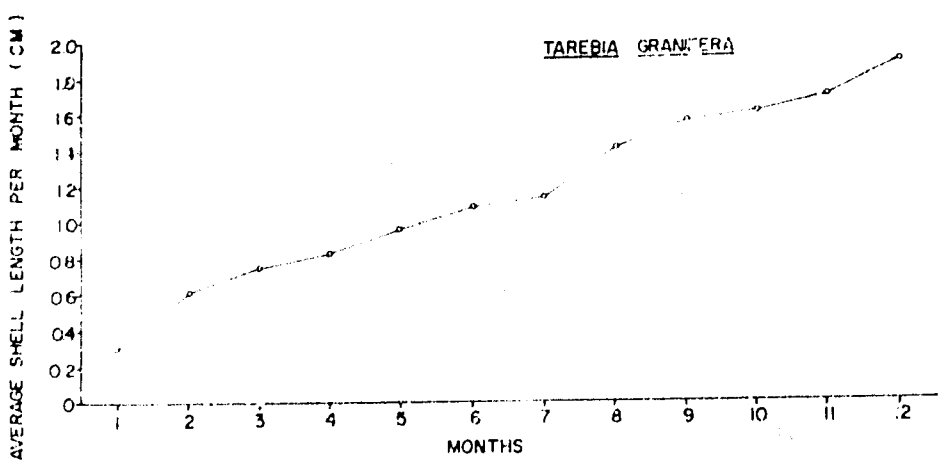


Fig. 2. Growth table for *Tarebia granifera*

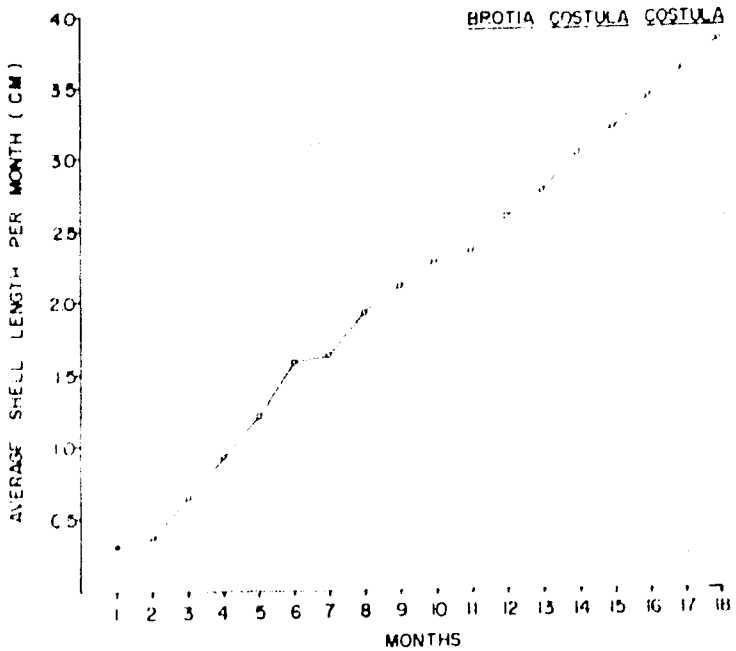


Fig. 3. Growth table for *Brotia costula costula*

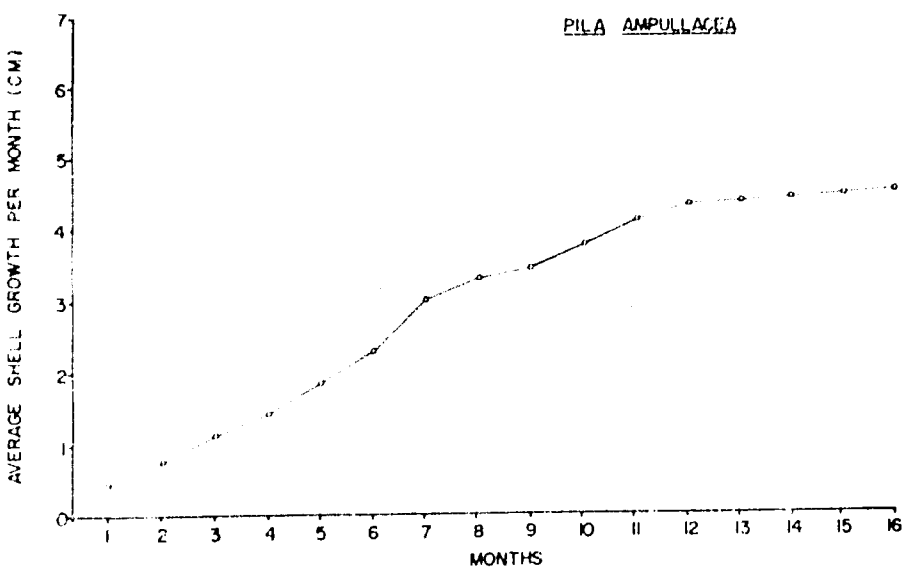


Fig. 4. Growth table for *Pila ampullacea*

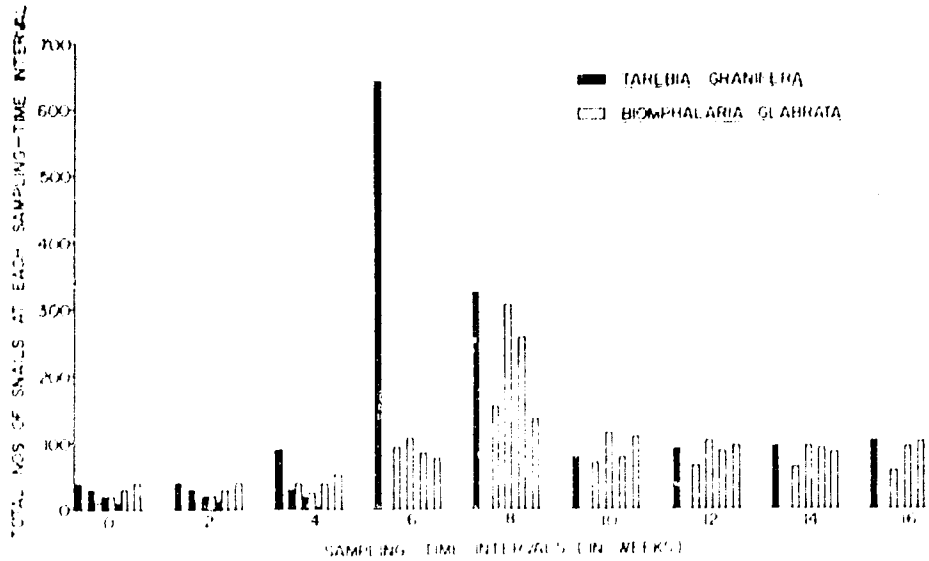


Fig. 5. Biological control study between *T. granifera* (control) and *B. glabrata* (target) under laboratory condition.

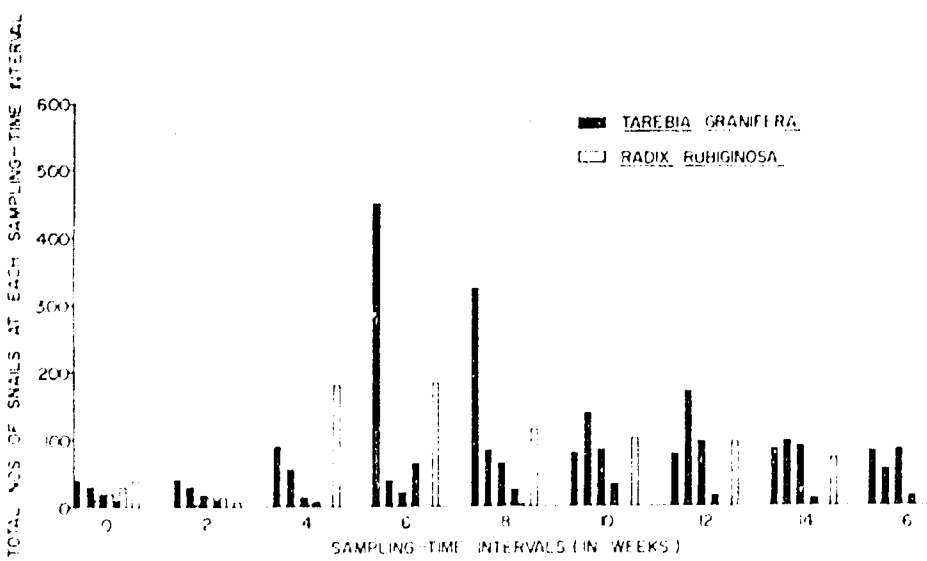


Fig. 6. Biological control study between *T. granifera* (control) and *R. rubiginosa* (target) under laboratory condition.

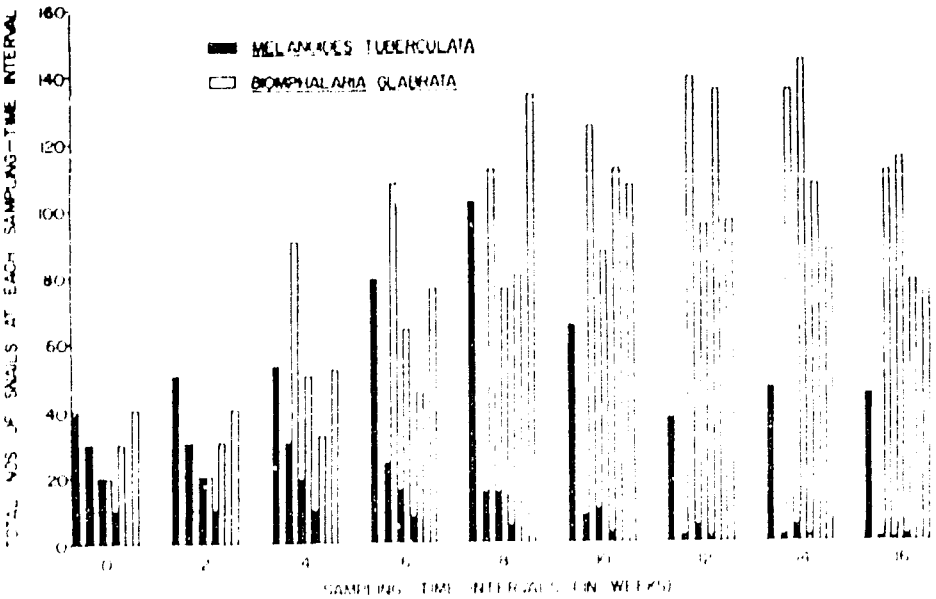


Fig. 7. Biological control study between *M. tuberculata* (control) and *B. glabrata* (target) under laboratory condition.

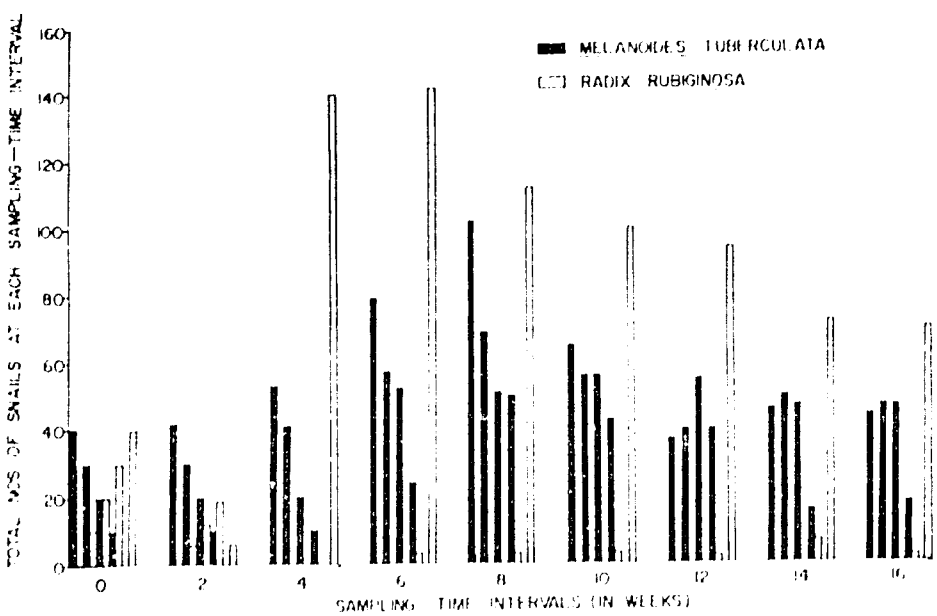


Fig. 8. Biological control study between *M. tuberculata* (control) and *R. rubiginosa* (target) under laboratory condition.

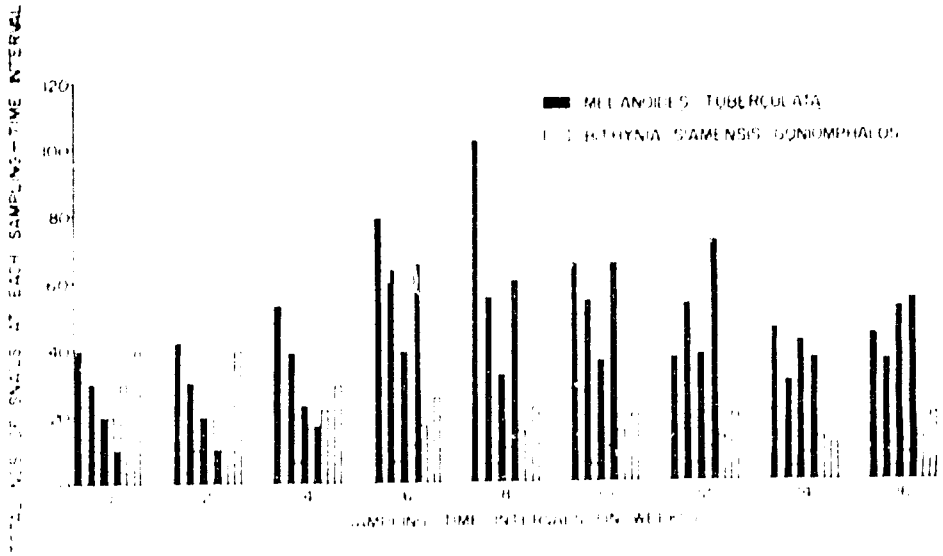


Fig. 9. Biological control study between *M. tuberculata* (control) and *B.s. goniomphalos* (target) under laboratory condition.

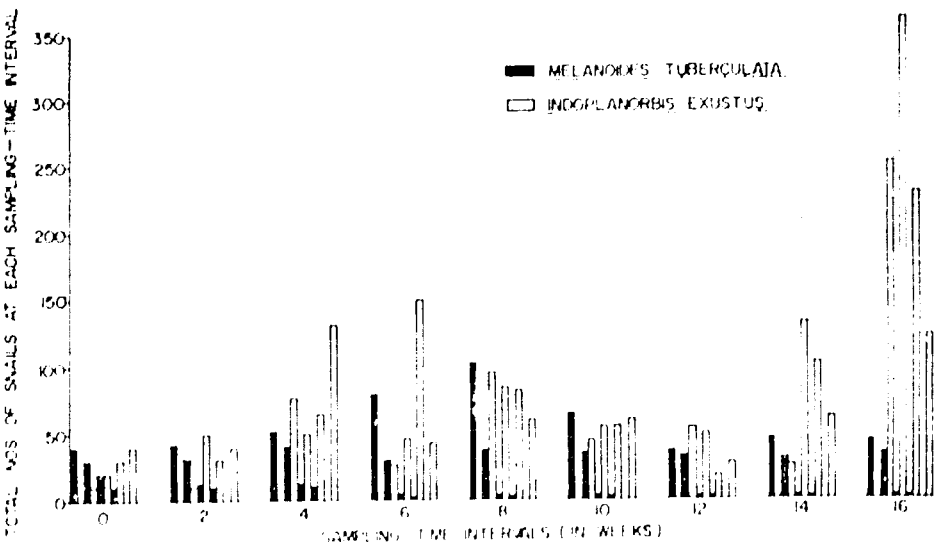


Fig. 10. Biological control study bwtween *M. tuberculata* (control) and *I. exustus* (target) under laboratory condition.

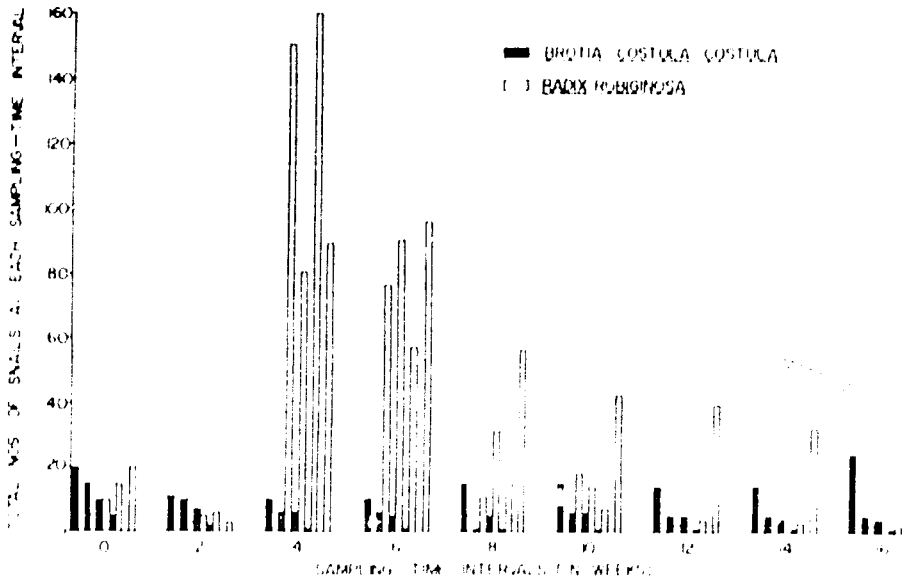


Fig. 11. Biological control study between *B.c. costula* (control) and *R. rubiginosa* (target) under laboratory condition.

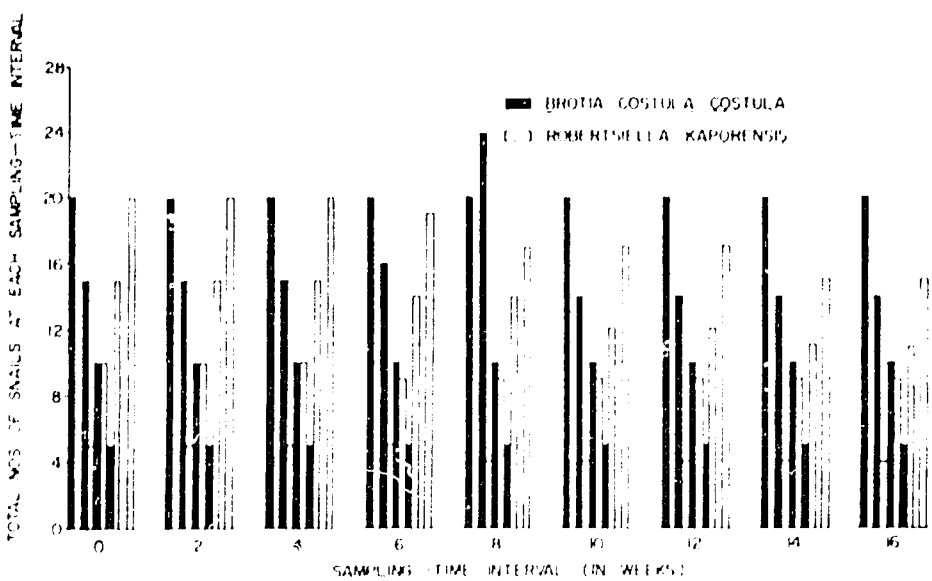


Fig. 12. Biological control study between *B.c. costula* (control) and *R. kaporensis* (target) under laboratory condition.

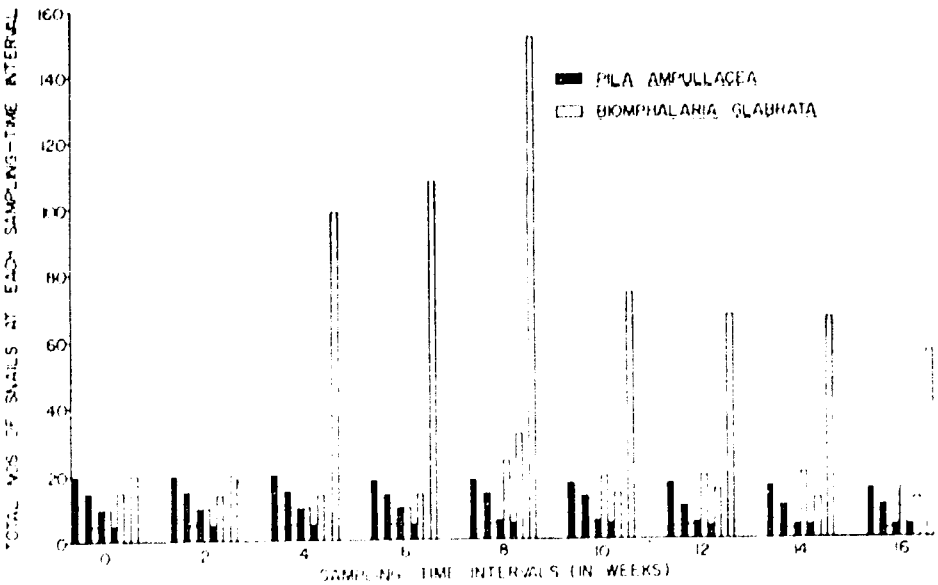


Fig. 13. Biological control study between *P. ampullacea* (control) and *B. glabrata* (target) under laboratory condition.

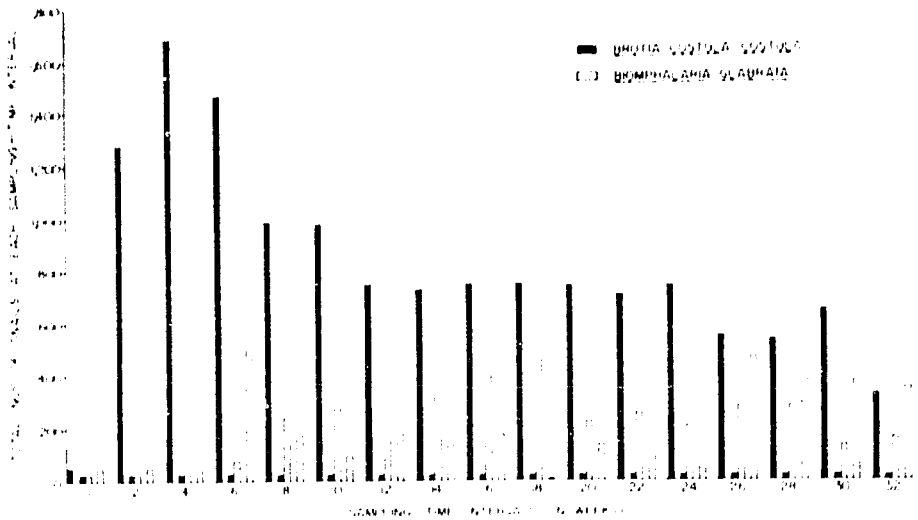


Fig. 14. Biological control study between *B.c. costula* (control) and *B. glabrata* (target) under simulated field condition.

Fig. 15. Biological control study between *B.c. costula* (control) and *I. exustus* (target) under simulated field condition.

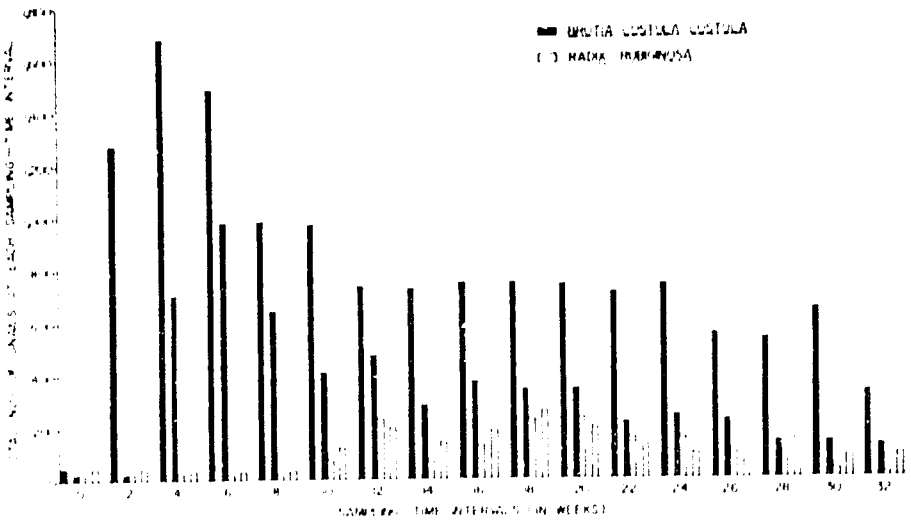


Fig. 16. Biological control study between *B.c. costula* (control) and *K. rubiginosa* (target) under simulated field condition.

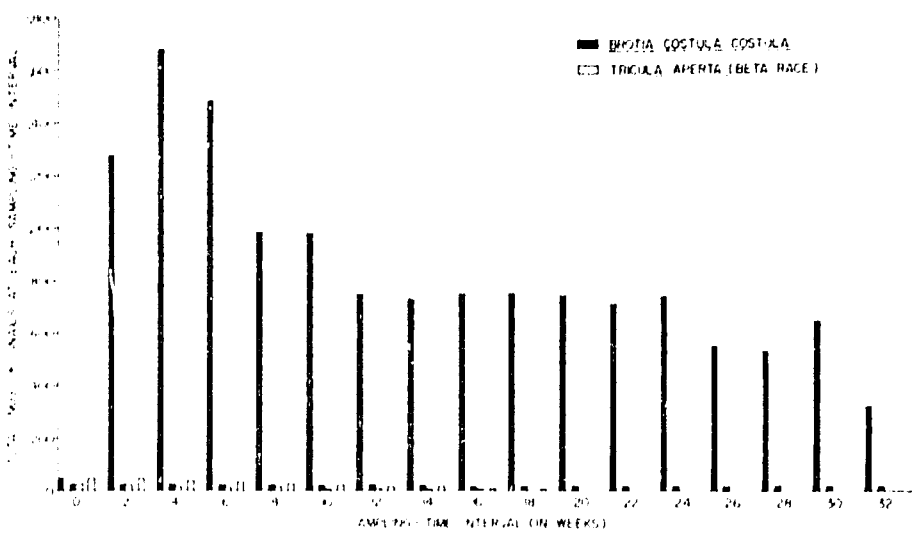


Fig. 17. Biological control study between *B.c. costula* (control) and *T. aperta* (target) under simulated field condition.

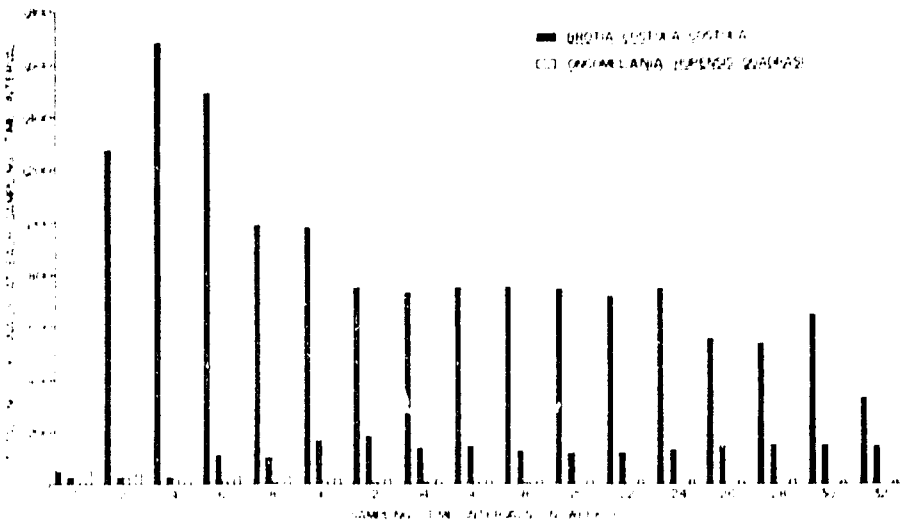


Fig. 18. Biological control study between *B.c. costula* (control) and *O.h. quadrasi* (target) under simulated field condition.

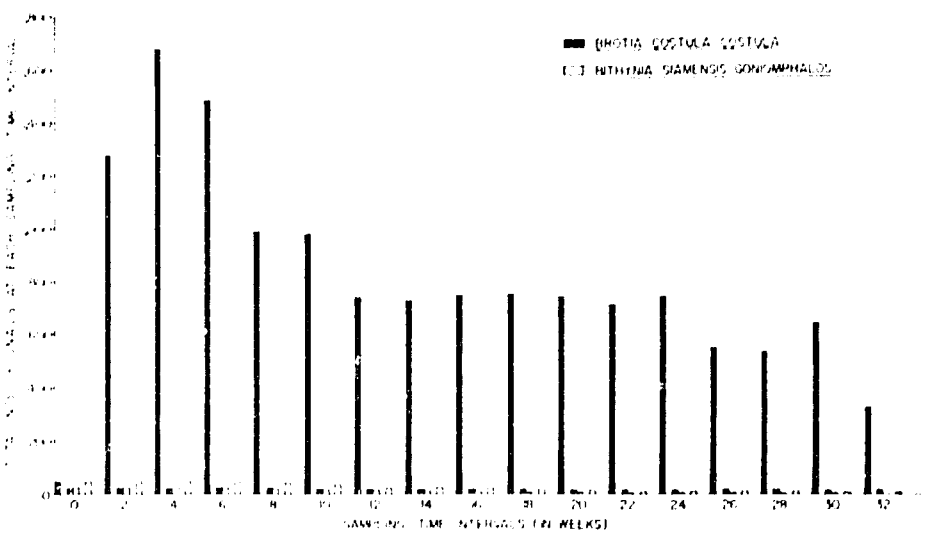


Fig. 19. Biological control study between *B.c. costula* (control) and *B.s. goniomphalos* (target) under simulated field condition.

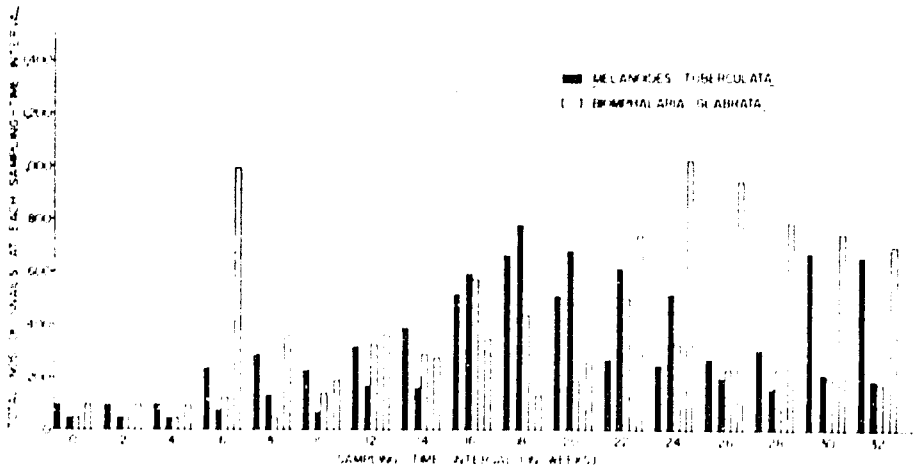


Fig. 20. Biological control study between *M. tuberculata* (control) and *B. glabrata* (target) under simulated field condition.

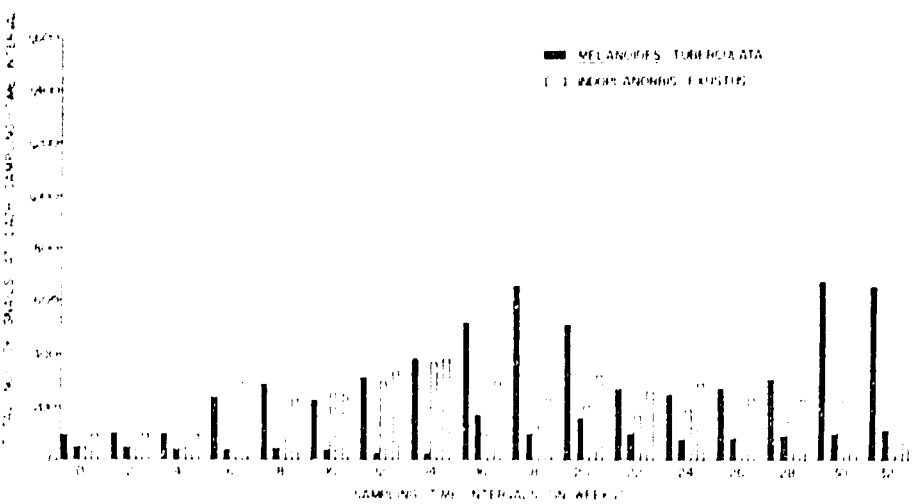


Fig. 21. Biological control study between *M. tuberculata* (control) and *I. exustus* (target) under simulated field condition.

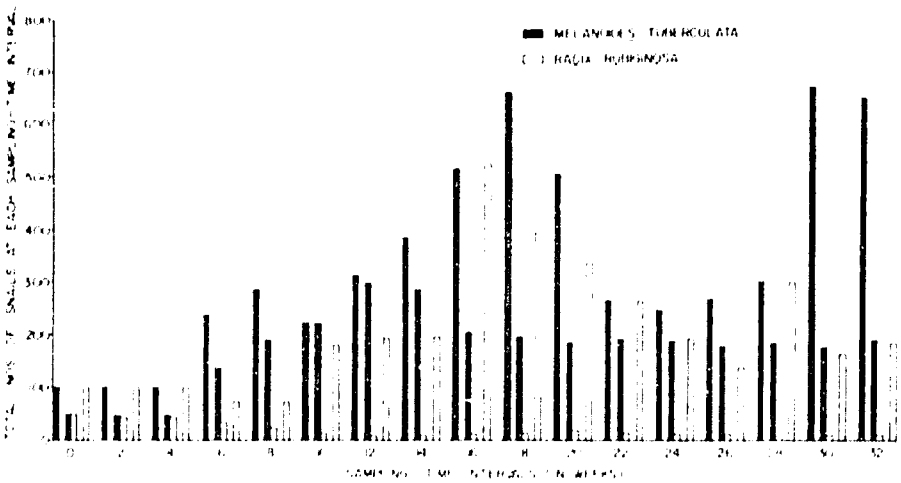


Fig. 22. Biological control study between *M. tuberculata* (control) and *R. rubiginosa* (target) under simulated field condition.

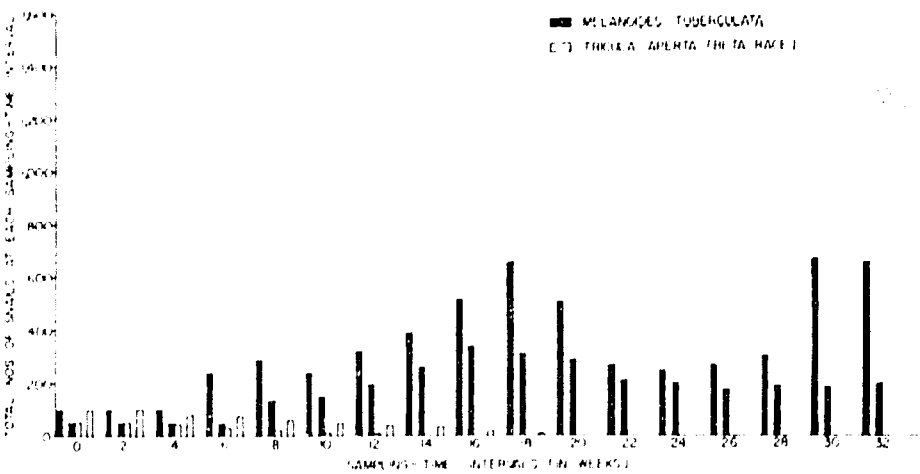


Fig. 23. Biological control study between *M. tuberculata* (control) and *T. aperta* (target) under simulated field condition.

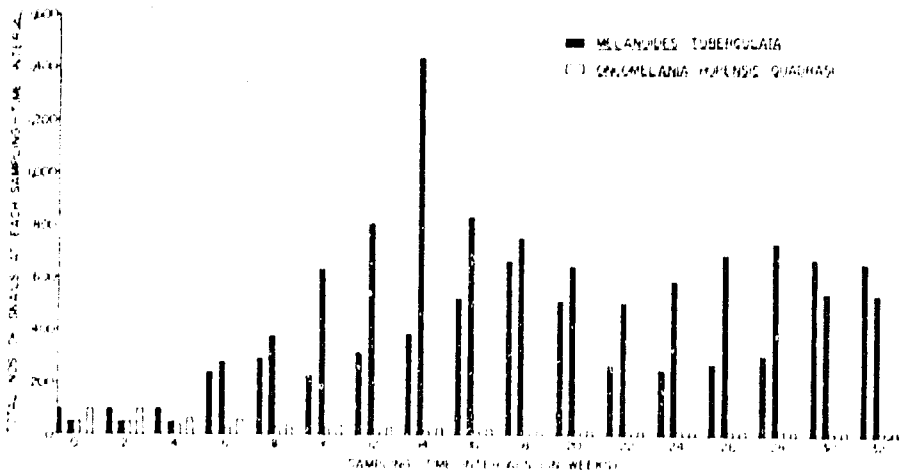


Fig. 24. Biological control study between *M. tuberculata* (control) and *O.h. quadrasi* (target) under simulated field condition.

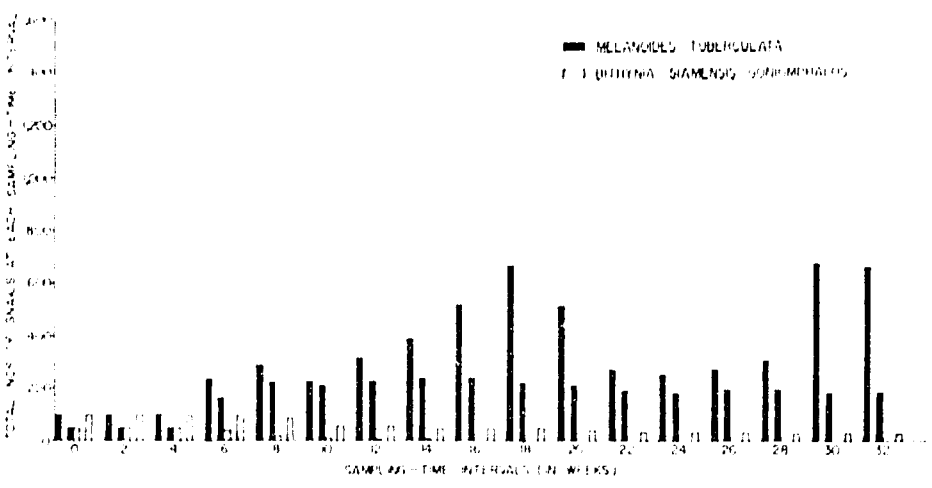


Fig. 25. Biological control study between *M. tuberculata* (control) and *B.s. goniomphalos* (target) under simulated field condition.

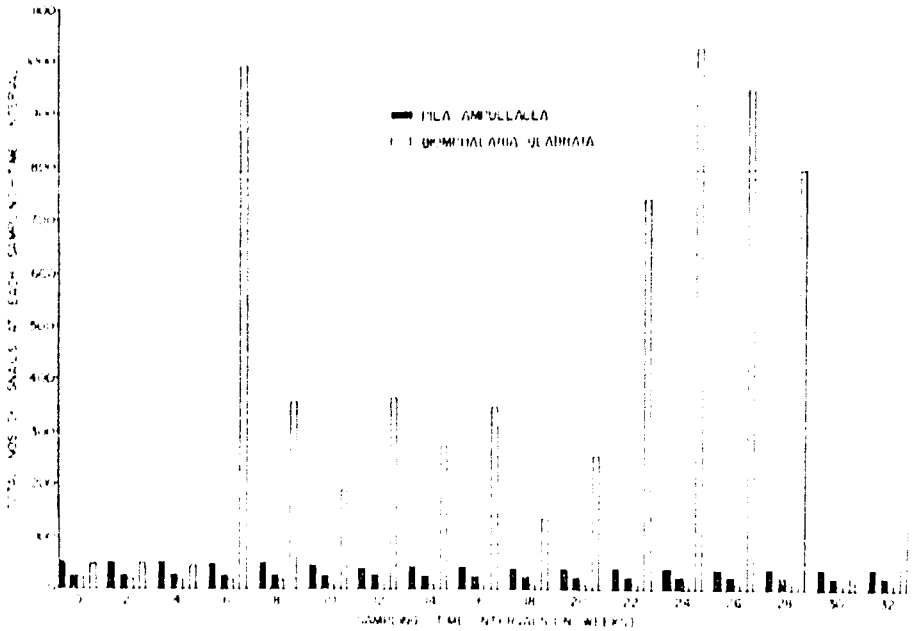


Fig. 26. Biological control study between *P. ampullacea* (control) and *B. glabrata* (target) under simulated field condition.

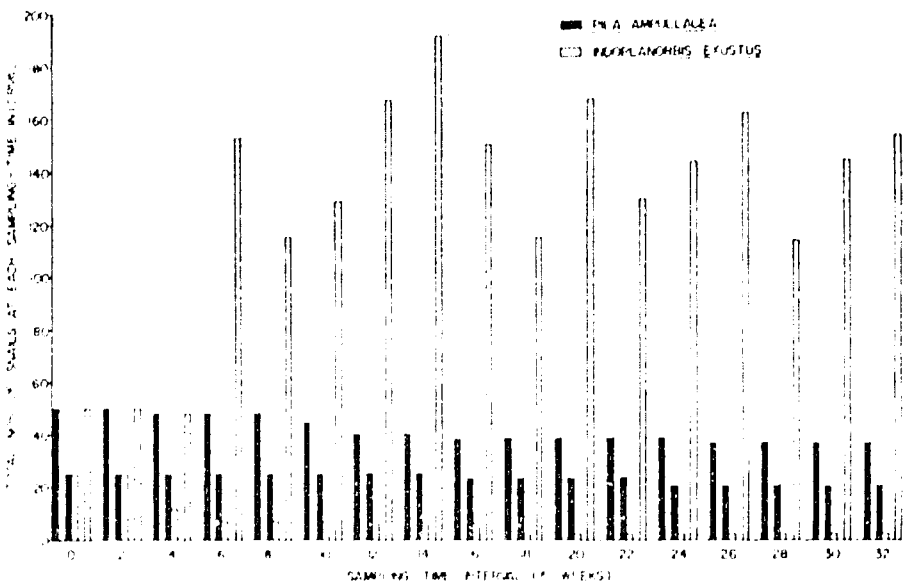


Fig. 27. Biological control study between *P. ampullacea* (control) and *I. exustus* (target) under simulated field condition.

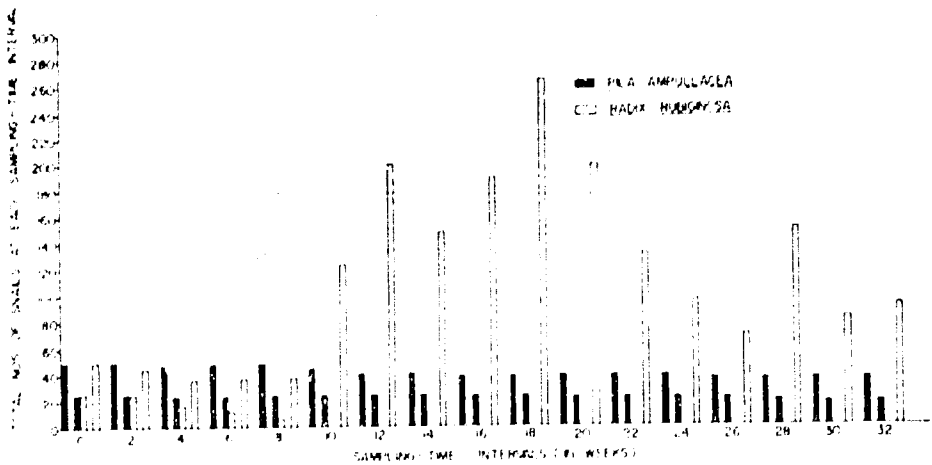


Fig. 28. Biological control study between *P. ampullacea* (control) and *R. rubiginosa* (target) under simulated field condition.

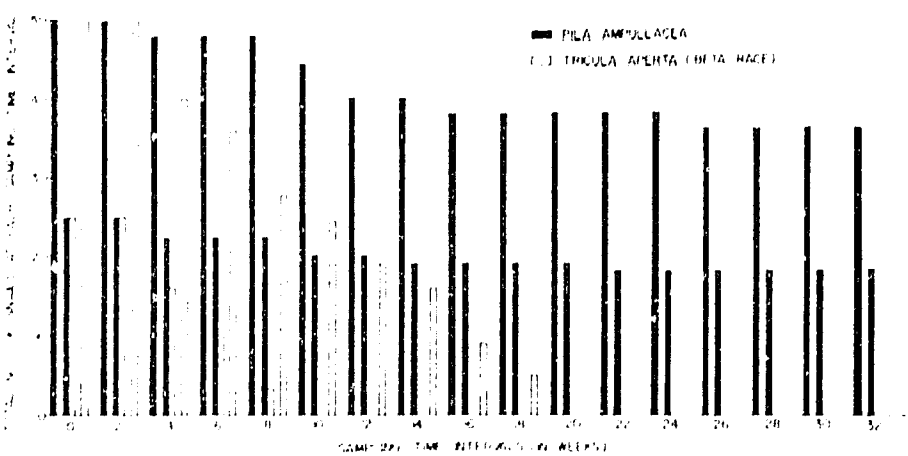


Fig. 29. Biological control study between *P. ampullacea* (control) and *T. aperta* (target) under simulated field condition.

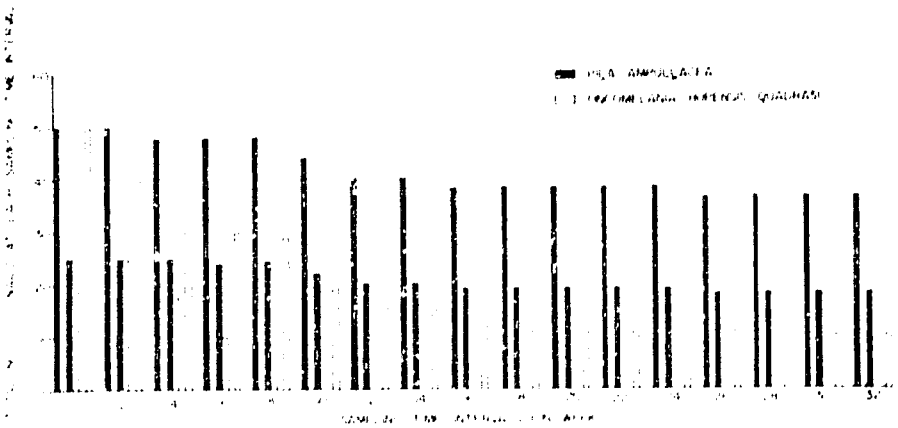


Fig. 30. Biological control study between *P. ampullacea* (control) and *O.h. quadrasi* (target) under simulated field condition.

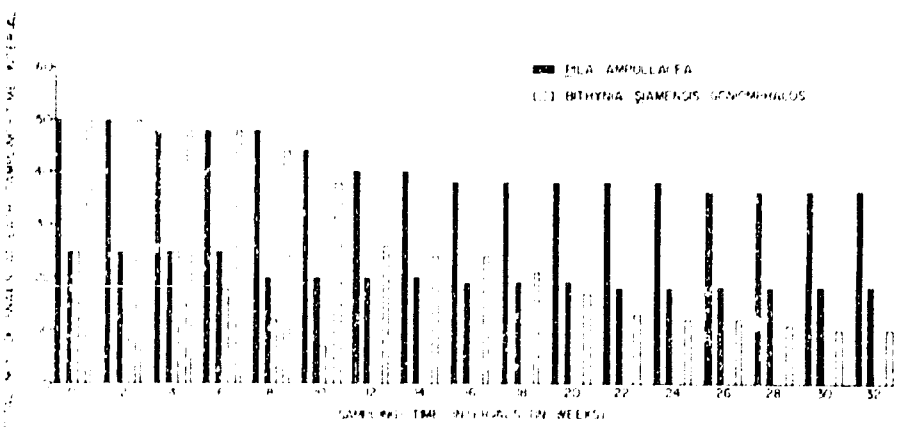


Fig. 31. Biological control study between *P. ampullacea* (control) and *B.s. goniomphalos* (target) under simulated field condition

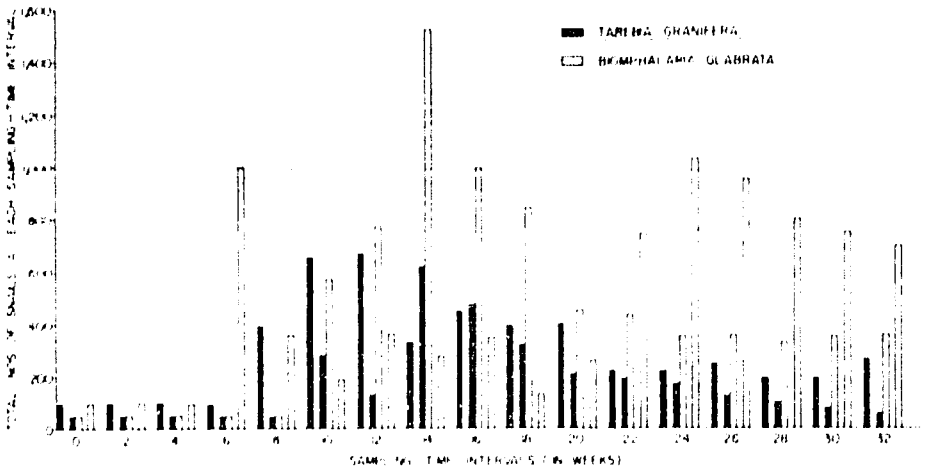


Fig. 32. Biological control study between *T. granifera* (control) and *B. glabrata* (target) under simulated field condition.

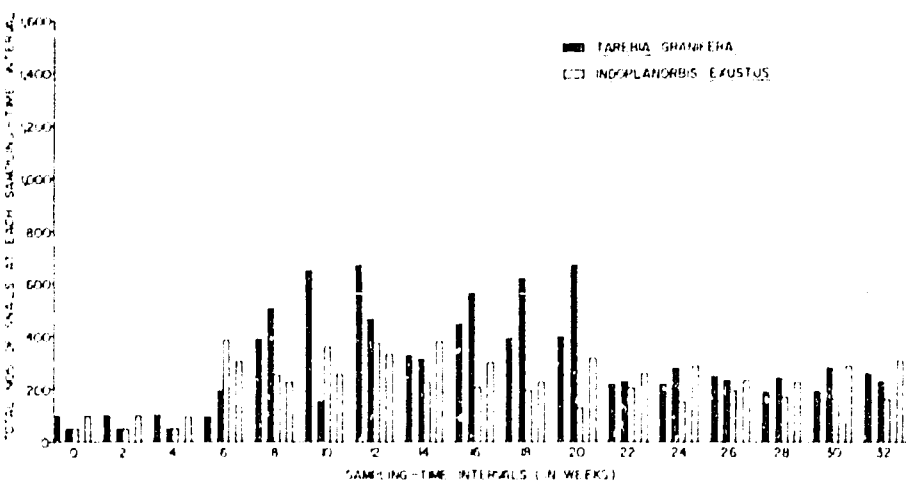


Fig. 33. Biological control study between *T. granifera* (control) and *I. exustus* (target) under simulated field condition.

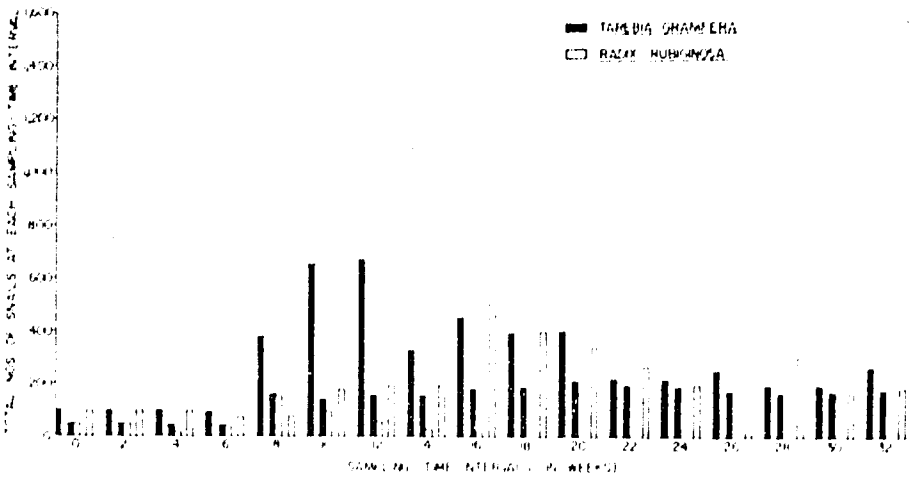


Fig. 34. Biological control study between *T. granifera* (control) and *R. rubiginosa* (target) under simulated field condition.

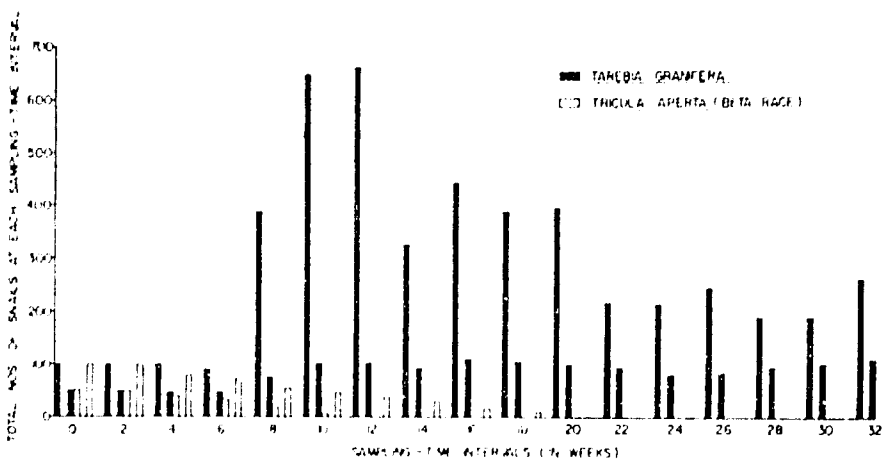


Fig. 35. Biological control study between *T. granifera* (control) and *T. aperta* (target) under simulated field condition.

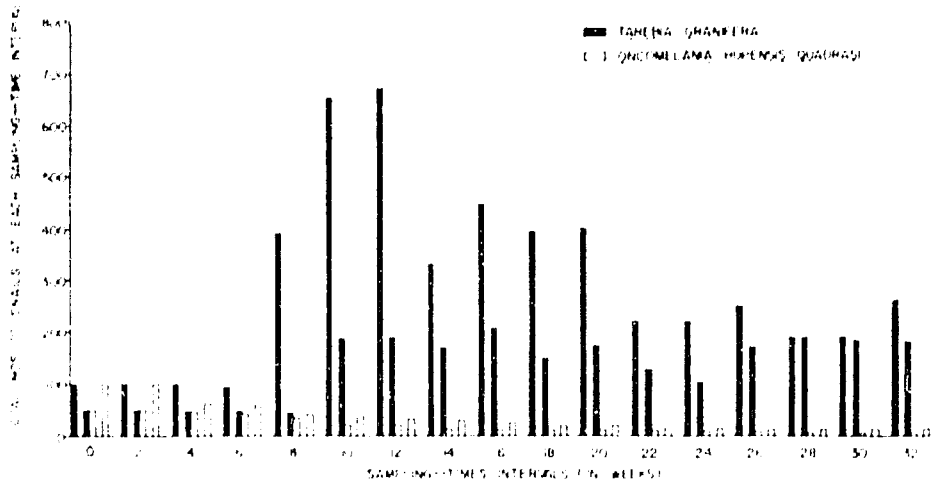


Fig. 36. Biological control study between *T. granifera* (control) and *O.h. quadrasi* (target) under simulated field condition.

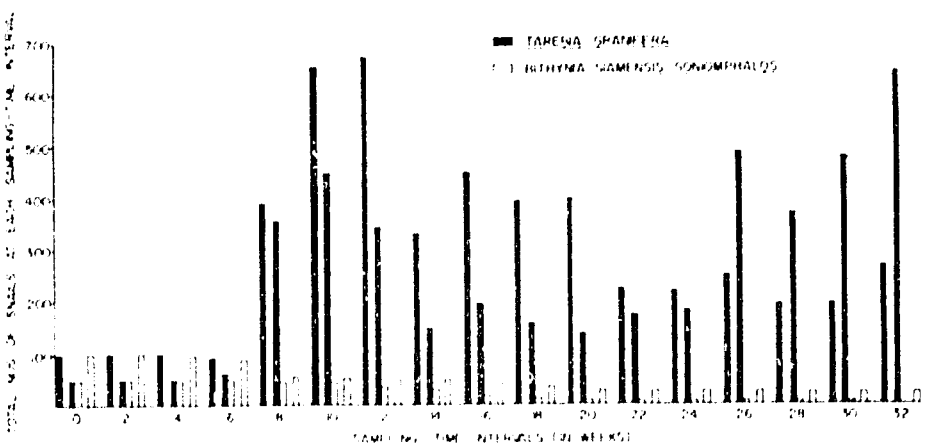
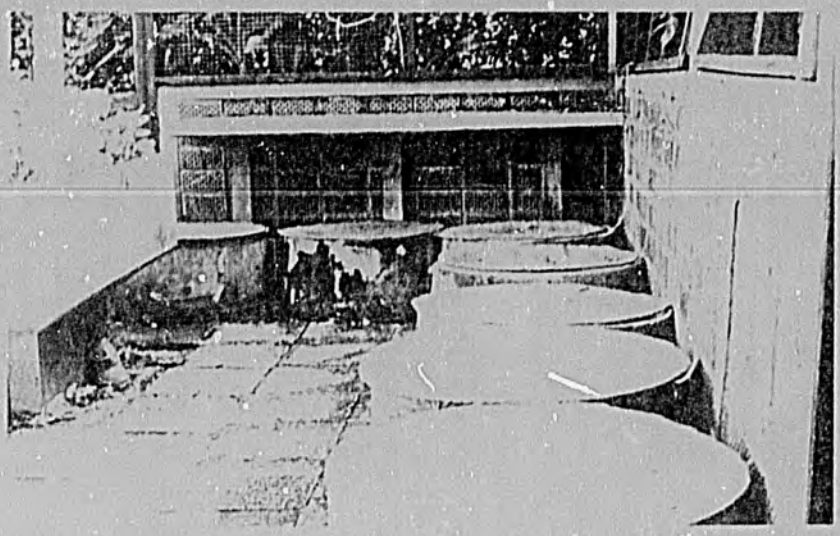


Fig. 37. Biological control study between *T. granifera* (control) and *B.s. goniomphalos* (target) under simulated field condition.



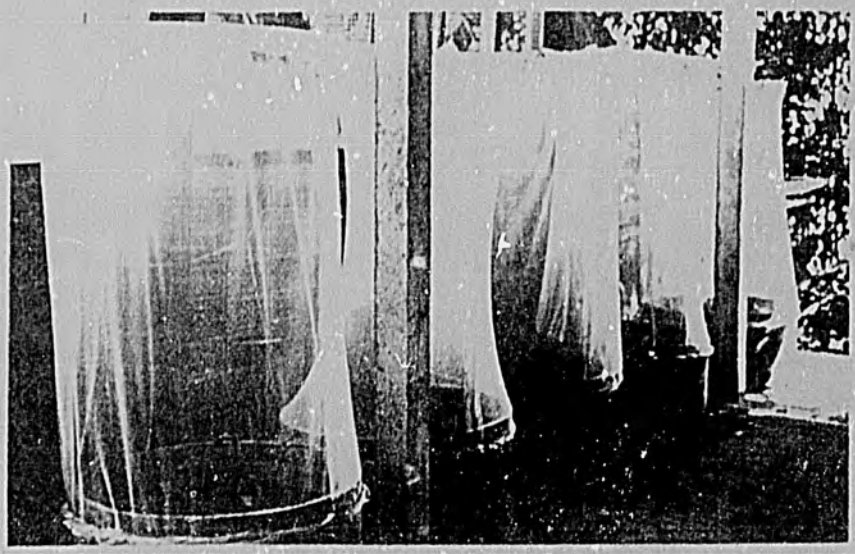


Figure 38. Top showing cement tanks used for testing biological control effects of target snails byu snails. Bottom showing cement tanks for testing biological control effects of target snails by sciomyzid fly larvae.

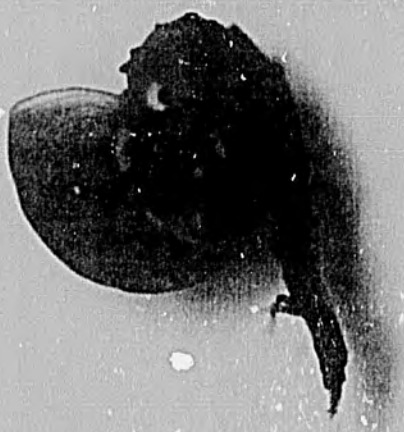


Figure 39. Top showing lampyrid fly larvae attacking pulmonate snails, *Radix rubiginosa*, the intermediate hosts of *Schistosoma incognitum* and *Fasciola gigantica* in Thailand. Bottom showing lampyrid fly attacking *Indoplanorbis exustus*, the intermediate hosts of *Schistosoma spindale*.

Figure 40. Top showing lampyrid fly larvae attacking *Biomphalaria glabrata*, the intermediate host of *Schistosoma mansoni*. Bottom showing lampyrid fly larva attacking operculate snails, *Bithynia siamensis goniomphalos*, the first intermediate host of the human liver fluke *Opisthorchis viverrini* in Thailand.