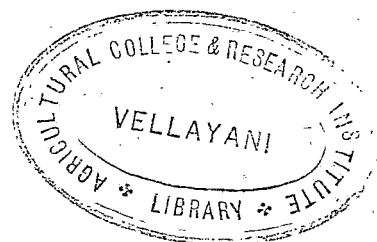


**STUDIES ON
THE EFFECT OF REFRIGERATION ON THE DEVELOPMENT OF
Trichospilus pupivora FERRIERE
(EULOPHIDAE)**

BY

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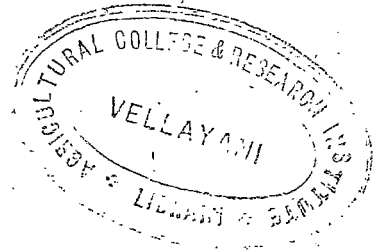


THESIS

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C E R T I F I C A T E

This is to certify that the thesis herewith submitted contains the results of bonafide research work carried out by Shri N.J. Narayanan under my supervision. No part of the work embodied in this thesis has been submitted earlier for the award of any degree.

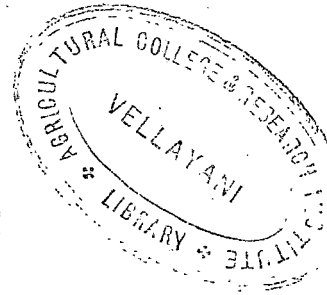
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A C K N O W L E D G E M E N T



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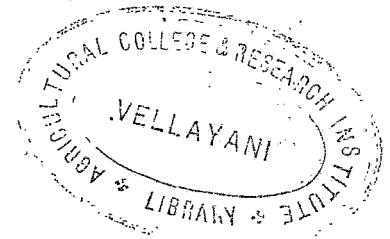
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I N T R O D U C T I O N

In the technique of biological control of insect pests, the most important phase is the mass breeding of the parasitic and predacious species of insects under artificial conditions. Among the problems which one may have to face in the mass multiplication and utilization of parasites for applied biological control, are, lack of adequate and continuous supply of host materials, preservation of parasites in viable state for use during lean periods and shipment of the parasites to long distances. Extension of the period of existence of the parasitic organism, provides a fairly satisfactory solution of these problems.

One method by which extension of the period of living existence of the parasite can be accomplished, is by the use of low temperatures. It is well known that when temperature is lowered, the life activities of organisms including their rate of development are retarded or arrested, the magnitude of retardation and the period for which this condition can be maintained are depending on the temperature. Considerable amount of work has been done by workers like Lesovoi (1929); Evans (1930); Peterson (1930) Tucker (1931) Schredd and Garman (1934) Kivit (1936) Subrahmonium (1937) Panasyuk (1937) Bymun (1937) Meier (1938) Hama (1938) Lapina (1939) Berry (1939) Chatterjee (1939, 1944) Ahamad and Gulamullah (1939, 1941, 1944) De Bach (1943) Ahamad and

Mathur (1946) Wilkes (1946) Gomes (1947) Vasiliev (1947) Romanova (1951) Venkataraman and Govil (1952) Maslenikova (1959) Lung et al (1960) Szmidt (1960) Bjergovic (1963) and others on the effect of low temperature on the rate of mortality, fecundity, viability, longevity, sex ratio and morphological alterations of different species of parasitic insects (Vide review of literature).

Trichospilus pupivora Ferriere (Eulophidae) has been proved to be an effective tool in the biological control of Nephantis serinona Meyr, the coconut leaf caterpillar, by workers like Ananthanarayanan (1934) Jayaratham (1941) and Ramachandra Rao et. al. (1948). In Kerala, this parasite has been employed to control the pest from 1941 onwards (Jones 1948). From 1955 onwards a scheme is functioning in this state to mass-breed the different parasites of N. serinona at various centres and liberate them systematically to control the pest and at present this work is being done at 7 centres in the state. The problems mentioned earlier have often to be confronted in the mass breeding of T. pupivora and the desirability of extending the living period of the parasite has been felt persistently. It is in view of this that the present investigations have been undertaken.

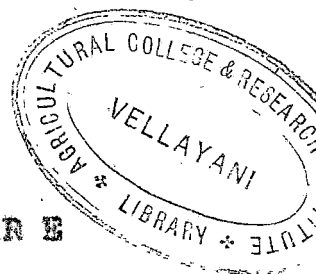
The aim of these studies is to explore the possibility of using low temperature to retard the rate of

development and thus extend the overall life duration of T. nupivora. No information is available on this aspect. In the studies presented, the effect of refrigeration at 10°C on development of T. nupivora within the host pupa of Phytometra peponis has been ascertained by refrigerating two stages of the parasite, the egg stage (24 hours after parasitization) and the pupal stage (8 days after parasitization). The extent to which the host pupa P. peponis can be kept at this temperature without undergoing deterioration, also has been ascertained. The results appear to be quite useful.

Literature on the effect of low temperatures on parasitic insects has been reviewed.

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REVIEW OF LITERATURE



Effect of low temperature on parasitic insects has received much attention from various workers in the field of parasitology. This is mainly because at low temperature, development of the parasite is retarded and development period extended and these are features which find much use in practical biological control. In the following pages a review of literature bearing on this aspect is given.

Lecovoi (1929) appears to be the first to initiate studies on the effect of low temperature on parasites. He found that it was possible to retard the development of the parasite Trichogramma evanescens within the parasitized host eggs of Lepidoptera when they were kept under low temperature, on the 5th and 6th day of parasitization and they could be preserved viable for months in this way. Of the parasitized eggs of Parathra brassicae L. kept under low temperature, 45, 15 and 2 per cents produced parasites when kept for 3, 4 and 4½ months respectively. The emerged parasites were weak and unable to oviposit. The parasitized eggs of Phalera luecephala L., which possess a thick chorion could be kept for 6 months in viable condition. Evans (1930) observed in Australia that Trichogramma evanescens emerging from hibernating parasitized eggs of

colding moth, were too weak to oviposit on fresh host eggs. This fact was substantiated by subjecting parasitized eggs to temperature fluctuations of 1° C to 10° C and then using the emerging parasites to parasitize fresh host eggs. Contrary to his earlier results, he could obtain healthy parasites emerging from eggs kept at 2° C. Tucker (1931) found that eggs of Diatrea saccharalis parasitized by Trichogramma minutum, kept at 40° F to 45° F produced on the 6th and 7th day an emergence of parasites varying between 80% and 90% with an average of 84%.

Peterson (1930) conducted experiments with parasitized eggs of the Angoumois grain moth, Sitotroga cerealella Olio, Oriental Fruit Moth, Laspeyresia molesta Busk, Mediterranean Flour moth Ephestia kuhniella Zell and common bag worm Thyridopteryx ephemeraeformis H for studying the effect of refrigeration on the sex ratio, longevity and productivity of the parasite Trichogramma minutum Riley. He found that the parasitized eggs of the Angoumois grain moth would not survive longer than 4 to 6 weeks, and the adult parasite not longer than two weeks at 40° F. However he was able to overwinter out of doors yellow species of Trichogramma in the eggs of Oriental Fruit Moth and Ephestia kuhniella during an entire winter from November

to April. The parasite under refrigeration did not live longer than those overwintering in nature. In the eggs of Thyropodteryx ephemeraeformis H., the viability of the parasite was maintained even up to 6 months. The mortality of the parasites in the bag worm under refrigeration for 3 to 6 months was as high as 75 per cent as against a negligible mortality in those kept for 40 days. The susceptibility of the parasitized eggs to low temperature depended on the age of the parasitized egg. The greatest survival was noticed among eggs in which the parasite was about one half developed. Eggs nearly mature and ready to hatch, suffered high mortality. In the case of newly parasitized eggs also, considerable to complete mortality occurred. Temperature below freezing point, averaging 27° F were much more detrimental than those immediately above 34° F. Most satisfactory survival was obtained between 40 and 45° F with fair humidity. Species possessing yellow females was able to withstand longer and more severe refrigeration than the species possessing darker females when reared in bag worm eggs. It was also observed that when reared in bagworm eggs, sex ratio of the parasite which was normally 2 males to one female, did not change as a result of refrigeration.

Thompson and Parker (1933) found in Eulimeria crassifemur, an important parasite of European Corn Borer,

a retardation of development as a result of refrigeration at 18° C. The cocoon of the larvae kept at 27° C produced adult in 11 days whereas those kept in 18° C produced parasites only on 22nd day.

Schread and Garman (1934) conducted investigations to find out the reason why Trichogramma sp. reared in the eggs of Sitotroga cerealella could not be held successfully in ordinary refrigeration for any great length of time. With 2 days' pre-refrigeration development and 8 days refrigeration at 37° F and 60 per cent R. H., the mortality was 12 per cent for the yellow strain, I. pretiosum and 45 per cent for dark species, I. minutum. A 5 days refrigeration under the same conditions gave 34 per cent mortality in yellow species and 32 per cent for dark species. Four days pre-refrigeration development and 8 days refrigeration, gave 22 per cent mortality. In the case of 3 day pre-refrigeration development, 90 per cent mortality occurred when refrigerated continuously for 40 days. For an equal length of refrigeration preceded by 5 days pre-refrigeration development, there was 84 per cent mortality in the yellow strain. The least mortality at the termination of a 40 days refrigeration, development was 50 per cent for dark species, with 4 days pre-refrigeration development. But the mortality of the yellow species for the same period of refrigeration and pre-refrigeration period was 77 per cent. With 4 days pre-refrigeration development

and 7 days refrigeration at 44°F, the mortality was only 4 per cent which was considered to be a normal emergence under ordinary conditions without refrigeration. But with the same pre-refrigeration development and refrigeration at 44°F for 40 days, mortality was less than 66 per cent whereas, under the same conditions, refrigeration for 60 days, cent per cent mortality was obtained. At 47°F the yellow species survived longer than the dark species and there was less mortality for equal periods of refrigeration. With 3 days' pre-refrigeration development and 7 days refrigeration, 97 per cent of T. pretiosum (yellow) emerged, whereas in the case of dark species it was 92 per cent. Under the same conditions, but 40 days refrigeration, emergence was only 10 per cent in the case of T. pretiosum and 48 per cent in the case of T. minutum. With 4 days pre-refrigeration development followed by 7 days refrigeration, the yellow species produced 96 per cent emergence and the dark species 76 per cent. When the period of refrigeration lengthened to 40 days, the emergence was 80 per cent and 81 per cent respectively for yellow and dark species. He found that the mortality of the parasites was proportionately the same for two species, with 2 days pre-refrigeration development.

At 49°F after one month, the emergence was 85 per cent for 4 days, 86 per cent for 3 days and 73 per

cent for 2 days pre-refrigeration development. For one week of refrigeration and 4, 3 and 2 days pre-refrigeration periods, the percentages of emergence were 92, 91 and 94 respectively. In the grain moth eggs, Trichogramma were capable of withstanding low temperature for long or short periods depending on the stage of development at the time of refrigeration. The yellow species (T. pretiosum) could survive longer than T. minutum at any single temperature except 37° F and a pre-refrigeration development of 4 days. Low temperature frequently produced a change in sex ratio and below 47° F, the sex ratio was upset, the change being evident in the generation following the refrigerated eggs.

He found that refrigeration had its effects on the wings of the parasites emerging. The deformity of the wings, was found to be higher in black species than in the yellow species for the first 2 weeks of refrigeration but after that period there was no significant difference between the percentage of wing deformity for equal period of refrigeration between the several strains of the two species. T. pretiosum showed apparently 25% less wing deformity than T. minutum. The percentage of wing deformity was higher among the females of T. pretiosum.

Flanders (1936) in his investigations in Tetrastichus sp on the eggs of Gallerucella maculicollis,

a pest of Elm trees, found that under 38°F for 14 days in transit, the development of the host embryo and prepupal stage of the parasite, were inhibited.

Kivit (1936) found that Aphelinus mali could withstand the temperature as low as 21 and 25°C in November and December when they were liberated in south eastern parts of North Caucasus against Eriosoma lanigerum.

Lebedyanskaya, Medvedeva and Chernopanevkiana (1936) observed that the eggs of the Lymantrid Dasychira pudibunda, parasitized with T. evanescens when kept at 0-6°C for 7 to 8 months, gave 13 to 6 per cent there being no emergence when kept for longer periods. He concluded that the best results would be obtained when they were refrigerated 4 to 5 days after parasitization at a stage when larvae are half grown. Further it was found that the eggs of Loxostege sticticalis parasitized by T. evanescens W. were not affected by keeping them under snow for 7 days, the temperature of snow being as low as 11°C. The effect of low temperature on adult parasites was also observed and they found that the adults did not suffer mortality even after keeping them for 24 hours under a temperature not lower than 6.5°C. But when the exposure was to 20°C mortality occurred.

Subramoniam (1937) found that the flour moth eggs when refrigerated at 5 to 11°C for 30 to 35 days, five days after parasitization with Trichogramma, gave only partial emergence. The parasites emerged were capable of parasitizing fresh Flour Moth eggs. Long days of exposure to cold reduced the percentage of emergence of parasites. However in one instance, he recorded the survival of parasites even after 90 days refrigeration. But these parasites were inactive and weak and died shortly after emergence. As soon as the parasitized eggs were placed under low temperature, the development of the parasite within the egg ceased and development was resumed when it was taken out and kept exposed under the ordinary condition.

Reeks (1937) in his investigations found that Microgaster fuscipennis Z, a cocoon parasite of Dioxon polytomum, could oviposit even at 52° F when they were kept in field cages. He had also recorded the ability of the parasite to withstand the severe winter in Qubec. Forty three colonies of Microgaster adults were removed from Dioxon cocoons collected from Park Reserve in the fall of 1935 and similar collections in September 1936 thereby showing that Microgaster can withstand the rigorous winter with its low temperature.

Bynum (1937) observed in Hawaii the emergence of

of Pseudococcus nterryi F, after 30 days from Pseudococcus boninsis (grey sugarcane mealy bug) the host, collected after the temperature had dropped to 24° F and he was able to recover the parasites in the summer following an exceptionally cold and wet winter. In the case of adult parasites confined in a tube and placed in the open where the temperature was 24° F, there was no mortality. Panassyuk (1937) found that Trichogramma evanescens produced larval diapause in spring and autumn when the temperature dropped to 11°C in the Province of Kiev. In spite of the diapause, the parasite produced 8 generations every year. Hama (1937) conducted experiments on the cold storage of eggs of Ephestia cautella parasitized with Trichogramma japonica which is an egg parasite of Chilo simplex the rice stem borer, in Japan. He observed that the eggs about 20 hours old were suitable for cold storage and those 22 hours old were satisfactory host materials after being stored at 8°C for over a month though they did not hatch after 2 weeks exposure. His experiments proved that the refrigeration of parasitized eggs reduced the percentage of emergence of adult parasite and their fecundity.

Johansso (1937) detected that the egg stage of Isostasius punctiger N. lasted 45 to 50 days at a temperature of 16 to 17°C and that of Lentacis tipulae 55-60 days both being the parasites of wheat gall midges,

Contarinia tritici and Sitodiplosis mesellana. He found that the parasite did not show any sign of development taking place below 11°C , thereby showing that cold has a retarding effect on the developmental activities of the parasite.

Meier (1938) discovered that Anhelinus mali, hibernated when the temperature dropped to 13°C were then able to withstand temperature as low as 25°C and resumed development when the mean temperature reached 7°C . He showed that the best conditions for storing eggs of Sitotroza cerealella parasitized by Trichogramma evanescens, were $1-2^{\circ}\text{C}$ and 90% R.H. When the parasitized eggs with mature larvae were refrigerated under these conditions for 1, 2, 3 and 4 months the emergences of adult parasites were 92.2, 66.1, 51 and 52 per cent respectively. In the case of eggs containing the pupae of the parasite, the corresponding emergences were 60.1, 45, 32 and 15 per cent. When the parasitized eggs of Eohestia kubniella were stored under the same conditions, the percentages of parasite emergences were correspondingly lower and more emerged after storage for 4 months.

Hama (1938) had found that when stored at $5-10^{\circ}\text{C}$ and 60 per cent R.H. the percentage of parasitism and parasite emergence were high for Trichogramma japonica

in eggs of Ephesia kuehniella, but eggs that had been kept at a lower R.H. were unsuitable for parasitism. The fecundity of the female parasites reared from the eggs were not affected by humidity at which the latter had been kept but decreased when they had been kept in cold storage for long periods. The progeny of the female parasites reared in eggs that had been kept in cold storage, was quite normal.

McLeod (1938) observed that in Canada development of Encarsia formosa was retarded at low temperatures. Lund (1938) in his studies on the longevity and productivity in Trichogramma evanescens, found abnormally high proportions of female progeny at 15°C. McLeod (1938) found that in Aphelinus marlatti, the developmental period increased more rapidly below 60°F and completely reduced the vitality. Lapina (1939) observed that Trichogramma evanescens in its immature larval stage, in the eggs of Sitotroga cerealella and Ephesia kuehniella when stored at low temperature, the sex ratio was 1:3 while the proportion was 1:1 when the pupal stage was subjected to the same low temperature. Talenhorst (1939) in his experiments had shown that refrigeration had different effects on longevity on male and female parasites of Ichneumon tersius G, a parasite of Pine Geometrid. The male and female parasites when fed on dilute honey, survived respectively for 57 and 90 days at 10°C, 54 and 65 days at 15°C, 25 and 52 days at 20°C and 12 and 44 days at 25°C

25°C. Berry (1939) observed that Ephealtes jurionellae L, a pupal parasite of European Pine Shoot Borer, Rhyacionia buoliana developed normally when subjected to high temperature. In an unheated insectory, the life cycles was longer than when it was in the field. Female parasites liberated successfully at normal winter temperatures. During the severe autumn of 1934-35 there was 10% mortality when the parasites were held in wooden boxes in an unheated insectory where the temperature dropped to 11°F. A few of the late emerging males had always survived the winter. In the laboratory, very low mortality to the species occurred during winter at temperatures, ranging from 32 to 38°F. It was also found that the female parasites reared at 80°F or above began to oviposit soon after emergence while those insects developed at lower temperature of 50°F to 65°F did not oviposit for a week or two. Further, the parasites reared at high temperatures and then refrigerated at 40°F for 7 days, could not oviposit until 4 to 7 days after they were subjected to 70° to 80° F. The adults developing in October when the temperature was low, would not oviposit in the following spring until they had been subjected to 50°F to 75° F, for a period of 14 days.

Chatterjee (1939) found that longevity of Ananteles machaeralis was prolonged for 2 and 1 day respectively for male and female at 80-90°F to 26 and 10 days respectively at 74°F. Sauer (1939) observed that Ephealtes

demorphus in parasitized cocoons of Macrocentrus could be kept for one month at 10-15° C and R.H.70%. Vevai (1942) noted that Anhidius matricariae N, a Braconid internal parasite of Myzus persicae, took 3-4 days at 70°F and 7-9 days at 55°F to hatch. The parasite became sluggish at lower temperatures below 40° F.

Rivany(1942) found that Clausenia purpurea Ishi produced lesser number of eggs at temperatures below 20°C. Some individuals survived in Pseudococcus comstockii for 5 days at 0-5°C. When exposed to 4-7°C for 6-40 days, the mortality of pupae was 10 per cent but the number of offsprings reared from the survivors decreased as the exposure increased. In the case of adults 50 percent lived for 4 weeks at 15 to 20°C but they were motionless below 15°C. Reproduction was negligible at 16°C to 20°C and ceased altogether at lower temperatures. Callan(1942) noted that Theresia claripalpis, parasitizing Diatrea spp when placed in cold storage at 65°F - 75°F for a week, its development was retarded and produced no harmful effects and he used this method for shipment of the parasite. Ahamed and Gulamullah (1939, 1941) observed that in Microbracon lefrovi, the threshold of development was at 12°C. In the laboratory continued exposure to temperatures 13°C or below proved fatal to both the parasite and its host, Farias fabia. In the case of eggs the usual incuba-

tion period at 16°C was 64 hours. Fresh eggs when refrigerated at 13°C failed to hatch though they could stand short exposures to temperatures below this limit. Eggs laid at $23-25^{\circ}\text{C}$ when exposed to 24 hours at 0°C showed 60-100 per cent mortality, whereas those exposed to 12 hours hatched completely. In the case of grubs, the normal period at 16°C is 6.4 days and continuous exposure below 12°C was fatal but the grub exposed for a period of 36 hours to 0°C and then brought back to 25°C , developed normally. The normal pupal stage averaged 12.16 days. A small per centage of pupae could develop at a temperature of $13-13.5^{\circ}\text{C}$ and the survivors took $1\frac{1}{2}$ to 2 months for development. It was further revealed that temperatures close to the threshold of development i.e. $13-16^{\circ}\text{C}$ were found to be the most suitable for storing the parasites. Any temperature lower than this when prevailing for long periods, was injurious to the species and caused high mortality. At $13-16^{\circ}\text{C}$ the average longevity of the adult male was 33-49 days and for female 45 to 73 days. The maximum duration of life was found to be 79 and 11 days for male and female respectively. Some of these females kept for over 2 months, copulated and laid a smaller number of eggs when brought back to suitable temperature. These experiments indicated the possibility of collecting the parasites when abundant and successfully storing them under low temperature for over 4 months to pass over

unfavourable conditions of environment. At temperatures close to the threshold of development, the fecundity of the host and the parasite was reduced, but the fecundity of the host was much more affected than that of the parasite. At 16°C the longevity of the adult was also lengthened.

De Bach (1943) noted that larvae of Hormoniella vitripennis W. on pupa of Musca domestica gave rise to adults in 9 days at 23°C and in 28 days at 15°C, pupated after 31 days at 10°C and showed no outward change after 21 days of refrigeration at 2 to 6°C. The fecundity of the adults emerging from those kept at -2 to 6°C was the highest and the lowest from those kept at 23°C or 15°C. The ratio of males to females was high in those exposed to low temperature. Adults of Muscidi furax obtained from larvae refrigerated at 4.4°C for 28 to 31 days or pupae for 25 days did not show any appreciable change in reproductive capacity or sex ratio of the progeny. Adults of both species with a normal longevity of 1-2 weeks, survived for 1-5 months when removed every 3-4 days and allowed to feed on honey, but mortality was high after the longer exposures. Adults of Pachycrepoides dubius A. and Microbracon sp. kept under similar conditions survived up to 4 months.

Schell (1944) observed that adults of Hadronotus ajax, an egg parasite of Anasa tristis, kept in cotton plugged

vials in the laboratory and fed daily with diluted honey, laid eggs as long as 12 days, at a temperature of 75 to 85°F, whereas those kept in light stoppered vials and refrigerated at 45°F survived for 5 to 6 weeks without food or water. Chatterjee (1944) noted that the adults of Euplectron parvulus F. survived for 13 days at 8°C to 15°C and the cocoons for 13 days and grubs of the parasites failed to develop and died under the same refrigeration.

Tashkir Ahamed and Gulamullah (1944) found that at 16°C, oviposition period of Melcha nararii in pupae of Earias sp. varied between 2 and 3 days, and that the longevity on an average, the maximum being 69 and 49 days respectively. Oviposition and fecundity were found to be the highest at this temperature. The mean incubation period at 16°C took 2.19 days and eggs laid at this temperature failed to hatch when kept at 13°C. The larval period at 10°C was found to be 8.6 days. The pupal period lasted 28.7 days at 13°C, as against 16.5 days at 25°C.

Silveria Guido and Condejohn (1946) working with Aphidius plantensis B, parasite of the aphid Toxoptera graminum, observed that the pupae of the parasite kept at 5°C produced only 1 percent emergence.

Ahamad and Mathur (1946) noted in Melcha ornatipennis C, a parasite of Scirpophaga nivella F,

that under controlled conditions of temperature and humidity, the egg stage lasted 45-48 days, while under 11°C it lasted for 13.5 days with an average viability of 50 per cent. At 15 to 35°C, the viability was cent per cent. Refrigeration at larval stage at 11°C for 13 to 18 days, resulted in mortality. At 15°C the larval stage was completed in 27.77 days. Refrigeration at 15°C was fatal to the pupal stage. At 11°C and 15°C, the insect was unable to complete its preimaginal development. At 11°C the average unfed male lived for 5.6 days and fed ones for 11 days. Under the same cold condition, a female lived for 7 and 4 days respectively. At 15°C the fed and unfed adult female parasite lived for 18.6 and 8.6 days respectively. The corresponding periods for males were 12.6 and 7.3 days respectively.

Wilkes (1946) succeeded in storing the cocoons of Phytodietus fumiferanae parasite of spruce worm, at 32 to 34°F for 8 months. Gomes (1947) showed that the pupae of Macrocentrus ancylivora Roh separated from the host Gnorimoschima operculella and kept at 5, 10.5 and 17.2°C and 40% R.H. for 15 days, gave an emergence of 10 and 9, 31 and 23, 34 and 40 per cent males and females respectively for the corresponding temperatures. Storage at low temperatures in the pupal stage had no harmful effect on the fecundity of the adult female or the sex ratio of the progeny.

Vasilev (1947) found that Lathromeris brachocida which parasitized Bruchus pisorum L., could be stored in eggs of Bruchus oltectum at 5 to 6°C for a long time so as to liberate them when the crop was in bloom in the field. Graham (1947) studying Pimpla turinellae L., a parasite introduced in Canada to control Rhyacionia buoliana S., on pine, showed that when the parasite was refrigerated at 42°F to 44°F for 3 days and at 32-34°F, for more than six months and then transferred to 74°F, there was failure of emergence from the last batch. The best emergence was from pupae that had been kept at 72°F for 5 days before being placed in cold storage.

Neilson (1949) observed that in Pleurotropis utahensis, a parasite of wheat stem fly, there was not such mortality among the adult parasites emerged from those refrigerated at 5 to 10°C for over 48 days. Larvae collected from Agropyrum smithii in the field, had an under cooling of about -22.5°C and the freezing weather did not appear to cause much mortality. Larvae collected from Brumus stubs stored in the laboratory at 6°C and later exposed to 77°F and 60 per centage R.H. pupated after a mean period of 18.5 days.

Romanova and Pechahoba (1951) noted that in Telenomus laeviusculus, egg parasite of Malacosoma nustria, the

development could be retarded during spring and summer by keeping the parasitized eggs at 4° C and 60-70 per centage R.H. Borg (1952) observed that Aphelinus mali overwintered usually in its larval stage within the dead host, giving practically little winter mortality of the parasite. The lowest temperature at which adult emerged was about 12-30° C. Venkataraman and Govil (1952) found that in Trichogramma evanescens the most suitable stage for cold storage was the prepupal stage at 4 days after parasitization and that at this stage, it could be successfully stored at a temperature of 10 to 12° C and 85 per cent R.H. for a period of 15 days, without affecting the viability, longevity and sex ratio of the parasite. The average per centage of viability decreased as the period of refrigeration was increased and upto 15 days of refrigeration, viability was not adversely affected. The longevity and fecundity decreased as the refrigeration period was increased, there being no effect upto 15 days of refrigeration. Sex ratio was not affected upto about 10 days refrigeration. Beyond this, there was a gradual increase in the per centage of females.

Zinna (1955) found that the development of Crataepoides russel, a parasite of Dicoryctria splendidella H., ceased when the temperature fell below

14° C. De Bach (1956) found that in the parasites Aphytis chrysomphali M. and Aphytis lingnanensis on Citrus Red scale, 50 per cent of the adults died by refrigeration at 50° F for 7 and 4 days respectively. Carpenter (1956) found that Lixophaga saccharalis, parasitic on Diatrea saccharalis survived the winter in 1953 when the minimum temperature was 25° F.

Kolubaju (1958) observed that in Ropalices sp. Roptocerus sp. and Rhopalophorus sp., the parasite of Ips typographus, there was only limited emergence when kept at 5-26° C and 65-85 per cent R.H. in summer and at 8-25° C and 65-75 per cent R.H. in winter. Evenhuis (1958) investigating on the ecological conditions of Eriosoma lanigerum and its parasite Aphelinus mali, found that the parasite completed its development in the mummified host in 44 days when the temperature was lowered to 15° C and in 14.5 days at 25° C. Wood Jr. (1958) found that Aphelinus nigritis parasitized its aphid hosts at temperatures as low as 42° F. Maslenikova (1959) observed that in Trichogramma evanescens W., refrigeration at 1-2° C for 1-2 months caused total mortality. It was also observed that 95-97 per cent of larvae entered diapause at 10° C, regardless of light conditions, 3-6 per cent at 20° C and none at 25° C. When the temperature was

altered to 23° C by day and 4-5° C at night, there was complete emergence of parasites in 17-19 days. Experiments on cold hardiness showed that cooling to -5 or -10° C caused only negligible mortality while lower temperature of -15 and -20° C caused complete mortality. Larvae in diapause, cooling to temperature down to -25° C caused negligible mortality. The mortality at -30° C and -35° C were 18 and 52 per cent respectively. The effect of longer exposure of parasitized eggs to -20° C for 48 hours or 120 hours showed complete mortality for normal larvae and negligible for those in diapause. It was concluded that larvae in diapause was the most suitable stages for prolonged storage under refrigeration.

Watson and Arthur (1959) had found that Orgilus obscurator and Crematus interuptor G., the most common parasite of Rhyaciona buoliana, in Ontario, established well where minimum winter temperature was lower than -10 to 0° C.

Lung et al. (1960) found that Aphelinus mali overwintered as a full fed larva within the mummified host body at temperatures as low as -12° C giving 80 per cent emergence. Pupation occurred in late March when the temperature reached 6 to 7° C and the emergence occurred at 10° C. The development period varied from 7 days in mid August to 114 days in Winter. Szmidt (1960) observed

that a reduction in temperature from 37.4° C to 13.6° C resulted in an increase of 23 days in the development period of Dirhienus alboannulatus R. with 50 per cent mortality of females. The development of the parasites reared in Gallaria mellonella varied between 18 to 60 days when kept at 27.2° C and 16.7° C respectively.

Bjegovic (1963) noted that in Achaetoneura aletiae, a Tachinid parasite of Fall web worm, the parasite pupae exposed to a low temperature of -10° C during overwintering, resulted in mortality.

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was used to close the specimen tubes.

f. Blotting paper: Small strips of blotting paper each of the size 6 cms. x 3.5 cms. were cut and inserted at the bottom of each tube to remove by absorption water that might be formed when the tubes were placed inside the refrigerator.

g. Trays: Small iron trays of the size 18 cms. x 14 cms. x 4 cms. were used to hold the specimen tubes when they were placed within the refrigerator.

h. Tube stand: 135 cms. x 38 cms. to hold 72 tubes.

i. Tube rack: 45 cms. x 37 cms. to hold 30 tubes.

j. Camel hair brush.

k. Diluted honey for feeding parasites.

2. METHODS

1. Rearing of *Phytometra (Plusia) peponis* F. (Noctuidae)

Caterpillars collected from snakegourd cultivated in the college farm and fields around were reared in the laboratory on snakegourd leaves. Rearings were done in hurricane chimneys closed with muslin cloth. Every morning chimneys were cleaned of the larval castings and other debris and fresh leaves supplied. The caterpillars in prepupal stage were removed to clean and dry petridishes. The petridishes and chimneys were occasionally cleaned in dilute mercuric chloride solution and after drying,

they were used again for rearing those which had not reached prepupal stage.

The pupae which formed out of the prepupae transferred to clean petridishes, were used for the experiments.

2. Maintaining a stock culture of *Trichospilus pupivora* F.

A stock culture of *T. pupivora* was maintained by breeding it on pupae of *P. peponis*.

Five female parasites which were less than 24 hours old since their emergence from the host, were introduced into each specimen tube. For this, the parasites which contained in specimen tubes are tapped down on a clean white paper spread on the work-table and covered over immediately with a dry clean, sterile petridish confining the parasites under it. The work-table lamp which is at the farther end of the table was then switched on. Then the side of the petridish towards the light was slightly raised until sufficient number of parasites moved out and started to the light source. The required number of the parasites were then led into a specimen tube with the help of a fine fresh camel-hair brush. The tube was then closed with a cotton wool plug and the parasites were then examined against light, with a hand lens to ensure that the five parasites introduced were females.

The freshly pupated host pupa was then gently cleaned with a camel-hair brush and introduced into the specimen tube containing the five female parasites. A small strip of filter paper dipped in dilute honey was then placed inside the tube as food for the parasites. The closed specimen tubes were then placed on tube racks, their bottom ends with the pupae towards light. This together with the narrowness of the tubes ensured parasitization to the maximum extent. The pupae were kept exposed to the parasites until all the parasites died or upto the time limit of parasitization described in each experiment.

The parasitized pupae were taken out from the tubes, cleaned and then removed to clean, dry and sterile specimen tubes (7.5 cms x 2.5 cms.) having filter paper strips inserted at the bottom. The specimen tubes were then closed with cotton wool plugs and placed either on tube rack or inside the refrigerator, according to the nature of the experiment.

3. Determination of the effect of refrigeration on development of *Trichospilus pupivora*.

The studies on the effect of refrigeration on *T. pupivora* were conducted at two different stages of the parasite viz. the egg and the pupal stage within the host pupa.

In the first case, fresh pupae less than 24 hours old were parasitized with female parasites, each for 24 hours and refrigerated as described for varying periods. At the end of each refrigeration period the respective sets of tubes containing the parasitized pupae, were taken out and kept on the tube stand in the laboratory. The date on which the fully developed adult parasite emerged from the shell of the host pupae was recorded as the date of emergence. When emergence of the parasite was complete, they were killed with chloroform and counted under a binocular microscope into males and females.

In the second case, the parasitized pupae were maintained under laboratory conditions for 8 days before being refrigerated for varying periods. Preliminary observation had shown that by eight days of parasitism all the parasite larvae within the host pupae had pupated. After refrigeration for the different periods, the host pupae with the parasite pupae within were transferred to laboratory and the dates and of emergence of adults observed.

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IV. DETAILS OF EXPERIMENTS AND RESULTS.

Experiment I.

Duration of pupal stage of *Phytometra peponis* F.
under laboratory conditions.

Experimental details:

Number of pupa used:	10
Age of pupa:	Below 24 hours.
Period of experiment:	9-9-63 to 18-9-63
Temperature during experiment:	Mnm. 78°F - 82°F Mxm. 82°F - 86°F
Relative Humidity during experiment:	90 % - 92 %

Procedure: Ten fresh Plusia pupae, one each in a specimen tube of 7.5 cms. x 2.5 cms. were kept on a tube stand after closing the mouth of the tubes with cotton wool plugs and dates of emergence and pupal period observed.

Results: The results of the experiment are presented in Table I. It can be seen from the table that the pupal stage of *Phytometra* varies from 7 to 9 days, the average pupal stage being 7.5 days.

TABLE I

Duration of pupal stage of *Phytometra peponis* F. under
Laboratory conditions.

Serial No.	Date of pupation	Date of moth emergence	Pupal period (days)
1	9-9-63	16-9-63	7
2	9-9-63	17-9-63	8
3	9-9-63	16-9-63	7
4	9-9-63	16-9-63	7
5	9-9-63	17-9-63	8
6	9-9-63	17-9-63	8
7	9-9-63	16-9-63	7
8	9-9-63	18-9-63	9
9	9-9-63	16-9-63	7
10	9-9-63	17-9-63	7
Total			----- 75 -----
average			7.5

Experiment 2:

Period of Development of *Trichospilus pupivora* F. reared
in *Phytometra peponis* F. under Laboratory conditions.

Experimental details:

Number of pupae used:

10

Age of pupae:

Below 24 hours

Number of female parasites used to parasitize a single pupa.	 	5
Age of parasite used:		Below 24 hours
Period of experiment:		20-9-63 to 9-10-63
Temperature during experiment:		Mm. 72° F - 82° F Mm. 82° F - 86° F
Relative Humidity during experiment:	 	90 % - 92 %

Procedure: The newly formed pupae of P. peponis were exposed to the parasites as described under Methods, in specimen tubes which were kept on tube racks in the laboratory and the development period observed.

Results: The results are given in Table II. It is seen that the period of development of the parasite in the host pupae varies from 17 to 22 days, the average period being 19.3 days. The number of adult parasites emerging from a host pupa varies from 243 to 464 with an average of 388.9, the sex ratio male : female being 1: 28.

Experiment 3.

Effect of refrigeration at 10° C for 7 days on pupa of P. peponis.

Experimental details:

Number of pupae used:	10
Age of pupa:	Below 24 hours.

TABLE II

Period of development of *T. pupivora* reared in *P. peponis* under
Laboratory conditions.

Sl. No.	Date of exposure of pupa to parasite.	Date of removal of parasites.	Date of emergence of parasite	No. of parasites emerged			Development period in days
				Male.	Female.	Total	
1.	20-9-63	21-9-63	7-10-63	14	302	316	17
2.	20-9-63	21-9-63	10-10-63	18	410	428	20
3.	20-9-63	21-9-63	8-10-63	12	358	370	18
4.	20-9-63	21-9-63	11-10-63	16	432	448	21
5.	20-9-63	21-9-63	12-10-63	18	439	457	22
6.	21-9-63	22-9-63	11-10-63	10	366	376	20
7.	21-9-63	22-9-63	11-10-63	14	441	455	20
8.	21-9-63	22-9-63	9-10-63	9	323	332	18
9.	21-9-63	22-9-63	10-10-63	12	231	243	19
10.	21-9-63	22-9-63	9-10-63	13	451	464	18
Total				136	3753	3889	193
Average				13.6	375.3	388.9	19.3
Sex Ratio : 1:28.							

Period of experiment:

22-9-63 to 9-10-63

Temperature during experiment:

Min. 72° F - 82° F

Max. 82° F - 86° F

Humidity during experiment:

90 % - 92 %

Procedure: Each pupa was put in a clean specimen tube 7.5 cms. x 2.5 cms. with blotting paper lining its bottom. The specimen tube was then closed with cotton wool plug and refrigerated.

Results: Results are given in Table III. It may be seen that all the pupae survive refrigeration for 7 days and that when taken back under laboratory conditions. The pupal period subsequent to refrigeration for 14 days varies from 6-7 days with an average of 6.5 days.

Experiment 4.

Effect of refrigeration at 10° C for 14 days on host pupa of *P. peponis*.

Experimental details:

Period of experiment conducted: 29-9-63 to 17-10-63

Temperature during experiment: Min. 72°F - 82°F

Max. 82°F - 86°F

Relative humidity during)
experiment:) 90% - 92 %

Rest of the details as in Experiment 3.

Results: The results of the experiment are given in Table IV. It will show that all the pupa survive refrigeration at 10°C for 14 days and emerge as adults when returned to Laboratory conditions. The pupal period subsequent to refrigeration for 14 days varies from 6-7 days with an average of 6.5 days.

T A B L E IIII

Effect of refrigeration at 10° C for 7 days on
pupa of *P. peponis*.

Sl. No.	Date of exposure of pupa to Low Temp.	Date of removal from Low Temp.	Date of emergence of moth.	Post refrigeration pupal period (days)	Total pupal stage in days	Remarks
1.	22-9-63	29-9-63	6-10-63	7	14	
2.	22-9-63	29-9-63	5-10-63	6	13	
3.	23-9-63	30-9-63	8-10-63	8	15	
4.	23-9-63	30-9-63	7-10-63	7	14	
5.	23-9-63	30-9-63	7-10-63	7	14	
6.	24-9-63	1-10-63	8-10-63	7	14	
7.	24-9-63	1-10-63	8-10-63	7	14	
8.	24-9-63	1-10-63	8-10-63	7	14	
9.	24-9-63	1-10-63	9-10-63	8	15	
10.	24-9-63	1-10-63	8-10-63	7	14	
Total :				71	141	
Average :				7.1	14.1	

T A B L E IV

Effect of refrigeration at 10° C for 14 days on host pupa of
P. neonis.

Sl. No.	Date of exposure of pupa to Low Temp.	Date of removal from Low Temp.	Date of moth emergence.	Post refrigeration pupal period in days	Total pupal period in days	Remarks
1	25-9-63	9-10-63	15-10-63	6	20	
2	25-9-63	9-10-63	16-10-63	7	21	
3	25-9-63	9-10-63	16-10-63	7	21	
4	25-9-63	9-10-63	15-10-63	6	20	
5	25-9-63	9-10-63	15-10-63	6	20	
6	25-9-63	9-10-63	15-10-63	6	20	
7	26-9-63	10-10-63	16-10-63	7	21	
8	26-9-63	10-10-63	17-10-63	7	21	
9	26-9-63	10-10-63	16-10-63	6	20	
10	26-9-63	10-10-63	17-10-63	7	21	
Total				65	205	
Average				6.5	20.5	

Experiment 5.Effect of refrigeration at 10° C for 21 days on pupae of *P. peponis*.Experimental details:

Period of experiment:	3-10-63 to 4-1-63
Temperature during experiment:	Min. 72° F - 82° F Max. 78° F - 86° F
Relative Humidity during experiment:	86 % - 95 %

Procedure: The pupae were refrigerated continuously for 21 days as in Experiment 4.

Rest as in Experiment 4.

Results: In Table V is given the results. It will be observed that the post-refrigeration pupal period varies between 6 to 8 days with an average of 6.8 days. All the pupae remain healthy and normal after continuous refrigeration for 21 days and healthy moths emerged from them.

Experiment 6.Effect of refrigeration at 10° C for 28 days on pupae *P. peponis*.Experimental details:

Period of experiment:	2-12-63 to 9-1-64
Temperature during experiment:	Min. 76° F - 84° F Max. 81° F - 86° F
Relative humidity during the experiment:	85% - 95 %

TABLE V.

Effect of refrigeration at 10° C for 21 days on pupa of
P. deponis.

Sl. No.	Date of exposure of pupa to Low Temp.	Date of removal from Low Temp.	Date of moth emergence.	Post refrigeration pupal period in days.	Total pupal stage in days	Remarks
1	3-10-63	24-10-63	30-10-63	6	27	
2	4-10-63	25-10-63	1-11-63	7	28	
3	4-10-63	25-10-63	31-10-63	6	27	
4	4-10-63	25-10-63	31-10-63	6	27	
5	5-10-63	26-10-63	2-11-63	7	28	
6	5-10-63	26-10-63	2-11-63	7	28	
7	5-10-63	26-10-63	1-11-63	6	27	
8	6-10-63	27-10-63	3-11-63	7	28	
9	6-10-63	27-10-63	4-11-63	8	29	
10	6-10-63	27-10-63	4-11-63	8	29	
Total				68	278	
Average				6.8	27.8	

Procedure: The pupae were refrigerated continuously for 28 days.
The rest as in Experiment 4.

Results: The results are presented in Table VI. It shows that out of 10 pupae moths emerged from eight only when returned

to laboratory conditions after refrigeration of them continuously for 28 days. The two pupae out of which moths did not emerge, when dissected open showed dead moths within. The post-refrigeration pupal period varied between 7 and 8 days with an average of 7.6 days.

TABLE VI

Effect of refrigeration at 10° C for 28 days on pupa of *P. pomonis*.

Sl. No.	Date of exposure of pupa to Low Temp.	Date of removal from Low Temp.	Date of moth emergence.	Post refrigeration pupal period in days.	Total pupal period in days	Remarks
1	2-12-63	30-12-63	--	--	--	Moth found dead within pupa
2	2-12-63	30-12-63	7-1-64	8	36	
3	2-12-63	30-12-63	6-1-64	7	35	
4	2-12-63	30-12-63	7-1-64	8	36	
5	2-12-63	30-12-63	6-1-64	7	35	
6	2-12-63	31-12-63	8-1-64	8	36	
7	2-12-63	31-12-63	7-1-64	7	35	
8	4-12-63	1--1--64	8-1-64	8	36	
9	4-12-63	1--1--64	8-1-64	8	36	
10	4-12-63	1--1--64	--	-	--	Moth found dead within pupa
Total				61	285	
Average				7.6	35.6	

Experiment 7.

Effect of refrigeration at 10° C for 35 days on pupa of P. peponis.

Experimental details:

Period of experiment:	4-12-63 to 16-1-64
Temperature during the experiment	Min. 76° F - 80° F Max. 81° F - 86° F
Relative Humidity during experiment.	89 % - 95 %

Procedure: The pupae were continuously refrigerated for 35 days.

Results: The results of the experiment are given in Table VII. It is seen that none of the pupae developed into moths when transferred into laboratory conditions after continuous refrigeration for 35 days. All the pupae when dissected out showed dead moth within them.

Experiment 8.

Effect on development of Trichospilus pupivora in pupa of P. peponis when host pupae exposed to the parasites for 24 hours are refrigerated at 10° C for 7 days.

Experimental details.

Number of host pupae used:	10
Age of host pupae:	Below 24 hours.
No. of female parasites used to parasitize a single host pupa	5

Procedure: Ten fresh pupae of P. peponis were exposed to parasitization by 5 females of T. pupivora, each in specimen tubes as detailed under Methods. After 24 hours of parasitization, the parasitized pupae were refrigerated at 10°C for 7 days as already described. At the end of seven days the parasitized pupae under refrigeration were taken out of the refrigerator and kept in the laboratory till adult parasites emerged.

Results: The results of the experiment are given in Table VIII. The data presented in the table show that the post refrigeration period of development of T. pupivora varies from 16 to 19 days, the average period being 17 days. The number of parasites emerged from individual pupa ranges between 294 and 456 with an average of 383.6 parasites per pupa. The ratio of male to female parasites is seen to be 1: 25.

Experiment 9.

Effect on development of T. pupivora in pupa of P. peponis when host pupae exposed to the parasites for 24 hours are refrigerated at 10°C for 14 days.

Experimental details:

Period during which experiment conducted. | 7-12-1963 to 11-1-1964.

Temperature during experiment. | Min. 76°F to 80°F
| Max. 81°F to 86°F

T A B L E VIII

Effect on development of T. pupivora in pupa of P. reponis when host pupae exposed to the parasites for 24 hours are refrigerated at 10°C for 7 days.

Sl. No.	Date of exposure of parasite to pupa	Date of exposure of parasitized pupa to Low Temp.	Date of removal of parasitized pupa from Low Temp.	Date of emergence of parasites.	post refrigeration development period of parasites in days.	Total developmental period in days.	No. of parasites emerged.			Remarks
							Male	female	Total	
1	6-12-63	7-12-63	14-12-63	31-12-63	17	25	12	416	428	
2	6-12-63	7-12-63	14-12-63	2-1-64	19	27	18	425	443	
3	6-12-63	7-12-63	14-12-63	1-1-64	18	26	13	309	322	
4	6-12-63	7-12-63	14-12-63	30-12-63	16	25	17	344	361	44
5	6-12-63	7-12-63	14-12-63	1-1-64	18	26	13	406	419	
6	6-12-63	7-12-63	14-12-63	31-12-63	17	25	9	287	296	
7	6-12-63	7-12-63	14-12-63	31-12-63	17	25	16	366	382	
8	6-12-63	7-12-63	14-12-63	1-1-64	18	26	15	399	414	
9	6-12-63	7-12-63	14-12-63	30-12-63	16	25	18	438	456	
10	6-12-63	7-12-63	14-12-63	31-12-63	17	25	17	293	315	

Total					170	255	148	3688	3836	
Average					17.0	25.5	14.8	368.8	383.6	
Sex ratio:					1:25					

Relative Humidity during
experiment . 89% to 95%

Procedure: Pupae of P. peponis parasitized by T. pupivora for 24 hours were refrigerated for 14 days continuously. Rest as in Experiment 8.

Results: The data presented in Table IX show that parasites emerged only from four out of the total of 10 pupae. The post refrigeration development period ranged from 17-19 days, the average being 17.75 days. The total number of parasites emerged varies from 201 to 359 from each pupa with an average of 251.25. The ratio of male parasites to female parasites is 1:26.

Experiment 10.

Effect on development of T. pupivora in pupa of P. Peponis when host pupa exposed to the parasites for 24 hours has been refrigerated at 10°C for 21 days.

Experimental details:

Period of experiment. 8-12-1963 to 10-1-1964.

Temperature during experiment. (Min. 76°F to 80°F
Max. 81°F to 86°F

Relative Humidity
during experiment. 89% to 95%

Procedure: The experiment was conducted just as Experiment 9 i with the exception that the parasitized pupae were refrigerated for 21 days. Rest as in Experiment 8.

T A B L E IX

Effect on development of T. univora in pupae of P. penns when host pupae exposed to the parasites for 24 hrs. are refrigerated at 10°C for 14 days.

Sl. No.	Date of exposure of parasite to pupa	Date of exposure of parasitized pupa to Low Temp.	Date of removal of parasitized pupa from Low. Temp.	Date of emergence of parasites.	Post refrigeration development period of parasites in days.	No. of parasites emerged				Remarks	
						Total development period	Male	Female	Total		
1	7-12-63	8-12-63	22-12-63	--						*	
2	7-12-63	8-12-63	22-12-63	--						*	
3	7-12-63	8-12-63	22-12-63	8-1-64	17	31	9	244	253		
4	7-12-63	8-12-63	22-12-63	--						*	
5	7-12-63	8-12-63	22-12-63	9-1-64	18	32	14	343	359		
6	8-12-63	9-12-63	23-12-63	--						*	
7	8-12-63	9-12-63	23-12-63	--						*	
8	8-12-63	9-12-63	23-12-63	--						*	
9	8-12-63	9-12-63	23-12-63	9-1-64	17	31	8	193	201		
10	8-12-63	9-12-63	23-12-63	11-1-64	19	33	8	225	233		
					Total	71		127	39	1005	1046
					Average	17.75		31.75	9.75	251.25	261.5
					Male : Female ratio 1:2						

* No emergence of parasites.

Results: Table X gives the results of the experiment. It will be observed that no parasites emerged from any of the parasitized pupae refrigerated for 21 days

Experiment 11:

Effect on development of *T. pupivora*, in pupae of *P. poponis*, when host pupae parasitized 8 days earlier are refrigerated at 10° C for 7 days.

Experimental details:

Period of experiment.	1-1-1964 to 25-1-1964.
Temperature during experiment.	Min. 76°F to 80°F Max. 82°F to 86°F
Relative Humidity during the experiment.	82% to 95 %

Procedure: Fresh pupae of *P. poponis* were parasitized with *T. pupivora* as described and kept under laboratory conditions, for 8 days. These were then refrigerated at 10°C continuously, for a period of 7 days after which they were again returned to the laboratory and emergence of adult parasites observed.

Results: Results of the experiment are given in Table XI. It may be seen that the post refrigeration development period of the parasite ranges between 4 and 7 days with an average of 5.7 days. The total period taken by the parasites to complete their life cycle, including the period of

T A B L E X

Effect on development of T. punivora of P. peponis when host pupa exposed to parasites for 24 hours
has been refrigerated at 10°C for 21 days.

Sl. No.	Date of exposure of parasites to pupa	Date of exposure of parasitized pupa to Low. temp.	Date of removal of parasitized pupa from Low. Temp.	Date of emergence of para- sites.	Remarks
1	8-12-63	9-12-63	30-12-63	-	No emergence of parasites.
2	8-12-63	9-12-63	30-12-63	-	
3	8-12-63	9-12-63	30-12-63	-	
4	8-12-63	9-12-63	30-12-63	-	
5	9-12-63	10-12-63	31-12-63	-	
6	9-12-63	10-12-63	31-12-63	-	
7	9-12-63	10-12-63	31-12-63	-	
8	9-12-63	10-12-63	31-12-63	-	
9	9-12-63	10-12-63	31-12-63	-	
10	9-12-63	10-12-63	31-12-63	-	

refrigeration averages 20.7 days. The number of adult parasites produced per pupa varies from 78 to 408, the average being 266.2. The male: female ratio of the parasites reared is 1:26.

Experiment 12.

The parasitising capacity of *I. pupivora* emerging from *P. peponis* refrigerated 8 days after parasitization at 10°C for 7 days.

Experimental details:

Period of experiment.	20-1-64 to 11-2-64.
Temperature during experiment.	Min. 76°F to 82°F Max. 82°F to 85°F
Relative humidity during experiment.	82% to 95%

Procedure: Five females from each set of parasites emerging from the 10 pupae in Experiment 11, were used to parasitize 10 fresh host pupae in specimen tubes and allowed to develop under laboratory conditions.

Results: The results of the experiment are presented in Table XII. It may be seen that they have parasitised the host pupae and developed in them normally, each pupa yielding on an average 262.3 parasites.

T A B L E X I.

Effect on development of T. pupivora, in pupae of P. peponis when host pupae parasitized 8 days earlier, are refrigerated at 10°C for 7 days.

Sl. No.	Date of parasitization.	Date of exposure of parasitized pupae to Low Temp.	Date of removal of parasitized pupae from Low Temp.	Date of emergence of parasites.	post refrigeration development period of parasites in days.	Total developmental period in days.	No. of parasites emerged.			Remarks
							Male	Female	Total	
1	1-1-64	9-1-64	16-1-64	22-1-64	6	21	8	294	302	
2	1-1-64	9-1-64	16-1-64	23-1-64	7	22	10	286	296	
3	1-1-64	9-1-64	16-1-64	20-1-64	4	19	9	309	218	
4	3-1-64	11-1-64	18-1-64	23-1-64	5	20	8	175	183	
5	4-1-64	12-1-64	19-1-64	25-1-64	6	21	12	78	90	
6	4-1-64	12-1-64	19-1-64	24-1-64	5	20	10	243	253	
7	4-1-64	12-1-64	19-1-64	25-1-64	6	21	14	341	355	
8	4-1-64	12-1-64	19-1-64	26-1-64	7	22	12	379	391	
9	4-1-64	12-1-64	19-1-64	24-1-64	5	20	13	408	421	
10	4-1-64	12-1-64	19-1-64	25-1-64	6	21	8	249	257	
Total						207	104	2662	2786	
Average						20.7	10.4	266.2	276.6	
Sex ratio						1: 26.				

Experiment 13.

Effectt on development of *T. pupivora* in pupa of
P. peponis when host pupae parasitized 8 days earlier,
are refrigerated at 10°C for 14 days.

Experimental details:

Period of experiment. 4-1-64 to 3-2-1964.

Temperature during the experiment. Min. 76°F to 80°F
Max 82°F to 86°F.

The Relative Humidity during experiment. 82% to 95 %

Procedure: Same as in Experiment 11 except that the parasitized pupae were refrigerated for 14 days. Rest as in Experiment 11.

Results. The results are given in Table XIII. The post-refrigeration development period of the parasite is seen to be from 5-7 days with an average of 6.2 days. The life cycle of the parasite including the refrigeration period takes on an average 28.2 days. The number of parasites emerging from a single pupa varies from 258 to 452 with an average of 357.3. The sexratio male: female being 1:31.

Experiment 14.

Parasitising capacity of *T. pupivora* emerging from
P. peponis refrigerated, 8 days after parasitization,
at 10°C for 14 days.

T A B L E X I I

The parasitizing capacity of *T. pupivora* emerging from *P. peponis* refrigerated 8 days after parasitization at 10°C for 7 days.

Sl. No.	Date of parasitization of pupa	Date of emergence of parasites	Total parasites emerged	Development period in days	Remarks
1	20-1-64	4-2-64	342	15	
2	22-1-64	8-2-64	282	17	
3	23-1-64	10-2-64	80	18	
4	23-1-64	8-2-64	79	16	
5	24-1-64	10-2-64	394	17	
6	24-1-64	9-2-64	269	16	
7	25-1-64	11-2-64	334	17	
8	25-1-64	9-2-64	401	15	
9	25-1-64	10-2-64	119	16	
10	26-1-64	11-2-64	323	16	
Total			2623	163	
Average			262.3	16.3	

TABLE XIII

Effect on development of T. univora in pupa of P. penns when host pupae parasitized 8 days earlier, are refrigerated at 10°C for 14 days

Sl. No.	Date of parasitization.	Date of exposure of parasitized pupa to Low Temp.	Date of removal from Low Temp.	Date of emergence of parasites.	Post refrigeration development period of parasites in days.	Total developmental period in days.	No. of parasites emerged.			Remarks
							Male	Female	Total	
1	4-1-64	12-1-64	26-1-64	31-1-64	5	27	13	373	386	
2	4-1-64	12-1-64	26-1-64	1-2-64	6	28	11	392	403	
3	4-1-64	12-1-64	26-1-64	2-2-64	7	29	15	442	457	
4	4-1-64	12-1-64	26-1-64	1-2-64	6	28	9	258	267	
5	4-1-64	12-1-64	26-1-64	2-2-64	7	29	8	301	309	
6	4-1-64	12-1-64	26-1-64	1-2-64	6	28	14	452	466	
7	5-1-64	12-1-64	26-1-64	1-2-64	6	28	11	272	283	
8	5-1-64	13-1-64	27-1-64	2-2-64	6	28	13	405	418	
9	5-1-64	13-1-64	27-1-64	3-2-64	7	29	11	321	332	
10	5-1-64	13-1-64	27-1-64	2-2-64	6	28	12	357	369	
Total					62	282	117	3573	3690	
Average					6.2	28.2	11.7	357.3	369.0	
Sex ratio:					1:31					

53

Experimental details:

Period of experiment. 31-1-1964 to 22-2-1964.

Temperature during experiment. Min. 76°F to 82°F
Max. 82°F to 86°F

Relative Humidity during experiment. 71% to 96%

Rest as in Experiment 12.

Results: Table XIV gives the results. It will be seen that the parasites develop normally taking 15 - 19 days (average 16.7 days) producing on an average 265.9 parasites per pupa.

Experiment 15.

Effect on development of *T. pusivora* in pupa of *P. neoonis* when host pupa parasitized 8 days earlier is refrigerated at 10°C for 21 days.

Experimental details:

Period of experiment: 6-1-1964 to 11-2-1964.

Temperature during the experiment. Min. 76°F to 82°F
Max. 82°F to 86°F

Relative Humidity during experiment. 82% to 95%

Procedure: As in Experiment 11, except that the parasitized pupae were refrigerated for 21 days. Rest as in Experiment. 11.

TABLE XIV

Parasitizing capacity of *E. univora* emerging from *P. neponis* refrigerated, 8 days after parasitization
at 10°C for 14 days.

Sl. No.	Date of parasi- tization of pupa	Date of emergences of parasites.	Total parasites emerged	Development period in days.	Remarks.
1	31-1-64	16-2-64	346	16	
2	1-2-64	18-2-64	218	15	
3	1-2-64	18-2-64	196	17	
4	1-2-64	19-2-64	384	18	
5	1-2-64	17-2-64	401	16	
6	2-2-64	17-2-64	264	15	
7	2-2-64	21-2-64	318	19	
8	2-2-64	19-2-64	106	17	
9	2-2-64	17-2-64	328	15	
10	3-2-64	22-2-64	98	19	
		Total	2659	167	
		Average	265.9	16.7	

Results: The results of the experiment are given in Table XV. It may be observed that the post-refrigeration development period of the parasite ranges from 4-7 days, averaging 5.6 days. The total development period of the parasite including refrigeration period is 34.6 days on an average. The number of adult parasites produced per pupa varies from 276 to 436, the average being 356.8. The male:female ratio of the parasite produced is 1:35.

Experiment 16.

Parasitizing capacity of *I. pupivora* emerging from pupae of *P. peponis* refrigerated 8 days after parasitization at 10°C for 21 days.

Experimental details:

Period of experiment : 8-2-1964 to 2-3-1964.

Temperature during experiment		Mmn. 80°F to 82 °F
		Mxm. 82°F to 88°F

Relative Humidity during experiment		71% to 96%

Rest as in Experiment 12.

Results: Table XVI gives the results. It may be seen that the parasites develop normally, taking 16-21 days, average being 18.3 days, producing on an average 301.3 parasites per pupa.

T A B L E X V

Effect on development of T. univora in pupa of E. japonis when host pupae parasitized 3 days earlier, and refrigerated at 10°C for 21 days.

Sl. No.	Date of parasitization.	Date of exposure of parasitized pupa to Low Temp.	Date of removal from low temp.	Date of emergence of parasites.	Post refrigeration development period of parasites (days)	Total development period in days.	No. of parasites emerged.			Remarks
							Male	Female	Total	
1	6-1-64	14-1-64	4-2-64	10-2-64	6	35	8	276	284	
2	6-1-64	14-1-64	4-2-64	9-2-64	5	34	11	436	447	
3	6-1-64	14-1-64	4-2-64	11-2-64	7	36	11	398	409	
4	6-1-64	14-1-64	4-2-64	10-2-64	6	35	9	388	397	
5	6-1-64	14-1-64	4-2-64	8-2-64	4	33	8	286	294	
6	6-1-64	14-1-64	4-2-64	9-2-64	5	34	10	371	381	
7	6-1-64	14-1-64	4-2-64	10-2-64	6	35	14	397	411	
8	7-1-64	15-1-64	5-2-64	11-2-64	6	35	13	406	419	
9	7-1-64	15-1-64	5-2-64	10-2-64	5	34	9	301	310	
10	7-1-64	15-1-64	5-2-64	11-2-64	6	35	8	309	317	
Total					56	346	101	3568	3669	
Average					5.6	34.6	10.1	356.8	366.9	
Sex ratio:					1	: 35				

T A B L E X V I

Parasitizing capacity of T. pupivora emerging from pupae of p. pepenis refrigerated, 9 days after parasitization, at 10°C for 21 days.

Sl. No.	Date of parasitization of pupa	Date of emergence of parasites	Total parasites emerged	Development period in days	Remarks
1	8-2-64	27-2-64	458	19	
2	9-2-64	29-2-64	409	20	
3	9-2-64	27-2-64	313	18	
4	10-2-64	28-2-64	347	18	
5	10-2-64	26-2-64	296	16	
6	10-2-64	29-2-64	189	19	
7	10-2-64	27-2-64	112	17	
8	11-2-64	27-2-64	97	16	
9	11-2-64	1-3-64	486	19	
10	11-2-64	2-3-64	526	21	
		Total	5015	195	
		Average	501.5	19.5	

Experiment 17.

Effect on development of *T. pupivora* in pupa of *P. peponis* when host pupa parasitized 8 days earlier is refrigerated at 10°C for 28 days.

Experimental details:

Period of experiment.		7-1-1964 to 19-2-1964
Temperature during experiment.	Mnm.	76°F - 80°F
	Mxm.	82°F - 86°F
Relative Humidity during experiment.		71% to 96%

Procedure: As in Experiment 11, except that the parasitized pupae were refrigerated for 28 days. Rest as in Experiment 11.

Results: The results of the experiment are given in Table XVII. It may be seen from the table that no adult parasites emerged from 2 of the 10 parasitized pupae, when returned to the laboratory conditions after refrigerating them continuously for 28 days. The two pupae out of which parasites did not emerge, when dissected open found to contain dead parasites in their pupal stage. The table also shows that the post-refrigeration development period of the parasites emerged ranged from 4 to 6 days averaging 5.4 days. The total development period of the parasite including refrigeration period is 41.4 days on an average. The number of

T A B L E X V I I

Effect on development of T. pupivora in pupa of P. peponis when host pupae parasitized 8 days earlier, are refrigerated at 10°C for 28 days.

Sl. No.	Date of parasitization.	Date of exposure of parasitized pupa to Low Temp.	Date of removal from low Temp.	Date of emergence of parasites.	Post refrigeration development period of parasites (days)	Total development period in days.	No. of parasites emerged			Remarks
							Males	Female	Total	
1	7-1-64	15-1-64	12-2-64	17-2-64	5	41	9	219	228	
2	7-1-64	15-1-64	12-2-64	16-2-64	4	40	8	348	356	
3	7-1-64	15-1-64	12-2-64	-	-	-	-	-	-	*
4	7-1-64	15-1-64	12-2-64	18-2-64	6	42	12	384	396	
5	7-1-64	15-1-64	12-2-64	-	-	-	-	-	-	*
6	8-1-64	16-1-64	13-2-64	19-2-64	5	41	10	113	123	
7	8-1-64	16-1-64	13-2-64	19-2-64	6	42	8	374	382	
8	8-1-64	16-1-64	13-2-64	18-2-64	5	41	9	395	404	
9	8-1-64	16-1-64	13-2-64	19-2-64	6	42	8	72	80	
10	8-1-64	16-1-64	13-2-64	19-2-64	6	42	11	295	306	
					Total	45	75	2200	2275	
					Average	5.4	9.4	275.0	284.4	
					Sex ratio	1: 29.				

* No emergence of parasites.

adult parasites produced per pupa varies from 80-404, the average being 284.4. The male: female ratio of the parasites produced is 1:29.

Experiment 18:

Parasitizing capacity of *T. pupivora* emerging from pupa of *P. peponis* refrigerated 8 days after parasitization at 10°C for 28 days.

Experimental details:

Period of experiment. 16-2-1964 to 6-3-1964.

Temperature at the time
of experiment. Min. 76°F to 82°F
Max. 82°F to 88°F

Relative Humidity
during experiment 77% to 90%

Rest as in Experiment 12.

Results. Table XVIII gives the results. It may be observed that the parasites develop normally taking 14 to 16 days (an average of 12.1 days) producing on an average 152.4 parasites per pupa.

Experiment 19.

Effect on development of *T. pupivora* in pupa of *P. peponis* when host pupa parasitized 8 days earlier is refrigerated at 10°C for 35 days.

Experimental details.

Period of experiment. 10-1-1964 to 8-3-1964.
Temperature during
experiment. Min. 76°F to 82°F
Max. 82°F to 88°F

T A B L E X V I I I

Parasitizing capacity of T. pupovora emerging from pupa of P. peponis refrigerated,
8 days after parasitization, at 10°C for 28 days.

Sl. No.	Date of parasiti- zation of pupa	Date of emergence of	Total parasites emerged	Development period in days	Remarks
1	16-2-64	2-3-64	189	15	
2	17-2-64	2-3-64	308	14	
3	18-2-64	4-3-64	80	15	
4	18-2-64	5-3-64	96	16	
5	18-2-64	4-3-64	549	15	
6	19-2-64	4-5-64	216	14	
7	19-2-64	6-3-64	267	16	
8	19-2-64	4-3-64	19	14	
		Total	1524	119	
		Average	190.5	14.9	

Relative Humidity during
experiment.

71% to 95%

Procedure:

As in Experiment 11, except that
the parasitized pupae were refrigerated
for 35 days. Rest as in Experiment 11.

Results. The results of the experiments are shown in
Table XIX. It may be observed that no adult parasites
emerged from any of the parasitized pupae when returned
to the laboratory condition after refrigeration of them
continuously for 35 days. The host pupae when dissected
open found to contain dead parasites in their pupal
stage.

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TABLE XIX

Effect on development of T. pupivora, in pupa of P. reponis, when host pupae parasitized 8 days earlier, are refrigerated at 10°C for 35 days.

Sl. No.	Date of parasitization	Date of exposure of parasitized pupa to Low temp.	Date of removal from Low Temp.	Date of emergence of parasites	Remarks
1	10-1-64	18-1-64	22-2-64	-	No emergence of parasite. Host pupa with dead pupae parasited.
2	10-1-64	18-1-64	22-2-64	-	
3	10-1-64	18-1-64	22-2-64	-	
4	10-1-64	18-1-64	22-2-64	-	
5	10-1-64	18-1-64	22-2-64	-	
6	10-1-64	18-1-64	22-2-64	-	
7	12-1-64	20-1-64	24-2-64	-	
8	12-1-64	20-1-64	24-2-64	-	
9	12-1-64	20-1-64	24-2-64	-	
10	12-1-64	20-1-64	24-2-64	-	

D I S C U S S I O N

I. The effect of refrigeration at 10°C on the pupa of *Phytometra peponis*.

Refrigeration of the alternate host of *Trichospilus pupivora* at 10°C, has shown that the pupa is able to tolerate the low temperature without any ill-effects, upto a period of 28 days. In 28 days, however 20 per cent mortality of the pupae occurs, while in 21 days, no mortality is observed. Continuous refrigeration for 35 days appears fatal to the pupa.

The average pupal period under laboratory conditions of development is 7.5 days and the post-refrigeration pupal period also approximates to that, with slight variation between 7.6 and 6.5 days. This indicates that during refrigeration for periods up to 28 days, practically no development of the pupa takes place and that when returned to laboratory conditions they revive and develop into moths normally. This is a useful finding because it shows that on occasions when excess of host pupae are available and not enough parasites to parasitize them, these pupae can be safely stored at 10°C upto a period of 21-28 days.

II. Effect of refrigeration at 10°C on the development of *T. pupivora* in pupa of *P. peponis*.

The effect of low temperature (10°C) on the

development of T. pupivora within pupa of P. peponis has been ascertained by refrigerating the parasites at two pre-imaginal stages viz. egg stage and pupal stage. Ananthanarayanan (1934) has observed from dissections of the parasitized pupa after various periods of parasitization, that 24 hours after exposure of the pupa to the parasites, the progenies within are in the egg stage and that after 8 days of parasitism, they are invariably in the pupal stage. Hence the refrigeration of the parasitized host pupae has been done 24 hours or 8 days after parasitism as the case may be. The results of these are discussed below:-

(a) Effect of refrigeration at 10°C on the development of T. pupivora in the host pupa of P. peponis exposed to the parasite for 24 hours.

Results presented (Table XX and Figure 1) show that continuous refrigeration at 10°C of the parasite eggs within the host pupae, for 7 days, does not adversely affect the viability of the eggs and that they develop normally when returned to laboratory conditions. The sex ratio of the parasites emerged also is not affected materially, the ratio being 1:25 as against the ratio 1:28 in the laboratory-reared parasites. When the parasite eggs within the host pupae are refrigerated continuously for 14 days, the parasites have been obtained only from 4 out of 10 pupae and in these pupae, the

number of parasites developing appears to be reduced the average number being 261.5 per pupa as against 388.9 parasites per pupa under laboratory conditions. The

T A B L E XX

Effect of refrigeration of eggs of *T. pupivora*
AT at 10°C

Period of refrigeration (days)	Average No. of parasites emerging per pupa.	Sex ratio Male: Female.	Post refrigeration development period (days)
Normal condition	388.9	1:28	-
7	383.6	1:25	17 17
14	261.5	1:26	17.75
21	-	-	-

refrigeration of the parasite in its egg stage for 21 days causes complete mortality in all the ten cases tried. In both cases of refrigeration for 7 days and 14 days, the post-refrigeration developmental period of the parasite, remains more or less similar to the normal egg period, thereby indicating that no development of the parasite takes place during the period of refrigeration.

These results thus show that *T. pupivora*, in its egg stage can withstand refrigeration at 10°C upto

seven days without any ill effects and upto 14 days with 60 per cent reduction in survival. Continuation of refrigeration beyond this period appears to be lethal totally.

(b) Effect of refrigeration at 10°C on the development of pupa of *T. pupivora* in the host pupa of *P. peponis*.

The results of these experiments are consolidated in Table XXI. It will be seen that continuous exposure to 10°C

T A B L E XXI
Effect of refrigeration on pupa of *T. pupivora*
at 10°C

Period of refrigeration. (days)	Average No. of parasites emerging per pupa	Sex ratio Male: Female	Post refrigeration development period (days)	Average number of parasites from second generation of refrigerated parasites.
Normal conditions	388.9	1:28	--	--
7	276.6	1:26	5.7	262.3
14	369.0	1:31	6.2	265.9
21	366.9	1:35	5.6	301.3
28	284.4	1:29	5.4	190.5
36	--	--	--	--

for periods upto 28 days, does not affect materially the revival of the parasites. Extension of the refrigeration

- Fig. 1. Effect of refrigeration on viability of T. pupivora in egg stage.
- Fig. 2. Effect of refrigeration on viability of T. pupivora in pupal stage.
- Fig. 3. Effect of refrigeration on sex ratio of T. pupivora in pupal stage.
- Fig. 4. Effect of refrigeration on the fecundity of progeny of T. pupivora.

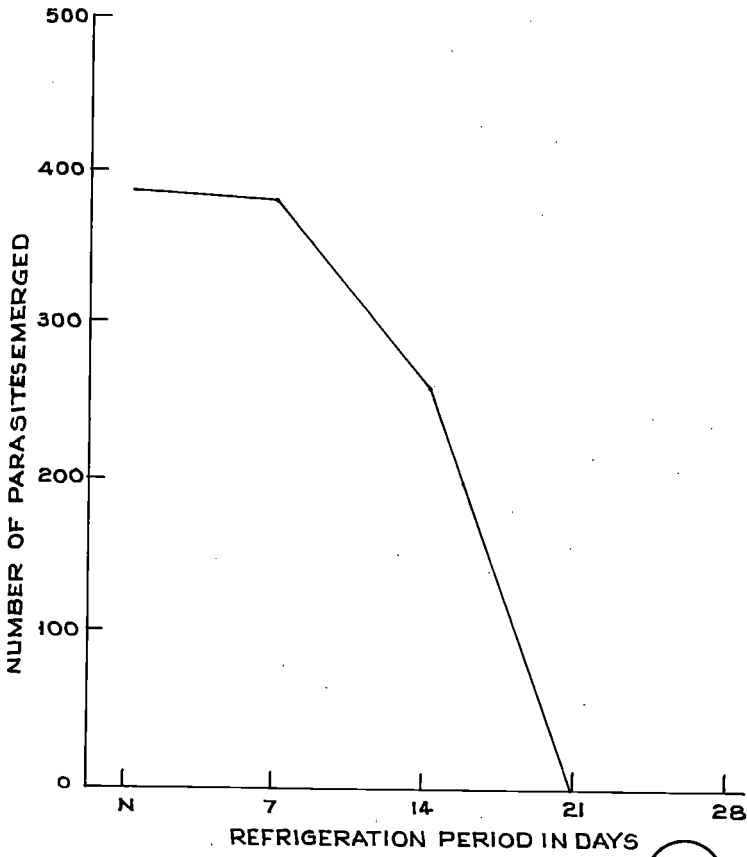
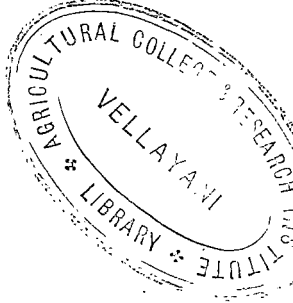


FIG. 1

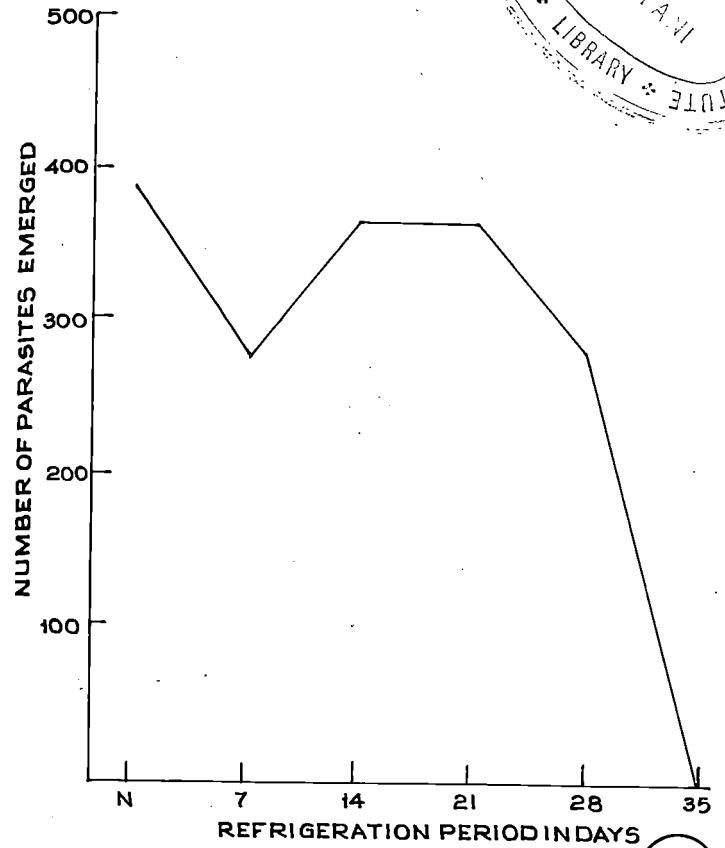


FIG. 2

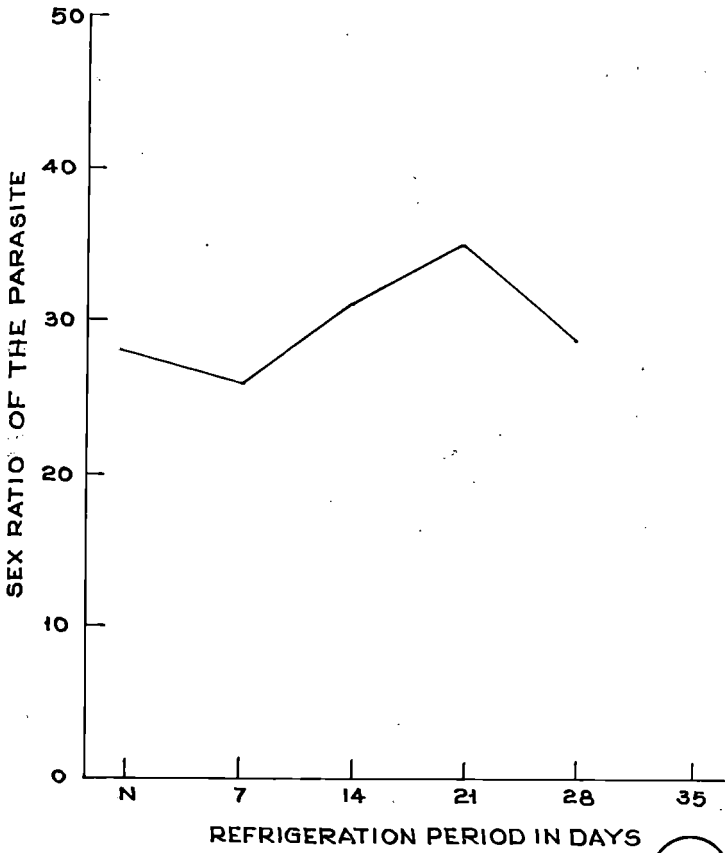


FIG. 3

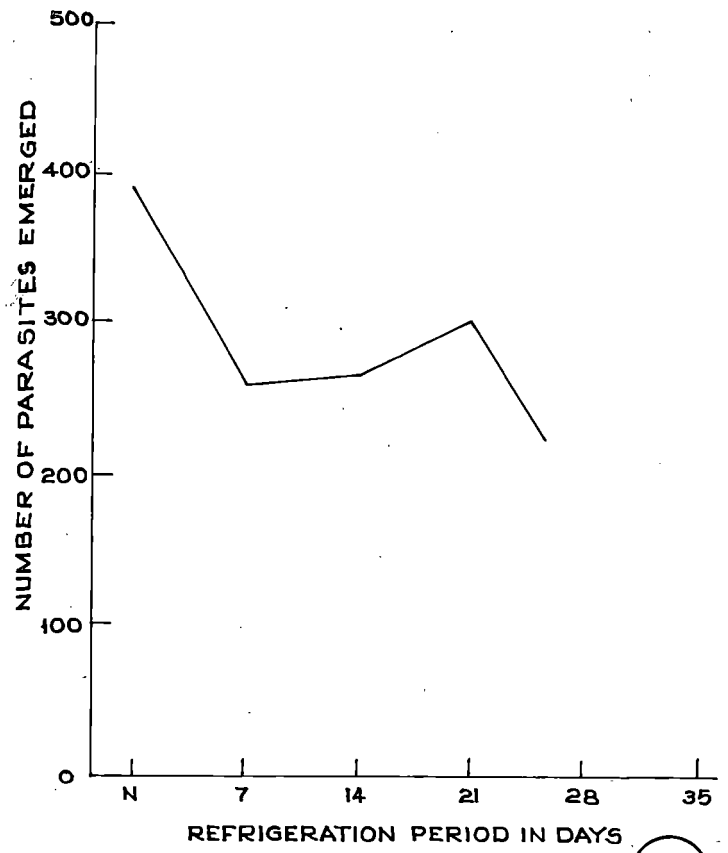


FIG. 4

to 35 days , however, results in the complete mortality of the parasites within the host pupae. There is indication that the number of parasites surviving low temperature, is slightly reduced, but this reduction does not appear to be of any significance and neither is it commensurate with the increasing periods of refrigeration. (See Fig.2).

The proportion of female parasites is seen to increase a little from 26 to 35 as the period of refrigeration increases from 7 to 21 days. However, this proportion again tends to dip as the refrigeration period is increased further (Fig.3) . Schread and Garman (1934) have also reported varying effects of Trichogramma minutum when exposed to low temperature.

As regards the post-refrigeration development period of the parasite pupae, it is seen that this period does not significantly vary from that of the unrefrigerated parasite pupa. This shows that the developmental activities within the pupae are completely stopped at 10°C.

Further, it is also observed that the reproduction capacity of the adult parasite is impaired to some extent as a result of refrigeration during the pupal stage. (Table XXI, Fig.4). A drastic reduction in the fecundity is evidenced in the parasites emerging from pupae refrigerated for periods over 21 days.

Overall position.

The results discussed above will show that it is advantageous to refrigerate T. pupivora, in its pupal stage, than in its egg stage. The maximum period for which the development of the parasite can be arrested by exposing the egg stage to 10° C is 14 days while this period in the pupal stage of the parasite is 28 days. It is also observed that the adult parasites emerging from pupae, refrigerated upto this period, are suitable for parasitizing fresh host pupae.

The present observation that the pupal stage of the parasite can tolerate more the ill-effects of exposure to low temperature than the earlier pre-imaginal stages, has parallels in the case of other parasites also. For example Chatterjee (1944) has observed that the pupal stage of the parasite Euplectron parvulus F., is more resistant to low temperature than its grubs. Further Gomes (1947) has shown that the pupal stage of the parasite Macrocentrus ancylihora when stored at low temperature has no harmful effect on the fecundity of the adult female or the sex ratio of the progeny.

It has thus become patent that it is possible to postpone emergence of T. pupivora to nearly one month without impairing the reproductive capacity of the emerging parasite, by keeping the parasitized

host pupae at 10°C after 8 days of parasitization .
Further the host pupa itself has been found to remain
viable upto 28 days at 10°C. These information are
useful when exigencies arise for storing the host
pupae or the parasites for future or delayed use.

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S U M M A R Y

Up-to-date literature on the effect of low temperature on parasitic insects has been reviewed.

Effect of refrigeration at 10° C on the survival of pupa of Phytometra peponis F., the alternate host of Trichospilus pupivora Ferriere has been ascertained.

The fresh pupa of P. peponis survives continuous exposure to 10° C upto 31 days, without any ill-effects and develops normally to moth when returned to laboratory. Continuous exposure to the low temperature for 28 days results in 20 per cent mortality among the pupae. There is no revival of pupae after 35 days of refrigeration. The post refrigeration development period of surviving pupae does not differ from the pupal period under laboratory conditions showing that the development of the pupae is completely arrested at 10° C.

Effect of refrigeration at 10° C on the development of Trichospilus pupivora within the hostpupa Phytometra peponis when refrigerated at the eggs stage (24 hours after parasitization period) at the pupal stage (8 days after parasitization) has been ascertained. The egg stage of the parasite survives exposure to 10° C for 7 days there being emergence from all the parasitized pupae. Exposure for 14 days results in the parasite failing to emerge from out of 10 host pupae while exposure for 21 days results in total

mortality of the parasite. Development of the egg appears to be completely arrested at 10° C.

The pupal stage of *T. pupivora* survives exposure to 10°c upto 28 days. exposure for 35 days results in complete mortality of the parasite pupae within the host pupa. Proportion of female parasites increases from 26 to 35 as the period of refrigeration is raised from 7 to 21 days. Further refrigeration results in a decrease of the proportion of females.

There is no significant reduction in the number of parasites surviving which is neither commensurate with the increasing periods of refrigeration. Exposure to 10°C results in the complete inhibition of the development of the parasite pupa. The reproduction capacity of the parasite emerging from refrigerated pupa is affected to some extent, a reduction being conspicuously seen in the case of refrigeration over 21 days.

The usefulness of these findings in storing the host pupae or the parasite for future or delayed use is indicated.

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