Storage studies of *Anaphes iole* Girault (Hymenoptera: Mymaridae) at different developmental stages, temperatures and light-dark regimes

Khalid ABDULLAH [1(✉)]
Joe J. ELLINGTON [2]
Sumbal YASMIN [1]

Summary

A series of experiments were conducted to study the storage life of *Anaphes iole* Girault (Hymenoptera: Mymaridae) parasitizing eggs of lygus bug *Lygus hesperus* Knight at different developmental stages, temperatures and photoperiods. Lygus eggs, parasitized by *Anaphes iole* were held for one, three, six and nine days and were stored at 10 and 4 ˚C, and two photoperiods (complete darkness and 09:15 (L:D)) for 20, 40, 60, and 80 days. The highest adult emergence resulted from parasitized lygus eggs held for six days at 29 ˚C and then stored for 40, 60 and 80 days. Adult emergence was better when eggs were stored at 10 ºC than at 4 ºC. Complete darkness gave better adult emergence than 9:15 (L:D) photoperiod. When six-day old parasitized lygus eggs were stored in complete darkness at 10 ºC for 20, 40, 60 and 80 days there were 62.90, 42.50, 29.60 and 8.35% emergence respectively. Significantly lower number of adult *A. iole* emerged from lygus eggs parasitized by *A. iole* then held for one, three and nine days after parasitization at 29 ˚C then stored at 4 ˚C, 9:15 (L:D) photoperiod, than from eggs parasitized and held for six days at 29 ˚C and then stored in complete darkness at 10 ˚C.

Key words

Anaphes iole; Lygus; storage; photoperiod; temperature; developmental stages; parasitiod

[1] Agriculture Research Institute, Dera Ismail Khan 29020, NWFP Pakistan
✉ e-mail: khalidabdullah99@yahoo.com
[2] Department of Entomology, Plant Pathology and Weed Sciences, New Mexico State University, Las Cruces NM, USA 88003

Received: February 17, 2009 | Accepted: July 1, 2009

Introduction

Lygus spp. are the most serious pests of alfalfa seed crop, cotton, vegetables, strawberries, and beans (Smith and Michelbacher, 1946; Lieberman and Knowlton, 1955; Wheeler, 1974; Yeargan, 1985; Flint and Clark, 1981). In North America, the most important species are *Lygus lineolaris* (Palisot de Beauvois), *L. hesperus* (Knight) and *L. elisus* Van Duzee (Stern et al., 1967).

Because lygus have many predators and parasites in nature such biological agents might offer an effective alternative control. Among the reported parasitoids, *Anaphes spp.*, *Erythmelus spp.*, *Polynema spp.* and *Telenomus spp.* are egg parasitoids of Lygus sp. *Anaphes iole* Girault is the principal lygus egg parasitoid in the Untied States (Gordon *et al.*, 1987; Sohati *et al.*, 1989; Ruberson and Williams, 2000). *Anaphes iole* in Arizona generally parasitized 21 to 32 % of the lygus egg population but reached as high as 89 % (Graham *et al.*, 1981a; Graham *et al.,* 1981b; Graham *et al.*, 1981c). It parasitize more than 64 % of Lygus population in strawberries (Udayagiri *et al.*, 2000)

Natural populations of *A. iole* do not generally regulate lygus populations below an economic threshold in seed alfalfa. However, it might be possible to achieve economic control of lygus with augmentative releases of *A. iole.* To get enough *A. iole* for field release, it is presently necessary to maintain an expensive parasitoid colony throughout the year. At normal rearing conditions (29 ˚C, 55 % RH), *A. iole* completes its life cycle in 12-15 days. For one or two summer releases, it is necessary to rear some 24 generations of *A. iole* per year. If we could store *A. iole,* rearing costs would be reduced.

There is a need to further document and determine the optimal conditions for the storage of *A. iole* in the laboratory.

Materials and Methods:

Colony maintenance

Lygus Colony: Lygus colonies were maintained as described by Patana and Debolt (1985) and Debolt and Patana (1985). Early nymphal instars were fed beans, while late nymphal instars and adults were fed artificial diet. Changes in artificial diet constituents were made, when needed. The ingredients were mixed and autoclaved at 121 ˚C for 20 minutes. The diet mixture was cooled and blended for two minutes and poured in diet packets. The diet packets were made by sealing three sides of pre cut 10x14 cm pieces of polyethylene (12.5 µ thickness) and Parafilm® with household electric heat sealer. These diet packets were stored in a refrigerator. The lygus rearing room was held at 29 ˚C and 55 % RH. Eggs were collected in 10x14 cm egg packets. The egg packets were made of the same material as the diet packets but filled with Gelcarin® gel. Lygus oviposited in the Gelcarin® gel packets. Three to four diet packets and one egg packet were placed (Parafilm® side down) on the top of the adult cage.

Anaphes Colony: The *A. iole* colonies were maintained in 20x20x20 cm clear plastic cages with 0.25 mm polyester mesh on two sides. One or two egg packets were placed in newly emerged parasitoid colonies for parasitization. After nine to 11 days the eggs turned black indicating *A. iole* pupae were inside the lygus eggs. The egg packets were cut open and the gel removed. The Parafilm® was stretched in luke-warm water to release the eggs from the Parafilm®. The eggs were strained from the water and placed in clear plastic cages, in environmental chambers (Atmar and Ellington, 1972), at 29 ˚C, 55 % RH and 10:14 (L:D) photoperiod. The environmental chambers were computer controlled and maintained temperatures within ± 2 ˚C and relative humidity of ±5 %. One 15 watt fluorescent tube, controlled with Dayton® timer, provided light in these chambers. Environmental conditions were printed out at 30 min intervals. *Anaphes iole* eggs took three to five days to emerge. Adult *A. iole* were fed a 5 % honey solution (Stoner and Surber DeBerry, 1992).

Experimental Procedure

Experiment 1: Effect of Photoperiod and Temperature on the Storage Period of Lygus Eggs Parasitized by *Anaphes iole*

This experiment was designed as a completely randomized experiment with a two-way treatment structure. Three temperatures and two photoperiods were the two treatment groups. The experiment was replicated three times. Treatment levels were as follows:

1. Temperature
   a. 18 ˚C
   b. 20 ˚C
   c. 22 ˚C
2. Photoperiod
   a. complete darkness
   b. 9:15 hours light:dark

Three environmental chambers were set at 18, 20 and 22 ˚C and 55 % RH. Each environmental chamber was divided into two sub-chambers (for photoperiods) with black cardboard. Half of each chamber was maintained with nine hours of light and 15 hours of darkness. The other half was held in complete darkness.

Twenty from freshly parasitoised eggs were counted, at random, and placed in 18, 30-ml plastic cups. The plastic cups were covered with a 0.25 mm fine polyester mesh and sealed with plastic glue. Three cups were placed in each sub-chamber of each environmental chamber. After 20 days, the cups were removed from the chambers and the emerged adults were counted. Adults which emerged during storage were not used for analysis. The cups were incubated at 29 ˚C and 55 % RH. After five to eight days, the number of emerged adults was counted again. A similar procedure was used for the 40 and 60 day storage experiments.

Ten cups each with 20 parasitized eggs were incubated at 29 ˚C, to determine the average emergence from un-stored eggs for comparisons purpose. Each storage period was considered to be a separate experiment and analyzed using 'FACTORS' MSTATC®.

Experiment 2: Effect of Low Temperature on the Storage of *Anaphes iole* Pupae

This experiment was designed as a completely randomized design, with two replications. The temperature treatments were -8, 0 and 8 ˚C for 20 days storage and an un-stored (control) at 29 ˚C. The experiment was carried out in complete darkness. Lygus eggs were parasitized by *A. iole*, and allowed to develop for seven days (to pupate) at 29 ˚C, 55 % RH and 10:14 (L:D) photoperiod. After seven days the Parafilm from the egg packets was stretched in warm water to remove the eggs. Ten parasitized lygus eggs were placed in each of 8, 30 ml plastic cups and covered with lids. Two cups were held for storage at each temperature, -8, 0 and 8 ˚C while un-stored cups were incubated at 29 ˚C in complete darkness. Emergence from the cups placed at 29 ˚C was completed after five to six days, and adults were counted. After 20 days, the cups from the -8, 0 and 8 °C chambers were removed and emerged adults were then counted. The cups were incubated at 29 °C 10:14 (L:D) photoperiod and 55 % RH. After four to six days the emerged adults were counted again and only adults that emerged after incubation were used for analysis. The data was analyzed by 'ANOVA-2' MSTATC®. Five percent level of probability was used for LSD test.

Experiment 3: Effect of Temperature, Photoperiod and Developmental Stage on Adult *Anaphes. iole* Emergence.

This experiment was designed in a split-split-plot design with temperature as main-plot treatments, photoperiod as sub-plot treatments and developmental stage by storage period as sub-sub-plot treatment combinations. The experiment was replicated five times. Main and sub-plot treatment levels were as follows:

1. Temperature
   a. 4˚ C
   b. 10˚ C
2. Photoperiod
   a. 9:15 light:dark
   b. complete darkness
3. Developmental stage
   a. one day after parasitization
   b. three days after parasitization
   c. six days after parasitization
   d. nine days after parasitization
4. Storage Period
   a. 20 days
   b. 40 days
   c. 60 days

Two environmental chambers were held at 4 ˚C and 10˚C. Each chamber was divided into two sub-chambers, using black card-board. One sub-chamber in each main chamber was subjected to nine hours light and 15 hours darkness, while complete darkness was maintained in the other.

Parasitized eggs were removed from the Parafilm® and held for one, three, six and nine days (developmental stage) at 29 ˚C. 20 eggs were placed in 60 30-ml plastic cups after each interval (one, three, six and nine days). To avoid desiccation, a small piece of moist filter paper was placed in each cup. Five cups from every developmental stage and every sub-chamber were removed after 20 days and incubated at 29 °C. The total number of adults emerged were counted. The same procedure was repeated for storage period of 40 and 60 days. The adults that emerged before incubation were discarded and only those adults that emerged after incubation were counted. The data was analyzed using 'Proc GLM' (SAS® Coorporation, 1985). Level of probability used was α=0.05, where applicable.

Experiment-4: Effect of Low Temperatures, Photoperiod and two Developmental Storages on the Extended Storage of *Anaphes iole*.

The experiment was designed as a split-split plot. The experiment was replicated at the sub plot level three times. The main and sub plot assignments were as follow:

1. Temperature
   a. 4 ˚C
   b. 10 ˚C
2. Photoperiod
   a. 9:15 light: dark
   b. complete darkness
3. Storage Period
   a. 20 days of storage
   b. 40 days of storage
   c. 60 days of storage
   d. 80 days of storage

Two environmental chambers were set at 10 °C and two chambers were set at 4 °C. Each environmental chamber was divided into two sub chambers using cardboard. Photoperiods (15 hours dark and nine hours light, and complete darkness were set in each sub-chamber. Twenty-five ml (5 cm diameter and 1 cm high) Gelman©, airtight, petri dishes were used for this experiment. These petri dishes came with blotting paper. The blotting paper was fixed to the bottom of the petri dishes with double sided Scotch® tape. One half ml distilled water was sprayed on the blotting paper, 20 lygus eggs were placed in each petri dish and the petri dishes were sealed firmly. After three days of parasitization the eggs were counted and placed in all 192 petri dishes to overcome the visual bias of the previous experiment.

Developmental stage three and six days were assigned randomly to the petri dishes. Twelve petri dishes (three for each of the four storage periods) were placed in each sub-chamber of each environmental chamber three days after parasitization, while the remaining petri dishes were held for three more days at 29 °C, 55 % RH, 10:14 (L:D) and then shifted to the sub-chambers of each environmental chamber. After 20 days of storage in the environmental chamber, three petri dishes were removed and held for four to six days in a colony chamber (29° C 55% RH) for incubation. The adults which emerged were then counted. The same procedure was repeated after 40, 60 and 80 days of storage. The data was analyzed using 'Proc GLM' (SAS® Corporation, 1985).

Results and discussions

Experiment 1: Effect of Photoperiod and Temperature on the Storage Period of Lygus Eggs Parasitized by *Anaphes iole*

Table 1 shows the number of *A. iole* that emerged after 20 and 40 days of storage at three temperatures and two light regimes. There was no emergence after 60 days. There was no significant temperature or light effect on adult emergence at any of the storage periods. Non-significant differences between treatments may have resulted because the temperatures were not very different from the normal rearing temperature (29 °C) and the differences between the three temperatures was not large i.e. 2 °C. Adult emergence at all of the tested temperatures and storage periods was 0-13.3 %, while emergence from the un-stored (incubated at 29 °C) eggs was 0-10 %. Low emergence probably occurred because eggs were exposed to air with 55 % humidity. Eggs in stems or gel packets are maintained under much higher moisture conditions.

Table 1. Mean[1] adult emergence of *Anaphes iole* from 20 parasitized lygus eggs placed at different temperatures and photoperiods for 20 and 40 days storage

Temperature	20 days		40 days	
	9:15 (L:D)	Darkness	9:15 (L:D)	Darkness
18 °C	0.67a	1.33a	0.67a	1.00 a
20 °C	2.33 a	0.67 a	1.67 a	0.67 a
22 °C'	1.67 a	0.67 a	1 .00 a	1.00 a

[1] Mean of three samples; Means having same letters in a row or column are non significant at both 1 % and 5 % levels of significance.

Experiment 2: Effect of Low Temperature on the Storage of *Anaphes iole* Pupae

Table 2 shows the number of adult *A. iole* that emerged from the pupae stored at three temperatures for 20 days and an un-stored (control). No emergence occurred from pupae stored at -8 and 0 °C, 75 % emergence occurred from pupae stored at 8 °C, and 90-100 % emergence occurred from the un-stored eggs held at 29 °C (control). Significantly higher numbers of adults emerged from eggs held at 8 °C than eggs held at 0 °C and -8 °C. Adult emergence from the un-stored (control) pupae was significantly higher than those stored at the other temperature.

Table 2. Mean[1] adult emergence of *Anaphes iole* from ten parasitized lygus eggs placed at different temperatures for storage in complete darkness and an un-stored (control)

Temperature	Number of adults emerged
–8 °C	0.0 c
0 °C	0.0 c
8 °C	7.5 b
29 °C[2]	9.5 a

[1] Mean of two samples; [2] Un-stored control; Means followed by different letters are significantly different from each other at 5 % level of probability.

Experiment 3: Effect of Temperature, Photoperiod and Developmental Stage on Adult *A. iole* Emergence

The emergence rate of *A. iole* from lygus eggs in the production colony (un-stored parasitized lygus eggs at 29 °C with 55 % RH and 10:14 (L:D photoperiod) was 6.19 to 0.523 adults/20 eggs. The three-way interaction between developmental stage, storage period and temperature was significant. Comparisons were made among developmental stage, storage period and temperature combinations, averaged over photoperiod. Because this three way interaction was significant, two way interactions between developmental stage, storage period and temperature and main effects involving developmental stage, storage day and temperature were misleading, and were not extended. Because no other three way interaction was significant, two-way interactions involving photoperiod were examined. The two-way interaction between photoperiod and development stage was significant and also examined. Comparisons were made between photoperiod and developmental stage combinations, averaged over temperature and storage period. Although the interaction between temperature and photoperiod was not significant, the P value (0.0602) was close to the probability level used in these studies ($P \leq 0.05$), and was also examined. Comparisons were made between temperature and photoperiod combinations, averaged over storage period and developmental stage.

Interaction between development stage, storage period and temperature

Table 3 shows the number of adult *A. iole* that emerged from 20 parasitized eggs from the four developmental stages, three storage periods and two temperature combinations averaged over photoperiod. One and three days developmental stages behaved similar at 4° C and 10° C, across all three storage periods, i.e., 20, 40 and 60 days for adult emergence. At six days developmental stage, 20 days of storage gave significantly more adult emergence than 40 and 60 days of storage at 4 °C. However, adult emergence for the same developmental stage at 10 °C was significantly lower at 20 days of storage than 40 and 60 days of storage, although, 40 and 60 days of storage were similar to each other. Comparing storage periods within the six day developmental stage across temperatures, adult emergence at 40 and 60 days of storage at 10 °C was significantly higher than 20 days of storage at 10 °C and 40 and 60 days of storage at 4 °C. Nine days of development gave significantly higher adult emergence than any other developmental stage/temperature combinations, at both temperatures after 20 days of storage. Adult emergence for the same developmental stage dropped to almost zero for both temperatures after 40 and 60 days of storage.

In general, significantly more adults emerged at 10 °C than at 4 °C, across the most developmental stages and storage periods. Up to 60 days of storage, developmental stage of six days at 10° C temperature gave better emergence than any

Table 3. Mean' number of adult *Anaphes iole* emerged from 20 lygus eggs held at two temperatures, four developmental stages and three storage periods

Development stage	20 days		40 days		60 days	
	10 °C	4 °C	10 °C	4 °C	10 °C	4 °C
one day	5.50 bcd	2.60 fgh	4.80 cde	3.00 efgh	4.80 cde	1.60 hi
three days	2.60 fgh	1.80 ghi	3.80 defg	2.40 fgh	4.20 def	1.40 hi
six days	4.00 def	7.00 b	6.60 bc	4.20 def	7.20 b	1.80 ghi
nine days	18.40 a	19.20 a	0.20 i	0.20 i	0.00 i	0.00 i

[1] Mean of ten samples; Means followed by same letters in a row or column are not significantly different at 5 % level of probability.

other developmental stage/temperature combination. The data showed little or no emergence after 40 and 60 days of storage after nine days of development. Either the insects failed to emerge due to temperature stress or the insects emerged in the cold chambers before they were shifted to the normal rearing chamber (29 °C). After nine days of development, the parasites inside the host eggs may be over developed to the point that they emerged even at 10 °C. Growth may have also been temporarily arrested at one, three and six days of development at 10 °C and then resumed when lygus eggs were placed in the rearing chamber at 29 °C. This idea was supported by Guzman and Peterson (1986) and Gautam (1986). Guzman and Peterson (1986) suggested that 3rd instar larvae or early-formed pupae of *Muscidifurax zaraptor* Kogan & Legner and *Spalangia cameroni* Perkins, which parasitize filthfly, can be stored at 10 °C, while early and extremely late developmental stages could not be stored at low temperatures (0 °C). Gautam (1986) reported that 10 °C proved better than 5 °C for the storage of *Telenomus remus* Nixon parasitizing *Spodoptera litura* (Fab). Devi *et al*. (1983) stated that an extremely low temperature (5 °C) killed the pupae of *Trichogramma spp.* inside the *Coccyra cephalonica*.

Because lygus eggs turn black nine days after parasitization by *A. iole* a visual bias may have occurred in selecting test eggs after nine days of development. Eggs were selected randomly for other developmental stages, *i.e.* one, three and six days after parasitization.

Interaction between storage period and photoperiod:

Table 4 shows the mean number of adults that emerged from 20 eggs after being subjected to three different storage periods and either complete darkness or a 9:15 (L: D) photoperiod. The means were averaged over temperature and developmental stage.

Complete darkness and 9:15 (L:D) photoperiod gave non-significant (P=0.0725) adult emergence from each other after 20 days of storage but the adult emergence was significantly higher than in all other storage periods/photoperiods. Adult emergence was significantly better in complete darkness than in a 9:15 (L:D· photoperiod, when parasitized eggs were stored for 40 days. Storage for 60 days resulted in similar adult emergence for both photoperiods and was significantly lower than storage for 20 days (either photoperiod) and 40 days in complete darkness.

Table 4. Mean[1] number of adult *Anaphes iole* emerged from 20 parasitized lygus eggs held at two photoperiods and three storage periods

Storage period	Complete darkness	9:15 light:dark
20 days	8.10 a	7.18 a
40 days	4.20 b	2.10 c
60 days	2.55 c	2.70 c

[1] Mean of 40 samples; Means having different letters in a row or column are significantly different at 5 % level of probability.

Table 5. Mean[1] number of adult *Anaphes iole* emerged from 20 parasitized lygus eggs stored at two temperatures and two photoperiods

Photo period	10 °C	4 °C
Complete darkness	5.43 a	4.46 ab
9:15 (D:L)	4.92 b	3.17 c

[1] Mean of 60 samples; Means having different letters in a row or column are significantly different at 5 % level of probability.

In general, complete darkness was better for parasitized egg storage than a 9:15 (L:D) photoperiod.

Interaction between photoperiod and temperature:

Table 5 shows the mean number of adult *Anaphes iole* that emerged after storage at two temperatures and two photoperiods. The means were averaged over developmental stage and storage periods. The data in Table 5 shows that complete darkness significantly improved adult emergence within each temperature (10 and 4° C). The number of adults, which emerged at 10 °C, was similar to that, emerged at 4 °C in complete darkness. Adult emergence at 10 °C was significantly better than at 4 °C when eggs were stored in a 9:15 (L:D) photoperiod. Parasitized eggs storage was best in complete darkness at 10 °C.

Experiment 4: Effect of Low Temperatures, Photoperiod and two Developmental Storages on the Extended Storage of *Anaphes iole*

There was a significant three-way interaction among developmental stage, storage period and temperature. The only significant interaction involving photoperiod was between photoperiod and storage period.

Interaction between developmental stage, storage period and temperature.

Table 6 shows the mean number of adult *A. iole* which emerged after three and six days of parasitization, two temperatures and four storage periods, averaged over photoperiods. When parasitized eggs were stored at 10 °C, for 20 and 80 days both developmental stages tested were non significantly different from each other. When parasitized eggs were stored for 40 and 60 days at 10 °C, six days of development gave significantly greater adult emergence than three days of development.

Three days of development gave significantly lower numbers of adult emergence than six days of development when parasitized eggs were stored at 4 °C for 20 and 60 days. No significant difference in adult emergence was found between two developmental stages (three and six days) when parasitized lygus eggs were stored at 4 °C for 40 and 80 days. In general, six days after parasitization was a good developmental stage for storage and 10 °C was the best storage temperature for parasitized lygus eggs. Longer storage resulted in less adult emergence.

Table 6. Mean[1] number of adult *Anaphes iole* that emerged from 20 parasitized lygus eggs at two developmental stages, two temperatures, and four storage periods

Storage period	Three days		Six days	
	10 °C	4 °C	10 °C	4 °C
20 days	14.67 a	8.33 b	12.58 a	13.29 a
40 days	3.08 e	6.50 bcd	8.50 b	8.17 bc
60 days	0.83 ef	1 .91 ef	5.92 cd	5.67 d
80 days	0.25 f	0.16 f	1 .67 ef	2.42 ef

[1] Mean of 12 samples; Means followed by different letters in a row or column are significantly different at 5 % level of probability.

Table 7. Mean[1] number of adult *Anaphes iole* which emerged from 20 parasitized lygus eggs at two photoperiods and four storage periods

Storage periods	Complete darkness	9:15 (L:D)
20 days	11.98 a	12.45 a
40 days	5.29 c	7.83 b
60 days	2.83 d	4.33 cd
80 days	0.95 e	1.29 e

[1] Mean of 24 samples; Means followed by different letters in row or column are significantly different at 5% level of probability.

Interaction between photoperiod and storage period

Table 7 shows the number of adult *A. iole* that emerged after different storage periods in complete darkness and a 9:15 (L:D) photoperiod, averaged over temperature and developmental stage. There were no significant differences in adult emergence between complete darkness and a 9a15 (L:D) photoperiod, within any storage period, except after 40 days of storage, where a 9:15 (L:D) photoperiod gave significantly higher numbers of adult parasitoid emergence than complete darkness. In general, adult emergence significantly decreased with increasing length of storage.

Emergence results were best for prolonged storage if *A. iole* were held for six days at 29 °C before low temperature storage. The optimal low temperature storage was 10 °C. The best emergence generally occurred after storage in complete darkness rather than in a 9:1 5 (L:D) photoperiod. Emergence was the highest when *A. iole* larvae or pupae were stored for 20 days. Emergence decreased significantly as the storage period increased. When six day old parasitized lygus eggs were stored for 20, 40 and 60 days, at 10 °C, 62.9%, 42.5% and 29.60% emergence occurred respectively. After 80 days, emergence dropped to 10%. Significantly fewer *Anaphes iole* emerged from parasitized lygus eggs held for one , three and nine days at 29 °C and then stored at 4 °C and a 9:15 (L:D) photoperiod than from six day old lygus eggs stored in complete darkness at 10 °C, except when nine day old parasitized lygus eggs were stored for 20 days at 4 or 10 °C.

References

Anonymous. 1982. Alfalfa-growing area of the western United States. U.S. Department of Agriculture, Agriculture Research Service; Tech. Bull No. 1 651 , pp-2.

Anonymous. 1983. Biological control of insects laboratory, Tucson, AZ. U.S. Department of Agriculture, Agriculture Research Service; Research Report. Jan-June, 1983. 84p.

Anonymous. 1969. Rearing cotton insect parasites in the laboratory. U.S. Department of Agriculture, Research Prod. Report. No. 109, pp-11 .

Atmar, J.W. and J.J. Ellington. 1972. Construction and control characteristics of environmental simulation chambers. J. Econ. Entomol. 65: 401-404.

Boivin, G. 1986. *Anaphes sordidatus* (Girault) (Hymenoptera: Mymaridae), an egg parasite of the carrot weevil, *Listronotus oregonensis* (Le Conte). Canadian Entomol. 33: 393-394.

Boivin, G. 1988. Laboratory rearing of *Anaphes sordidatus* (Hym.: Mymaridae) on carrot weevil eggs (Col.: Curculionidael. Entomophaga. 33: 245-248.

Clancy, D.W. and H.D. Pierce. 1966. Natural enemies of some lygus bugs. J. Econ. Entomol. 59: 853-858.

Collins, R.D. and E. Grafius. 1986. Biology and life cycle of *Anaphes sordidatus* (Hymenoptera: Mymaridael, an egg parasitoid of the carrot weevil (Coleoptera: Curculionidae). Environ. Entomol. 15: 100-105.

Crosby, C.R. and M.D. Leonard. 1914. An egg parasite of tarnished bug *Lygus pratensis* L. Canadian Entomol. 46: 181-182.

De Berry, J.R. 1992. An evaluation of *Anaphes iole* (Hymenoptera : Mymaridae) for insecticide resistance and efficacy. MS thesis submitted to NMSU, New Mexico. 36pp.

Debolt, J.W. and R. Patana. 1985. *Lygus hesperus*, Hand book of insect rearing. Vol. 1. Elsevier Sc. Publisher, B.V. Amsterdam. 329-338.

Devi, S.A.I.; B.J. Divakar and A.D. Pawar. 1983. A note on the rearing and storage of *Trichogramma embryophagum* (Hartig) and *Trichogramma cacoeciae pallidum* Meyar. Plant Prot. Bull. 35: 34.

Fleming, R. and A. Retnakaran. 1985. Evaluating single treatment data using Abbott's formula with reference to insecticides. J. Econ. Entomol. 78:1179-1181 .

Flint, M.L. and J.K. Clark. 1981. Integrated pest management for Alfalfa Hay. Uinv. Cali. USA.

Gautam, R.D. 1986. Effect of cold storage on the adult parasitoid *Telenomus remus* Nixon (Scelionidae: Hymenoptera) and the parasitized eggs of *Spodoptera litura* (Fabr.) (Noctuidae: Lepidoptera). J. Entom. Res. 10: 125-131.

Gautam, R.D. 1987. Cold storage of eggs of host, *Spodoptera litura* (Fabr.) and its effects on parasitism by *Telenomus remus* Nixon (Scelionidae: Hymenoptera). J. Entom. Res. 11: 161-165.

Gordon,R., J. Ellington, G.F. Faubion and H. Graham. 1987. A survey of insect parasitoids from alfalfa and associated weeds in New Mexico. Southwestern entomolo. 12: 335-350. Graham, H.M. 1987. Summary and recommendations, Economic importance and biological control of Lygus and Adelphocoris in North America. U.S. Department of Agriculture, Agriculture Research Service; ARS64, December 1987 pp-94.

Graham, H.M. and C.G. Jackson. 1981. Population of mirids and their parasites in block cut alfalfa fields. Biological control of insects laboratory, Tucson, AZ. U.S. Department of Agriculture, Agriculture Research Service; Report of Research. July-Dec. 1981 . pp-12.

Graham, H.M.; C.G. Jackson and J.W. Debolt. 1981a. Population of Lygus, Nabis and three cornered hoppers and parasites of Lygus on alfalfa near Tucson. Biological control of insects laboratory, Tucson, AZ. U.S. Department of Agriculture, Agriculture Research Service; Report of Research. Jan-June. 1981: 7-15. 66pp

Graham, H.M.; C.G. Jackson and J.W. Debolt. 1981b. Population of Lygus and their parasites on Russian thistle near Tucson. Biological control of insects laboratory, Tucson, AZ. U.S. Department of Agriculture, Agriculture Research Service; Report of Research. Jan-June. 1981 pp-16.

Graham, H.M.; C.G. Jackson and J.W. Debolt. 1981c. Lygus and their parasites in the Yuma area. Biological control of insects laboratory, Tucson, AZ. U.S. Department of Agriculture, Agriculture Research Service; Report of Research. Jan-June. 1981 : 17-20.

Graham, H.M., C.G. Jackson and J.W. Debolt. 1986. *Lygus* spp. (Hemiptera: Miridae) and their parasites in agricultural areas of southern Arizona. Environ. Entomol. 15: 132-142.

Grant, J.F. and R. Noblet. 1991. Response of *Brachymeria ovata* (Hymenoptera: Chalcididae) to live and freezer-stored pupae of *Anticarsia gemmatalis* (Lepidoptera: Noctuidae). Environ. Entomol. 20: 288-291.

Grant, J.F. and M. Shepard. 1987. Development of *Brachymeria ovata* (Say) (Hymenoptera : Chalcididae) in freezer-stored pupae of lepidopteran species. Environ. Entomol. 16: 1207-1210.

Guzman, D.R. and J.J. Peterson. 1986. Cold acclimation and prolonged low temperature survival of filth fly parasites (Hymenoptera: Pteromalidae). Environ. Entomol. 15: 936-942.

Hamerski, M.R.; R.W. Hall. and G.D. Kenney. 1990. Laboratory biology and rearing of *Tetrastichus brevistigma* (Hymenoptera: Eulophidae), a larval-pupal parasitoid of the elm leaf beetle (Coleoptera: Chrysomelidae) J. Econ. Entomol. 83: 2196-2199.

Hedlund, R.C. 1987. Foreign exploration for natural enemies of Lygus and Adelphocoris plant bugs; Economic importance and biological control of Lygus and Adelphocoris in North America. U.S. Department of Agriculture, Agriculture Research Service; ARS-64, December 1987: 76-81.

Huber, J.T. and Rajakulendran 1988. Re-description of and host-induced antennal variation in *Anaphes iole* Girault (Hymenoptera: Mymaridae), an egg parasite of Miridae (Hemiptera) in North America. Can. Entomol. 120: 893-901.

Jackson, C.G. 1986. Effect of cold storage of adult *Anaphes ovijentatus* on survival, longevity and oviposition. Southwestern Entomologist. 1 1 : 149-1 53.

Jackson, C.G. 1987. Biology of *Anaphes ovijentatus* (Hymenoptera: Mymaridae) and its host *Lygus hesperus* (Hemiptera: Miridae), at low and high temperatures. Ann. Entomol. Soc. Am. 80: 367-372.

Jackson, C.G., A.C. Cohen and C.L. Verdugo. 1988. Labeling *Anaphes ovijentatus* (Hymenoptera: Mymaridae) an egg parasite of *Lygus* spp. (Hemiptera: Miridae), with rubidium. Ann. Entomol. Soc. Am. 8: 91-99.

Jackson, C.G. and K. Lakin. 1981. Parasitism of *Polymers basilus* eggs in stem of Hymenothrix by *Anaphes ovijentatus*. Biological control of insects laboratory, Tucson, AZ. U.S. Department of Agriculture, Agriculture Research Service; Report of Research. Jan-June. 1981 pp-21.

Jackson, C.G. and H.M. Graham. 1983. Parasitism of four species of Lygus (Hemiptera: Miridae) by *Anaphes ovijentatus* (Hymenoptera . Mymaridae) and an evaluation of other possible hosts. Ann. Entomol. Soc. Am. 76: 772-775.

Jones, W.A. and C.G. Jackson. 1990. Mass production of *Anaphes iole* for augmentation against Lygus hesperus: Effect of food on fecundity and longevity. Southwestern Entomologist. 15: 463-468.

Johansson, C.G. 1975. Lygus bugs and their predators. U.S. Department of Agriculture. E.M 3440 (SR). May 1975: 1-2.

Legner, E.F. 1976. Low storage temperature effects on the reproductive potential of three parasites of *Musca domestica*. Ann. Entomol. Soc. Am. 69: 435-441 .

Lieberman, F.V. and G.F. Knowlton. 1955. Injurious insects and mites and their control. Utah Agric. Exp. Station Circular 135: 23-41 . 68

Madsen, R.R. and C.A. Johansson. 1976. Lygus bug damage and economic thresholds on alfalfa grown for seed. U.S. Department of Agriculture, E.M. 4026. April 1976: 1-8.

Patana, R. and J.W. Debolt. 1985. Rearing of *Lygus hesperus* in the laboratory. U.S. Department of Agriculture, Agriculture Research Service; Production Research Report. August 1985: 3-17.

Rajakulendran, S.V. and J.R. Cate. 1986. Laboratory rearing procedures for two egg parasitoids of cotton fleahopper *Pseudatomoscelis seriatus* (Reuter). Southwestern Entomologist. 11: 255-260.

Romney, V.E. and T.P. Cassidy. 1945. *Anaphes ovijentatus*, an egg parasite of *Lygus hesperus*. J. Econ. Entomol. 38: 497-498. SAS institute Inc. 1985.

SAS user guide: Basics. Version 5 Edition.

Ruberson, J. R., and Williams III, L. H. 2000. Biological control of *Lygus* spp.: A component of areawide management. Southwest. Entomol. Suppl. 23:96-110.

Cary, NC and Scales, A.L. 1973. Parasites of the tarnished plant bug in the Mississippi delta. Environ. Entomol. 2: 304-305.

Sillings, J.O. and D.B. Broersma. 1974. The parasites of the tarnished plant bug *Lygus lineolaris* in Indiana. Proced. North Central Branch-E.S.A. 29: 120-125.

Singh, R. and M. Srivastava. 1988. Effect of cold storage of mummies of *Aphis craccivora* Koch subjected to different pre-storage temperature on percent emergence of *Trioxys indicus* Subba Rao and Sharma. Insect Sci. Applic. 9: 655-657.

Smith, R.F. and A.E. Michelbacher. 1946. Control of lygus bugs in alfalfa seed fields. J. Econ. Entomol. 39: 638-648.

Sohati, P.H.; G. Boivin and R.K. Stewart. 1992. Parasitism of *Lygus lineolaris* eggs on *Coronilla varia*, *Solanum tuberosum*, and three host weeds in Southestern Quebec. Entomophaga 37: 515-523.

Sohati, P.H.; R.K. Stewart, and G. Boivin. 1989. Egg parasitoids of the tarnished plant bug, *Lygus lineolaris* (P. de B.) (Hemiptera: Miridae) in Quebec. Can. Entomol. 121: 1127-1128.

Stern, V.M.; R. Bosch; T. F. Leigh; O. D. McCutcheon; W. R. Sallee; C. E. Houston and M. J. Garber. 1967. Lygus control by strip cutting alfalfa. Univ. Cali.. Agri. Experi. Service. AXT-241 : 13pp.

Stitt, L.L. 1950. Lygus bugs and alfalfa production in the United States. Alfalfa improvement conference. vol. 12: 50-51.

Stoner, A. and D.E. Surber. 1969. Notes on the biology and rearing of *Anaphes ovijentatus*, a new parasite of *Lygus hesperus* in Arizona. J. Econ. Entomol. 62: 501-502.

Stoner, A. and D.E. Surber. 1971 . Development of *Anaphes ovijentatus*, an egg parasite of *Lygus hesperus* in relation to temperature. J. Econ. Entomol. 64: 1566-1567.

Udayagiri, S., Welter, S. C. and Norton, A. P. 2000. Biological control of *Lygus hesperus* with inundative releases of *Anaphes iole* in a high cash value crop. Southwestern Entomologist Supplement 23: 27- 38.

Wheeler, A.G. 1974. Studies on the arthropod fauna of alfalfa. VI. Plant bugs (Miridae). Canadian Entomologist. 106: 1267-1275.

Wynholds, P.F. and T.F. Leigh. 1976. Lygus resistance spurs research. Agrochemical Age. 19(5): 18-20.

Yeargan, K.V. 1985. Alfalfa: status and current limits to biological control in the eastern U.S. Biological control in Agri. IPM system. Academic press Inc. 521-536. 70pp.

---

acs74_56