BIOSTATISTICS OF *TRISSOLCUS VASSILIEVI* (HYM., SCELIONIDAE) DEVELOPED ON SUNN PEST EGGS COLD-STORED FOR DIFFERENT DURATIONS

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ABSTRACT: The common sunn pest (CSP) *Eurygaster integriceps* Puton is the most important pest of wheat and barley in Iran. A few egg pararsitoid from *Trissolcus, Telenomus, Ooencyrtus, etc.* attack CSP eggs. Often the host eggs are stored in cold temperatures for subsequent use with negative effects on the parasitoids which increases by time. This study focused on quantitative changes of biostatistics including parasitism rate, emergence rate, sex ratio, fecundity, immature development, longevity and body size of *Trissolcus vassilievi* (Mayr) reared on host eggs stored for different periods in 5±1°C. The results revealed that all above mentioned statistics were affected by storing with an increasing intensity by duration. Parasitism rate and sex ratio decreased more strongly than adult emergence rate. A maximum 35% decrease in fecundity was also observed with stronger effect in long term storages. An increasing effort to compensate the initial lag however was observed in first week of the reproductive life of those wasps that accessed to older eggs. Developmental time increased by a sigmoid trend by storage time. Head capsule width and hind tibia length as measurements of body size both decreased suddenly in those wasps that obtained from >2 week old eggs, but the width of the head capsule decreased more rapidly in females than males that may imply stronger fitness loss in females. As a conclusion host eggs stored < 1 month may use with negligible negative effects on the parasitoid efficacy, while longer storage times may cause serious negative syndromes.

KEY WORDS: *Trissolcus vassilievi*, *Eurygaster integriceps*, host storage, body size, sex ratio.

One basic problem in augmentation biological control programs is storage of biocontrol agents for subsequent use. Cold temperatures often are used in this purpose (van Driesche & Bellows, 1996). However holding egg parasitoids (Hym., Scelionidae) in cold conditions whether as pupa or as adult has no impressive results regarding high mortality during a short period (Asgari, 1995; Foerster et al. 2004; Kodan & Gurkan, 2004; Foerster & Doetzer, 2006). Storing host eggs and continuous rearing of these parasitods from *Trissolcus*, *Telenomus*, *Gryon*, and *Ooencyrtus* spp. upon them has been an alternative method with better results (Sklyarov, 1970; Gusev & Shmettser, 1975; Asgari, 1995; Kivan & Kilic, 2005). Unfortunately target host, the common sunn pest *Eurygaster integriceps* Puton (Hem., Scutelleridae) has obligatory reproductive diapause and may not lay eggs continuously in laboratory (Alexandrov, 1947). Alternative hosts have been a solution in some cases (Safavi, 1973; Asgari, 1995; Shahrokhi, 1997, Kivan & Kilic, 2005). However, storing sunn pest eggs in cold temperatures for subsequent use both for augmentation and research purposes has been frequently adopted by scientists (Sklyarov, 1970; Safavi, 1973; Gusev & Shmettser, 1975; Asgari, 1995; Kivan & Kilic, 2005). This makes timing of research events very strict. Some researchers (*e. g*. Asgari, 1995, Iranipour, 1996, Kivan & Kilic, 2005) stored host eggs for a few months and could catch and rear egg parasitoids successfully. Nevertheless gradual reduction in host quality occurs during storage. However few

quantitative data is present (Asgari, 1995; Kivan & Kiliç, 2005) about time horizon in which host eggs may use without any adverse impact on parasitoids' biostatistics. On the other hand, reaction of different egg parasitoids to duration of host storage may be different. For example it seems that *Ooencyrtus* species are less sensitive to host availability (Bazavar, 2013) or quality (*i.e.* they easily accept previously parasitized hosts both for superparasitism and multiparasitism; see Safavi, 1973, Iranipour, 1996, Ahmadpour et al. in press) than *Trissolcus* species or as another example *Trissolcus grandis* (Thomson) show behavioral flexibility in harsh conditions (Iranipour, 1996). Therefore, if we are going to use stored hosts for rearing or research purposes we need beware of reaction of the subjected species. Indeed every species is likely to have its own special characteristics in this relation (van Driesche & Bellows, 1996).

Although a relatively long list of natural enemies with special emphasize on egg parasitoids has been published by scientists (Safavi, 1973; Kozlov & Kononova, 1983; Radjabi & Amir Nazari, 1989; Iranipour, 1996; Modarres Awal, 1997; Radjabi, 2000; Sakenin et al., 2008; Samin et al., 2010a,b,c,d, 2011a,b), only a few of them have potential of using in applied biological control. *T. grandis* as dominant species of many parts of Iran (Radjabi & Amir Nazari, 1989) as well as *T. vassilievi*, the dominant species of Varamin - a typical region of sunn pest aggressions (Iranipour et al., 2011) - are the most obvious chooses. Both species have been used in augmentation programs in research scale (Asgari, 1995, 2011; Shahrokhi, 1997).

Safavi (1973) stated that keeping host eggs in 2-4°C may cease embryogenesis and continuation of the cold condition causes the fetus to kill. Such eggs can be used for parasitoid augmentation. In these temperatures eggs may store for several months and parasitic wasp may develop as long as their vitellus is intact.

Sklyarov (1970) obtained poor results in biological control of sunn pest due to using one year old stored eggs for rearing parasitoids. Gusev & Shmettser (1975) studied the optimum conditions for rearing *T. grandis* and *T. semistriatus* (Nees) in laboratory. Their results showed that storing host eggs caused an increase in male fraction to 37, 52, 84 and 97% following 1, 1.5, 2 and 2.5 months storing in cold conditions while no more than 20% of control wasps were males. On the other hand adult emergence rate from host eggs declined to 22% at the end of this duration compared to 86% in control. Depositing *E. integriceps* eggs at 4, 6 and -23°C and further offering them to *T. grandis* and *Telenomus chloropus* (Thomson), showed that adult emergence was high enough after 4.5 months and no significant differences in emergence rate was present from 3 to 7 months (Asgari, 1995). In other part of this study time horizon in which *Graphosoma lineatum* L. eggs remain acceptable for *T. grandis* and *T. vassilievi* following deposition in 4, 5 and 6°C was determined to be 2.5 months. The maximum parasitism rate was 96% for both species. Storing parasitized eggs in pupal stage of *T. grandis* in 6°C was led to 97.2% mortality after 1.5 months. On the other hand preserving female *T. grandis* in 4°C caused 69 and 95% mortality after 1 and 2 months respectively. Thus Asgari concluded that host egg storage is the best method of lengthening mass production duration of *Trissolcus* spp.

Kivan & Kiliç (2005) compared fresh and stored eggs of *E. integriceps*, *Dolycoris baccarum* (L.), *G. lineatum* and *Eurydema ornatum* (L.) in regards of *T. semistriatus* development and parasitism rate. Two low-temperature regimes (+6 and -20°C) were used in this study and 50 eggs per host species were exposed to females once a month. The results showed that those host eggs that stored at 6°C remained acceptable up to 2 months, while those ones stored at -20°C continued to support parasitism up to 4 months, although a decrease was observed in the

parasitoid performance with time as a lag in development. The greenhouse release of *T. basalis* (Wollaston) adults hatched from fresh eggs of *N. viridula* (L.) as well as eggs frozen at -25°C led to similar results by Ramos & Ferre (2010).

MATERIALS & METHODS

I- Stock cultures of the insects: Two populations of *T. vassilievi* were used in this study; one from northwest of Iran, Tabriz (38°N, 46°E with1360 m AMSL) and the other one from Varamin (35°N, 51°E with 918 m AMSL) in center of Iran with a 600 km distance between the two. Both populations obtained by field collections, as parasitized eggs of the host. The parasitized egg masses were transferred to glass tubes (1.5 ×10 cm) and maintained in laboratory (26±1°C, 50±5% RH and 16L: 8D photoperiod) up to when adults were emerged. After discrimination of the mentioned species a stock culture was established providing honey droplets as food and host eggs for augmentation.

Adult bugs of *E. integriceps* also were collected from wheat fields by a sweep net and maintained at the same condition in 50 pairs in rectangular plastic dishes (30 × 20 × 9 cm, L: W: H) containing wheat kernels, water supplies and folded papers as ovipositing substrate (Zomorrodi, 1961).

II- The experiments: The host egg clutches were removed daily from stock cultures and immediately were transferred to a refrigerator (277 liter internal capacity, Pars Company, Iran) with temperature settings of 5±1°C. Those eggs that stored for 1, 2, 4, 6 and 8 weeks accompanying with a control (fresh eggs) were exposed in glass tubes (1.5 ×10 cm) in 20 replications to one day old inexperienced mated female individuals of *T. vassilievi* for 24 h. Each replication was bearing one clutch including 14 host eggs. After this period female wasps were removed and host egg clutches were kept in a growth chamber (model IKH.RH Iran Khodsaz Company, Iran).

Parasitism rate (number of parasitized eggs per 14 eggs initially exposed), emergence rate (number of emerged wasps per parasitized hosts), immature development time (number of days spent within host egg) separately for females and males, and sex ratio (female% of the wasps emerged) was measured for overall individuals developed in the experimental environment. Then 20 pairs of females and males were selected randomly among the emerged wasps and adult longevity (number of days living after emergence) separately for females and males, and total fecundity of females (number of parasitized eggs in adult life span) as well as its distribution in three day intervals were recorded. All the experiments were carried out at 26±1°C, 50±5% RH, 16L: 8 D h photoperiod. Finally the parasitoid size was measured after death as width of head capsule from dorsal view and right hind tibia length (mm) using a stereomicroscope (Olympus, CH30, Japan) only for the chosen wasps. A scaled calibration slide (made by Graticules LTD. England, 100×0.01=1 mm) was used for converting number of divisions of a scaled ocular lens to unite length in mm.

III- The stable population growth parameter approximations: In order to make some predictions about the results of augmentation we required some estimates about stable population growth parameters. Fortunately in this study 3 day interval information of fecundity was present. So a rough approximation of net replacement rate (R_o), mean generation time (T) and intrinsic rate of increase (r_m) values obtained as:

$$R_o=SR.F \qquad \text{(eq. 1)}$$

$$T=D+\frac{\sum f_i x_i}{\sum f_i} \qquad \text{(eq. 2)}$$

$r_m=\ln(R_o)/T$ (eq. 3)

The SR, F, D, f_i and x_i are sex ratio, total fecundity, mean developmental time, three day fecundity of the i'th interval, and midpoint of the interval i respectively. All these components were estimated in the experiment condition, but no variance of them was available for statistical analysis. Two additional parameters including finite rate of increase (λ) and doubling time (DT) also were estimated as $\exp(r_m)$ and $\ln(2)/r_m$.

IV- Data analysis: A factorial experiment was performed on the basis of a completely randomized design (CRD) with two factors including the wasp populations in two levels (Tabriz and Varamin populations) and cold-storage durations of the *E. integriceps* eggs in six levels (fresh eggs or control, 1, 2, 4, 6 and 8 week stored eggs in 5±1 ºC). A third factor *i. e.* gender in two levels (female and male) was also included when body size, development or longevity were analyzed. Finally in fecundity analysis an additional split plot in time design was adopted for including 3-day interval fecundities as main plot and both the wasp population and storing duration as subplots. The mean comparisons were done using Tukey's HSD test (α= 0.05). Data analysis was done by SPSS.

RESULTS

I- Parasitism rate: No significant difference was observed in number of parasitized eggs between the two populations of the wasp (F=0.071; df=1, 228; *P*=0.791). So a common mean was estimated for both populations in each storing level. Nevertheless the number of host eggs attacked by wasps decreased with increasing duration of storage (F=45.38; df=5, 228; *P*<0.01; Fig. 1a). No significant decline however was observed until two weeks, but a significant decrease first time was observed in 1 month stored eggs and continuing the storage caused a further decline in parasitism in 8 week-old eggs. It seems that a significant decrease occurs in parasitism each month. The overall trend of this decline is so that one less egg will parasitize per 1.2 weeks (8.5 days) storing in cold condition. This trend will lead to zero parasitism after 16 weeks (4 months).

II-Emergence rate: The number of host eggs from which an adult wasp successfully was emerged decreased with increasing duration of storage (F=7.48; df=5, 218; *P*<0.01). The fall in emergence rate was observed only at the end of the experiment (the week 8) with a 12% decrease proportional to fresh eggs (fig. 1b). Difference between wasps of the two population also was significant (F=6.03; df=1, 218; *P*=0.015). The difference between wasps was negligible (<3%) up to two weeks, then increased in weeks 4 and 6 (7.6-10.1%) with more sever response of Tabriz wasps and then converged again in week 8 by a delayed response of Varamin wasps. In spite of different pattern of response of the two populations, the interaction between wasps and storing period was not significant (F=1.20; df=5, 218; *P*=0.308).

III-Sex ratio: The sex ratio was not significantly different between the populations (F=0.159; df=1, 218; *P*=0.690), but influenced by storage periods (F=17.18; df=5, 218; *P*<0.01). The general pattern of sex ratio changes with storing duration in both populations of wasps was so that 80-90% of progeny were female in ≤ 1 month stored hosts while it significantly declined to 50-60% in longer storage periods (Fig. 1c).

IV-fecundity: The total fecundity of wasps of the two populations was largely similar in all treatments (F=0.291; df=1, 228; *P*=0.590), but considerably influenced by storage duration (F=210.92; df=5, 228; *P*<0.01). A gradual

decreasing trend was obvious even in 2 week old eggs and fecundity continued to decline with longer storage. The total fecundity reached to 2/3 of the fresh eggs 1.5 months after storing in cold condition. It means 182 reached to 118. The inspection of parasitism trend of *T. vassilievi* in 3 day intervals of oviposition period revealed that those wasps that encountered ≤1 week old eggs showed their maximum performance in the first 3 day interval followed by a non-significant change in the second one. The fecundity of the remaining intervals of these wasps had a decreasing trend by time. Those wasps that encountered to older host eggs, revealed a different pattern. Depending on storing time of host eggs, they revealed 55-80% performance of the control wasps in the first 3-day interval of their oviposition period, but they further enhanced their reproductive efforts to compensate their lower initial performance. As a result they performed 11.5-19% higher parasitism in the second interval and 12.5-39% in the 3rd one in comparison to the first interval. Hence their reproductive curve reached to a peak at the third interval while exceeding that of the control, followed by a further decline afterward (Fig. 2). These patterns were similar in both populations.

V-development: The developmental time of immature stages of *T. vassilievi* was significantly different between parasitoid populations (F=15.90; df=1, 2346; $P<0.01$), genders (F=758.09; df=1, 2346; $P<0.01$) and storage periods (F=1873; df=5, 2346; $P<0.01$). Neither two-way nor three-way interactions were significant (wasp × sex: F=0.111; df=1, 2346; $P=0.740$, wasp × storage: F=0.130; df=5, 2346; $P=0.986$, sex × storage: F=1.924; df=5, 2346; $P=0.087$, wasp × sex × storage: F=0.577; df=5, 2346; $P=0.718$). The developmental time increased with a sigmoid trend with increasing duration of storage in all treatments (both sexes of both populations). As a result difference between all treatments (except 6 and 8 weeks) placed in different groups of Tukey's HSD. The maximum lengthening in development time was 32% in 8 week stored eggs proportional to control (Fig. 3). Development time of both populations was very near to each other in all treatments but the minor differences were still significant. Thus Tabriz wasps developed slightly slower than Varamin wasps. Moreover males developed nearly one day sooner than females.

VI- longevity: Like previous variable, longevity also was affected by all factors included in the experiment (F=18.91; df=1, 456; $P<0.01$ for population, F=81.54; df=1, 456; $P<0.01$ for gender and F=19.04; df=5, 456; $P<0.01$ for storage period). No interaction was significant again (wasp × sex: F=1.465; df=1, 456; $P=0.227$, wasp × storage: F=0.577; df=5, 456; $P=0.718$, sex × storage: F=1.782; df=5, 456; $P=0.115$, wasp × sex × storage: F=0.218; df=5, 456; $P=0.955$). The longevity was decreased 10-15 days during 8 weeks of host storage both in males and females. The females' mean longevity was 3-15 days longer than males in different treatments. On the other hand longevity in Varamin wasps was 1-8 days (average 3 days for females and 5 days for males) longer than Tabriz wasps (Table 1).

VII-body size: One probable effect of host quality on parasitoid is in its body size that in turn affects the fitness components like fecundity, development rate *etc.* Right hind tibia length as well as head capsule width were measured in this context. Both variables were affected by all the experiment factors (F=75.28; df=1, 456; $P<0.01$; F=152.81; df=1, 456; $P<0.01$; F=274.51; df=5, 456; $P<0.01$ for tibia length of the two populations, genders and storing periods and F=39.35; df=1, 456; $P<0.01$; F=247.95; df=1, 456; $P<0.01$; F=81.48; df=5, 456; $P<0.01$ for width of the head capsule for the same factors respectively). Interaction between sex and both factors of wasp population and storing duration also were significant for both variables (F=19.83; df=1, 456; $P<0.01$; and F=12.89; df=1, 456; $P<0.01$ for sex × population respectively for tibia length and head capsule width and F=2.58; df=5,

456; *P*=0.025; and F=25.12; df=5, 456; *P*<0.01 for the same variables for sex × duration). General pattern of these changes was so that head width was broader and tibia length shorter in females, body size decreased -in females more- by storing duration and Varamin wasps were a little larger than Tabriz wasps. Head capsule width was around 0.6 mm in females of control wasps (0.596 and 0.610 mm in Tabriz and Varamin wasps respectively) and 0.05-0.06 mm less broad in males. Storing host eggs for two months lead to head capsule decrease 14.7-16.0% in females and 4.3-5.5% in males. This may imply a stronger effect on females. Trend in hind tibia shortening was more similar between females and males of both populations (12-13% decrease in all treatments at the end of 8 weeks). It must be pointed out that a sharp decline in body size appeared in those wasps that emerged from host eggs stored more than 2 weeks (Fig. 4).

VIII- stable population growth parameters: The stable population growth parameters values are summarized in table 2. A 2.5 time difference in R_o, 40% in r_m and 17% in T was observed among treatments.

DISCUSSION

Cold storage is a common action for maintaining natural enemies produced over demand for subsequent use with varying results in different species (van Driesche & Bellows, 1996). Such efforts have had poor results in telenomin egg parasitoids (Asgari, 1995; Foerster et al., 2004; Kodan & Gurkan, 2004; Foerster & Doetzer, 2006). Alternatively host egg storage under such a condition has shown more desirable results (Lazarov et al., 1969; Sklyarov, 1970; Popov, 1974; Gusev & Shmettser, 1975; Correa-Ferreira & Moscardi, 1993; Asgari, 1995; Awadalla, 1996; Kivan & Kilic, 2005). In this study the effect of the recent action was explored on *T. vassilievi* egg parasitoid of *E. integriceps*. As expected this action caused negative effects on produced wasps with increasing intensity with longer storages, as earlier scientists also reported for the other species (above mentioned references). Our main focus was on research purposes rather than mass rearing so we had special emphasize on evaluation of quantitative impacts on the parasitoid biostatistics. Yet the results can exploit for applied purposes. Indeed many statistics estimated here are used as criteria in quality control of biocontrol agents. For example body size is an indicator of a parasitoid quality (larger parasitoid higher parasitism) (King, 1987; Godfray, 1994; Arakawa et al., 2004). Many entomologists have shown that body size is related to fitness indices such as fecundity, survival and development time (Sagarra et al., 2001;Allahyari et al., 2004; Silva et al., 2011). This is also true with respect to *T. vassilievi*. Also some researchers showed that host quality is related to sex allocation in female parasitoids (Pak & Oatman, 1982; Waage, 1986; Awadalla et al. 1996). The general pattern of this decision for a foraging parasitoid is so that she allocates more suitable hosts to her daughters (Waage, 1986). The tendency of *T. vassilievi* to produce more sons in longer stored host eggs confirms such a pattern that already also has been seen in *T. grandis* and *T. semistriatus* with higher intensity (Gusev & Shmettser, 1975). However in contrast to *Anastatus bifaciatus* Forcroy (Hym., Eupelmidae) this tendency is not absolute. No female progeny has been obtained on sunn pest eggs attacked by the later species apparently due to small size of the host egg (Iranipour et al., 1998). The reason of such discrimination between hosts in sex allocation has been stated that is higher fitness loss in females over males (van Driesche & Bellows, 1996). Body size measurements of *T. vassilievi* support this hypothesis.

An interesting point in our results is avoiding of *T. vassilievi* females from maximum clutch in stored host eggs. Taking into account that *T. vassilievi* can

parasitize more than one host clutch (each one including 14 eggs) per day (unpublished data; also may be deducted by averaging three day fecundities), we will find her exploitation has been reduced actually to less than half a clutch in the longest storage treatment. This may suggest presence of a suppressant or alternatively absence or reduction of a stimulant that may percept by receptors of ovipositor tip (Okuda & Yeargan, 1988). The compensatory effort of a parasitoid to regain her lost opportunity observed by Bazavar (2013) both in *T. grandis* and *Ooencyrtus fecundus* Ferriere & Voegele in response to no-access durations which is in full agreement to our results on *T. vassilievi*. Nozad Bonab & Iranipour (2012) showed that development rate of *T. grandis* is a function of total protein content of host eggs. Hence, reducing speed of development of *T. vassilievi* in older eggs also may suggest a parallel decrease in protein content of the cold-stored eggs maybe via breaking protein molecules. Interestingly emergence rate less effectively influenced by cold-storing. This may imply that sufficient nutrient although with worse quality is still present for entire development. These results are in partial agreement to Asgari (1995) and Kivan & Kiliç (2005) statements on *Telenomus chloropus, Trissolcus grandis*, and *T. semistriatus*.

All previous studies (Bazavar, 2013; Rafat et al., 2013; Ahmadpour et al., in press; Nozad Bonab et al., in press) reveal a characteristic type I survivorship curve of the egg parasitoids of sunn pest. High emergence rates may confirm this generalization in *T. vassilievi* too. So no immature mortality is necessary to include in calculating R_o values. In exact estimations of R_o on the other hand both age specific survivals and sex ratios have to include. In this study however due to no access to these data minor biases are possible. First source of bias is a common sex ratio used as average of the total cohort. This corresponds to equal sex ratios in all ages *i. e.* $S_x=S_{x+1}=...S_{\omega-1}=S_\omega$. Previous works show that the sex ratio decreases by age. This is not however an important case because common sex ratio is a weighted mean of all ages and because fecundity itself declines sharply at senescence, later sex ratios contribute small weights. The second source of bias is recording fecundity data in 3 day intervals instead daily records. This corresponds to equal fecundities in all three days of an interval. Although this may create bias in estimates, this bias may not be important because fecundity of a day more likely resembles that of yesterday or tomorrow. This explanation also may be true in the *T* calculations. As a result we expect only minor biases in r_m estimates as well. Comparison to results of the other scientists confirms accuracy of our estimates. For example R_o estimates as SR.F in *O. fecundus* lead to 0.5-4% bias in different treatments (Ahmadpour et al., in press). Developmental time and *T* estimates are also very similar in both species. As a result r_m estimates are also the same in both.

As a conclusion we may result that cold-storing for a week has no negative effect on biostatistic of *T. vassilievi* except a minor significant increase in development time that is less than 0.2 days, although this negligible difference has been significant. In this sense we can use one week old stored eggs for experiments with negligible bias in results. Continuing storing host eggs one week more will lead to significant fecundity loss (5%) in addition to one day delay in development. No statistic was affected >10% in this treatment, therefore 2-week old eggs also may use in research studies with less than 10% bias in results. However longer storage may cause considerable bias in majority of the statistics. These deviations are considerable in research contexts, because high precision and accuracy is needed.

In terms of mass production with particular reference to inundation programs the situation will be deeply different. To demonstrate this claim we will follow a simple model bellow. First let's we consider only one wasp population (Tabriz

population) because the results for both are deeply similar. Regarding the r_m value in a stable population no more than 5% change will occur monthly in number of wasps produced between control and those wasps that continuously has been reared in 1 week old eggs, while this difference will be 1.7, 3.7, 8 and 8.8 times in remaining treatments in similar horizon of time. Of course this is provided that no resource limiting factor such as density is present *i. e.* the rate of parasitoid/ host is low enough to prevent interference or exploitation competition (Maybe in mass production condition these forces are more critical than host-storage effects). Since sunn-pest egg parasitoids have a short releasing interval in field (Shahrokhi, 1997; Asgari, 2011), a seasonal activity of a biofabric rather may be expected. Hence a stable population is rarely possible in biofabric. The most common situation then is holding a small population semi-active out of season and starting mass production in an appropriate time to obtain a population large enough for inundative release in a determined time. Now we come back again to our model. In the mentioned situation we will have separate generations rather than overlapped stable populations. Let's the initial number of female wasps for beginning augmentation be N_o. Each female in a generation will increase R_o-times through T days. If desired number of females at the end of production assumed to be N_e and number of generation required to achieve this be n, then the time required for satisfying the above condition will be nT and the final population after this period will be $N=N_oR_o^n$. N is the number of females that are available for release that will be satisfactory if $N>N_e$. Because in inundation release no transition to the next generation is expected (van Driesche & Bellows, 1996), both male and female progeny are of the same value in terms of host egg mortality. The only thing that must be further considered is that the older eggs (8 week-old) will led to 2/3 parasitism after release, so if 8 week-old eggs are used in mass production the mentioned condition must be changed to: $N>1.5N_e$. The calculations based on the above mentioned model were illustrated in Figure 5 that may use for decision making. This reveals a maximum 100-times difference in number of female wasps produced in biofabric after five generations which will take 106 and 124 days respectively for control and 8-week old eggs.

Now suppose that we need 500 million wasps for release in 10 May. Further suppose we are going to start rearing only with a stock of 200 fertile females. The decision algorithm will be drawn by following this instruction. Because all figures in figure 5 are calculated per an individual initial female, so they will multiply by 200 when we are going to calculate number per 200 initial females. So it is better the desired number (500 million) divide by 200 for converting calculations to *per capita* counts. It means 2.5 million per an individual female is needed that corresponds to 500 million per 200 females. So we now may refer to figure 5 to decide how many generations are needed to achieve this. This amount of females are available in < 2 week-stored treatments only after three generations, while in other treatments we need to continue rearing a generation more with many extra females produced over demand. In order to manage additional costs due to further generations and extra females two options are available. The first one is handling initial number of females to reach 500 million after a determined number of generations (*i. e.* keeping number of generations constant). If initial number available is less than required, then we have to start a generation sooner with a small number to achieve the expected number. For example in above instance we need respectively 109, 115, 161, 300, 1314 and 1635 primary females in control, 1, 2, 4, 6 and 8-week old host eggs to achieve 500 million parasitic females after three generations. It is obvious that this amount is available for first three treatments (< 2 week old eggs), but in the other cases a further generation with small numbers is

needed. In this example however difference between 4 week old eggs is not considerable compared to previous treatments, but imposes additional costs for producer. Holding stock culture as large as 300 females/ generation will resolve the problem without increasing mass production costs. The difference of the two other treatments is however very more considerable as they need ≥ 5 times larger initial population to start. So, as long as the host eggs are not > 1 month-old the costs do not increase largely.

The second option is starting with any initial numbers of females available and stop whenever the concerned number or more is available. Extra females also may avoid again with handling initial number. The major difference between the two alternatives is number of generations in mass production. For example if we have to use the older eggs we can start with 1635 females and achieve the considered number of females after three generations or alternatively start with 25 females and gain the same number after four generations. It depends on producer decision and of course primarily to economic evaluations. The time required for both is 4T=99.2 days, with this major difference that in the first situation the first generation is rather a small scale culture for achieving 1635 females rather than a mass production. So we need start our production 100 days earlier *i. e*. at the end of January. If fresh eggs were always available we could start 3T=63.4 days earlier *i. e.* around the second week of March.

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Table 1. Adult longevity (±SE) of *T. vassilievi* on cold-stored eggs of sunn pest for different durations.

Period of storage (week)	Adult longevity (day) ±SE			
	Tabriz		Varamin	
	female	male	female	male
0	62.3±3.27a*	48.45±2.50a	65.95±2.56a	56.0±3.31a
1	62.15±2.11a	47.25±1.60a	65.25±2.16a	55.55±2.73a
2	52.8±1.08b	46.55±2.23b	54.65±3.17b	51.5±1.57b
4	53.2±3.02b	46.2±1.20b	54.3±2.87b	47.45±1.38b
6	50.7±1.62bc	43.75±2.69bc	54.1±2.44bc	47.25±1.45bc
8	47.15±1.88c	39.0±0.83c	51.55±0.65c	44.45±2.52c

*Means bearing the same lowercase letter in a column are not significantly different (Tukey's HSD, α= 0.05).

Table 2. Stable population growth parameters of *T. vassilievi* progeny developed in cold-stored eggs of sunn pest for different durations.

population	treatment	Parameter				
		R_0	r_m	λ	DT	T
Tabriz	Fresh egg	166.24	0.2421	1.274	2.86	21.12
	1 week old egg	163.35	0.2405	1.272	2.88	21.19
	2 week old egg	145.85	0.2251	1.252	3.08	22.14
	4 week old egg	118.63	0.1986	1.220	3.49	24.05
	6 week old egg	72.48	0.1730	1.189	4.01	24.76
	8 week old egg	67.37	0.1697	1.185	4.08	24.80
Varamin	Fresh egg	165.34	0.2434	1.276	2.85	20.98
	1 week old egg	144.80	0.2353	1.265	2.95	21.14
	2 week old egg	150.50	0.2267	1.254	3.06	22.12
	4 week old egg	125.23	0.2014	1.223	3.44	23.98
	6 week old egg	68.91	0.1729	1.189	4.01	24.48
	8 week old egg	64.74	0.1689	1.184	4.10	24.70

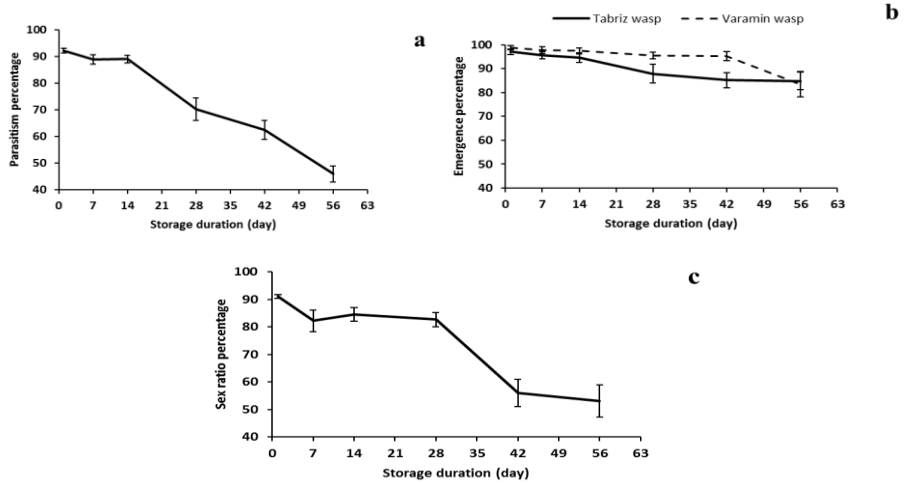

Figure 1. (a) Parasitism percentage, (b) emergence percentage and (c) progeny sex ratio (females %) of *T. vassilievi* on cold-stored eggs of sunn pest for different durations. Notice that parasitism rate and sex ratio curves have been pooled for both wasp populations due to non-significance.

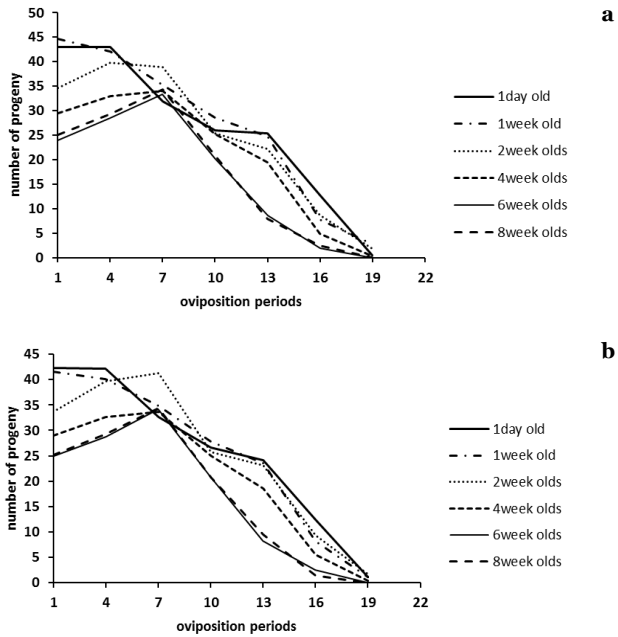

Figure 2. Fecundity distribution of *T. vassilievi* emerged from cold-stored sunn pest eggs over 3 day intervals of life span, a) Tabriz population b) Varamin population.

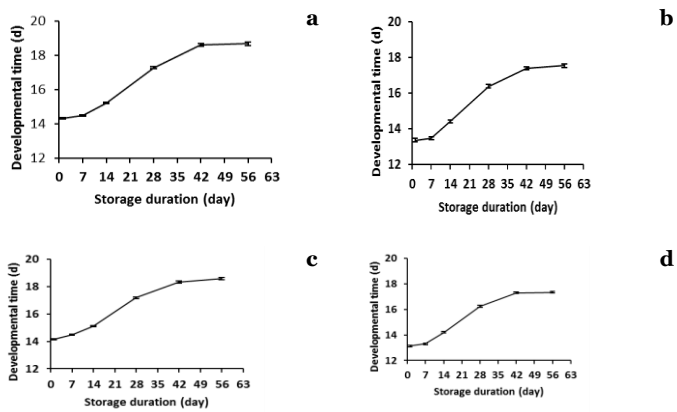

Figure 3. Developmental time of *T. vassilievi* on cold-stored sunn pest eggs for different durations. a) Tabriz females, b) Tabriz males, c) Varamin females, d) Varamin males.

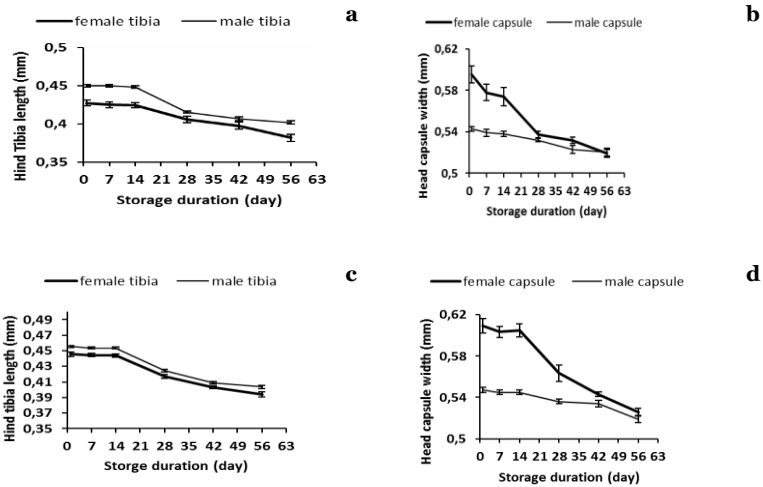

Figure 4. Body size of *T. vassilievi* obtained from cold-stored eggs of sunn pest, measured as right hind tibia length and head capsule width (mm ±SE). a) Tabriz population's tibia length, b) Tabriz population's head capsule width, c) Varamin population's tibia length, d) Varamin population's head capsule width.

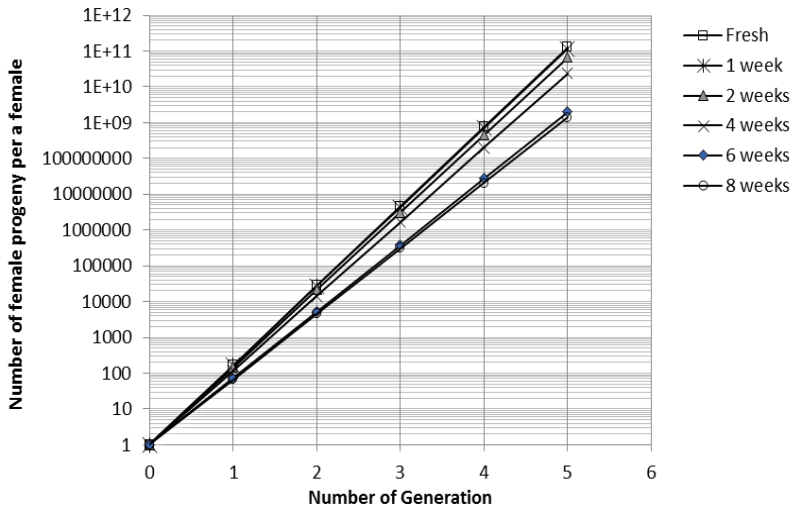

Figure 5. Hypothetical number of female progeny available per a single fertile female of *T. vassilievi* following rearing in biofabric through several generations.