COLD STORAGE OF THE ADULT STAGE OF *GONATOCERUS ASHMEADI*: THE IMPACT ON MATERNAL AND PROGENY QUALITY

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ABSTRACT
The effect of storage of adult *G. ashmeadi* at 2, 5 and 10°C on maternal and the unstored progeny fitness qualities was examined. The maternal generation did not survive five d exposure to 2°C and those stored at 10°C survived longer than those held at 5°C. Oviposition of the maternal generation continued for 13 d after storage at 10°C for 10 or 20 d and for eight d for the wasps stored for 30, 40 or 50 d. After storage for 60 d, the parasitoids did not oviposit for the first two d and then there was only a four d oviposition period. Cold storage reduced the fecundity of the parents and their F_1 progeny, but not the F_2 generation. After 20 d storage, the fecundity of the maternal generation decreased by 47% and for 60 d storage, it was reduced 90%. The longevity of F_1 parasitoids was also less than that of F_2 parasitoids. After 30 d storage of the parents, the longevity of the F_1 generation was reduced 49% as compared to a 10 d parental storage period. The F_1 progeny of the stored female parasitoids developed about one d more slowly than that of the F_2 and F_3 generations. Moreover, cold storage caused a reduction in the incidence of parasitism by parental generation, but this effect did not extend to F_1 and F_2 progeny. Emergence of F_1 parasitoids decreased as the length of storage of their parents increased. Further, the parental generation deposited more haploid than diploid eggs after storage for 20 d and after 50 d, production of males was increased by 132%. The sex ratios of the F_2 and F_3 generations did not vary with the storage duration of the ancestral generation.

INTRODUCTION
Cold storage can be a valuable tool in mass-rearing of biological control agents. It provides a steady supply of insects for research, yields flexibility and efficiency in mass production, allows synchronization of a desired developmental stage for releases, and facilitates availability to consumers (Leopold, 1998). Maternal survival and offspring fitness are of great concern when using cold exposure during production or storing of biological control agents. Some reports indicate that the quality of a variety of cold-stored pest and beneficial insects suffer by having reduced emergence, lifespan and/or reproduction (Leopold, 1998).

The egg parasitoid, *Gonatocerus ashmeadi* Girault, is one of the most common natural enemies of the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae). It accounts for 80-95% of the observed parasitism of sharpshooter eggs in California. As part of a control program, *G. ashmeadi,* along with two other mymarids, *G. triguttatus* Girault and *G. fasciatus* Girault, has been imported, mass-reared and released in California. However, there are two main problems that reduce the effectiveness of *Gonatocerus* spp. in control programs. One is that the native wasps do not build up rapidly enough to make an impact on the spring generation of *H. vitripennis* in the colder regions of central and northern California (Morse et al., 2005). Another problem is that effective mass production of these parasitoids can not be realized due to a shortage of *H. vitripennis* eggs caused by a reproductive diapause (Hummel et al., 2006), especially in the late winter season. Thus, it would desirable to store large numbers of parasitoids to meet these fluctuating demands.

Methodology for cold storage of *H. vitripennis* and *G. ashmeadi* has been developed to aid the mass rearing of *G. ashmeadi* (Leopold and Chen, 2005; Chen and Leopold, 2007; Chen et al. 2007). We have also examined a number of fitness factors of *G. ashmeadi* and progeny performance following the use of these storage protocols. Using chilling-killed *H. vitripennis* eggs as hosts for rearing *G. ashmeadi*, Chen and Leopold (2007) found that wasp progeny quality was severely reduced when their parents were reared on *H. vitripennis* eggs that had been stored for 60 days before parasitization occurred. Further, by stockpiling immature *G. ashmeadi* within *H. vitripennis* eggs, quality of maternal and first generations also suffered significantly with the length of storage duration (Chen et al. 2007). In this study, we examined the post storage quality of the parental, F_1, F_2 and F_3 generations of *G. ashmeadi* after storing the adult female parents to further understand chilling injury and its expression in this parasitoid.

OBJECTIVES
1. Determine the survival of adult parasitoid following cold storage.
2. Determine the effect of cold storage on fecundity and subsequent development of the progeny.
3. Determine progeny quality by examining some fitness factors including parasitism, emergence and sex ratio.

RESULTS AND CONCLUSIONS

Objective 1. Effect of storage temperature on survival of adult parasitoid.

The survival of adult parasitoids was examined every 10 d after storage in the incubators set at 2, 5, and 10°C with a 8L:16D photoperiod. Figure 1 shows that survivorship of *G. ashmeadi* varied with storage duration when storage was at 5 and 10°C. In addition, because all parasitoids died after 5 d storage at 2°C, their survivorship evaluation was excluded for this study. Temperature during storage significantly affected the survivorship of adult parasitoid (F= 11.01, df =1,108, P = 0.0012). However, under the same temperature, we found no significant difference in survivorship between female and male parasitoids (5°C, F= 2.69, df =1,54, P = 0.107; 10°C, F = 0.96, df =1,54, P= 0.331) although the females slightly outlived the males.

Objective 2. Post storage fecundity of parasitoid and development of progeny.

To determine the effect of cold storage on fecundity of the parental generation and the F_1 and F_2 progeny, 1 mated female parasitoid was introduced into a container having a total of 80 GWSS eggs (<24 h old) on euonymus leaves (*Euonymus japonica* Thumb.) and then held at 24°C and under a16L:8D photoperiod. Mated parasitoids made up six groups of adult females that were stored at 10°C for 10, 20, 30, 40, 50 and 60 d and five groups of F_1 or F_2 offspring obtained from the storage experiments involving the parental generation. Oviposition of *G. ashmeadi* after adult storage is shown in Figure 2. After storage for up to 50 d, the females oviposited on the first day after removal from storage. The duration of storage affected the length of ovipositional period. After storage for 10 and 20 d, oviposition of this parasitoid continued for 13 d, and for 30, 40 and 50 d storage, it lasted for 8 d. When storage was 60 d, oviposition was lacking for 2 d and then the eggs were deposited for only 4 d.

Lifetime fecundity of *G. ashmeadi* following cold storage of adult female parasitoid varied significantly with the length of storage duration (Table 1). After storage for 20 and 60 d, fecundity of the parasitoids was decreased by 47%, and 90%, respectively. The fecundity of the F_1 generation was also significantly affected by the length of storage of their parents. When the parents were stored for ≥ 20 d, the fecundity of their F_1 progeny decreased significantly. However, fecundity of F_2 generation was unaffected by storage duration of grandparental generation. A two-way ANOVA, with storage duration and generation as factors, revealed that lifetime fecundity of *G. ashmeadi* varied significantly with the length of storage of adult parasitoid (F = 9.64, df = 4,114, P < 0.0001) and generation (F = 40.43, df = 2,114, P < 0.0001). There was a significant effect of interaction between storage duration and generation on lifetime fecundity of this parasitoid when storage was ≥20 d (F = 3.32, df = 8,114, P = 0.0019). For 10 d storage, the lifetime fecundity of the parental, F_1 and F_2 generations was unaffected.

Development time of the F_2 and F_3 generations did not vary with the length of storage of parental generation (Figure 3A). However, progeny of F_1 generation developed significantly more slowly than that of F_2 and F_3 generations. There was ca. 1 d difference in progeny development between the F_1 and F_2 generations. Only the storage period for 50 d led to significant difference in development between the F_1 and F_2 and F_3 generations.

Longevity of F_1 and F_2 generations was shown in Figure 3B. The length of storage of the parental parasitoid had a significant influence on the longevity of F_1 parasitoids (F = 4.79, df = 4, 44, P = 0.003), but not on that of F_2 generation (F = 0.12, df = 4,35, P = 0.976). After parental females were stored for 30 d, the longevity of F_1 wasp was shortened by 49% when compared to that of wasps stored for only 10 d.

Objective 3. Effect of cold storage on the quality of maternal generation and progeny.

To determine the incidence of parasitism by the maternal and F_1 and F_2 generations, six excised leaves of the euonymus plant bearing a total of 80 GWSS eggs (< 24 h old) were provided for 1 wasp for 1 d at 24 °C and 16L: 8D photoperiod. Wasps were collected using a procedure described previously (Chen and Leopold, 2007). The GWSS eggs were kept at the same chamber to determine emergence rate and sex ratio of the offspring. Figure 3C shows the data on the incidence of parasitism by the P to the F_2 generation for up to 50 d of storage. Because the parasitoids did not deposit eggs during the first two days after storage for 60 d, they were excluded from this study. Our results showed that post storage parasitism by the parental generation varied significantly with storage duration (F = 7.14, df = 4, 40, P = 0.0002). The incidence of parasitism by the F_1 (F = 0.12, df = 4,45, P = 0.976) and F_2 (F = 0.73, df = 4,45, P = 0.574) generations was not affected by storage duration of the ancestral generation.

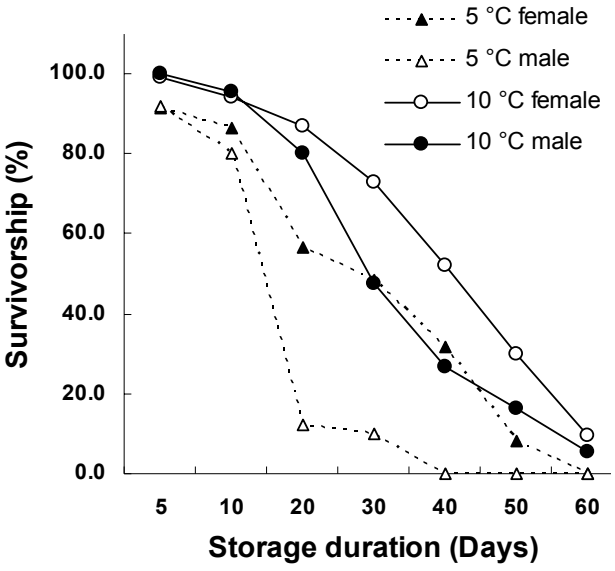

Figure 1. Survivorship of *G. ashmeadi* as a function of the length of adult storage. Each value represents means of 5 separate determinations.

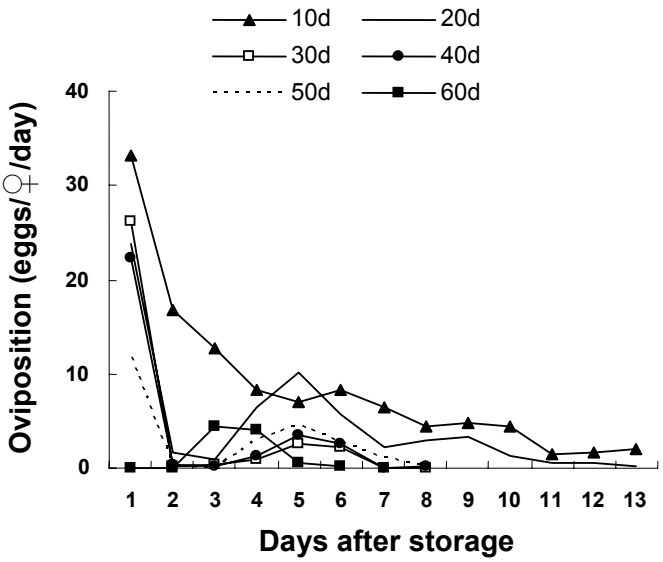

Figure 2. Oviposition of *G. ashmeadi* as a function of the length of adult storage. Each value represents means of 10 separate determinations.

Table 1. Lifetime fecundity of parental, F_1 and F_2 generations following cold storage of the parental generation.

	Parental (P) Storage Duration (Days)								
Gen	**10**	**20**	**30**	**40**	**50**	**60**	***F***	***df***	***P***
P	111.8 ± 23.7Aa	59.8 ± 24.2Cb	32.1± 8.4Cc	30.9 ± 10.2Cc	30.7 ± 20.8Cc	11.4 ±10.6d	16.5	4,34	<0.0001
F_1	106.7 ± 35.2Aa	74.1 ± 15.8Bb	61.7 ± 19.9Bb	61.6 ± 20.4Bb	59.2 ± 32.0Bb	/	5.5	4,43	0.0011
F_2	96.5 ± 15.8Aa	93.9 ± 32.6Aa	96.4 ± 13.1Aa	98.4 ± 29.3Aa	107.5 ± 47.7Aa	/	0.26	4,37	0.902
F	0.40	3.96	35.46	23.68	12.13				
df	2,16	2,23	2,23	2,25	2,27				
P	0.679	0.033	< 0.001	< 0.001	0.0002				

The emergence rate of the F_1 adults was significantly affected by the length of storage duration of parental generation (F = 11.52, df = 4, 38, P < 0.0001) (Figure 3D). However, adult emergence of the F_2 (F = 0.55, df = 4, 45, P = 0. 697) and F_3 (F = 0.08, df = 4, 45, P = 0.988) generations did not vary with storage duration of their ancestral generation

The length of storage duration of maternal generation significantly affected realized sex allocation of the F_1 progeny (F = 2.92, df = 2, 92, P = 0.034). The parental generation females produced more haploid than diploid eggs after storage for ≥20 d (Figure 4). After storage for 50 d, the male proportion of the wasp progeny was increased by 132.5%. Although the female proportion was slightly increased in the F_1 generation after their adult parents were stored for ≥20 d, the sex ratios of F_2 (F = 1.19, df = 4,33, P = 0.334) and F_3 (F = 0.36, df = 4,34, P = 0.834) generations did not vary with storage duration of the ancestral generation.

Chilling adult *G. ashmeadi* females for more than 10 days at 10°C or lower has a deleterious effect on fitness that carries over to the F_1 generation but not to subsequent generations. These observations should be taken into account when producing parasitoids as biocontrol agents for the GWSS.

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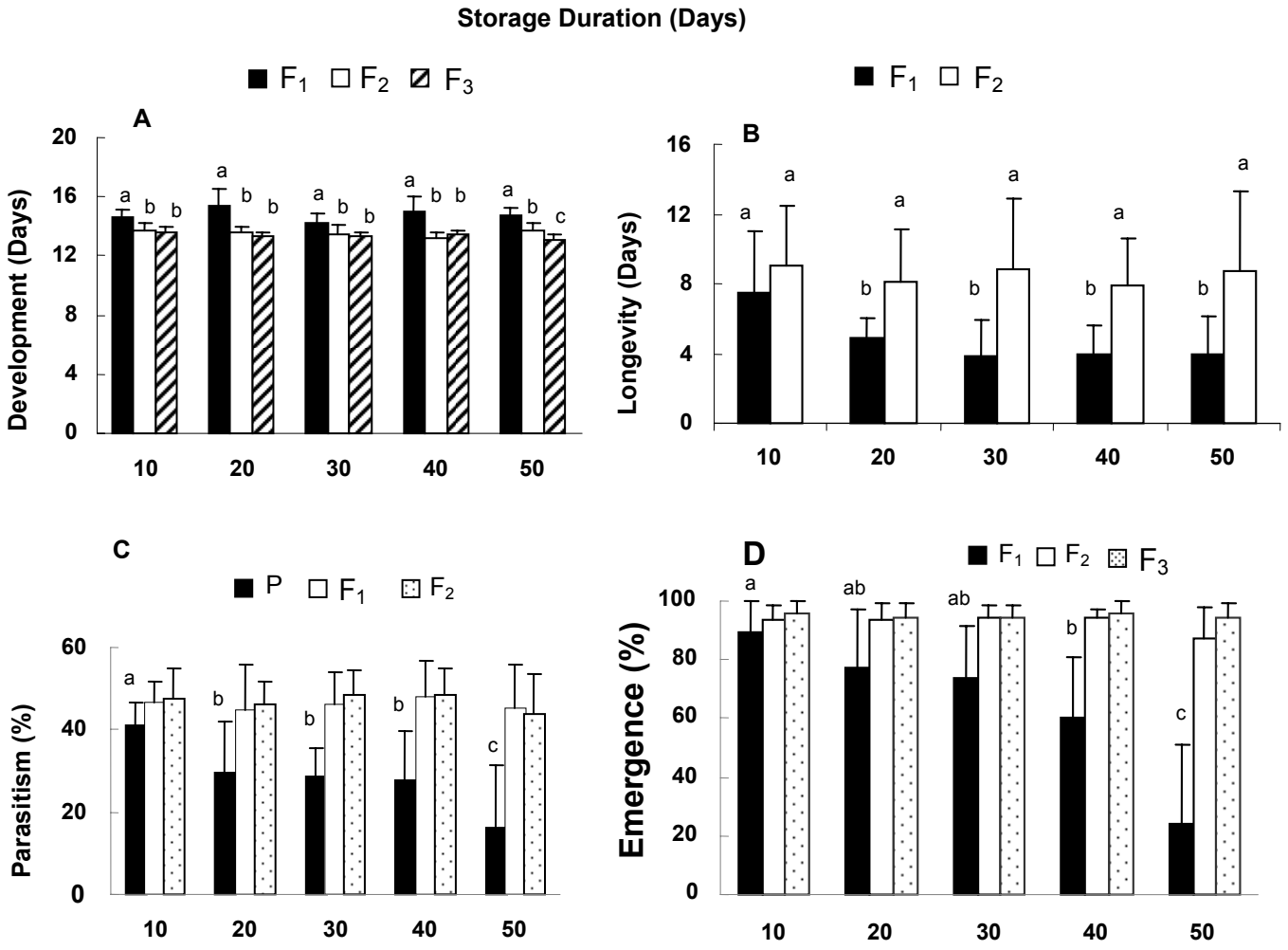

Figure 3. (A) developmental time of progeny of the F_1, F_2 and F_3 generations; (B) F_1 and F_2 longevity as a function of the P generation storage; (C) parasitism by parental, F_1 and F_2 generations; (D) emergence of the F_1, F_2 and F_3 generations as a function of storage of the P generation. Columns with different letters are significantly different from each other.

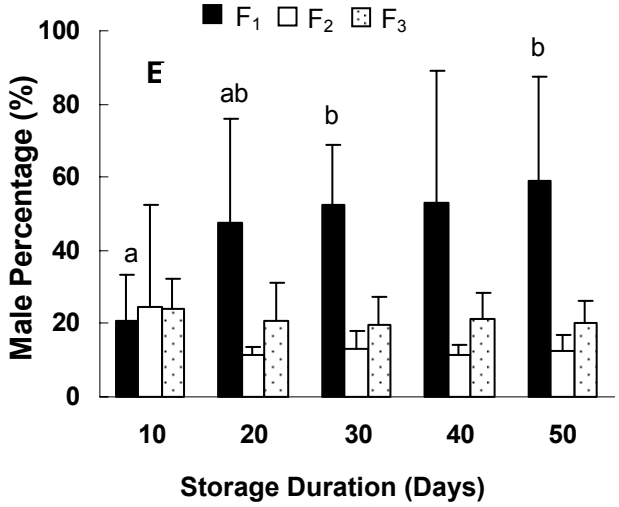

Figure 4. Sex ratios of the F_1, F_2 and F_3 generations of *G. ashmeadi* as a function of the length of storage of the P generation of mated females. Columns with different letters are significantly different from each other.