EFFECTS OF USING CONSTANT AND CYCLICAL STEPWISE-INCREASING TEMPERATURES ON PARASITIZED AND UNPARASITZED EGGS OF THE GLASSY-WINGED SHARPSHOOTER DURING COLD STORAGE

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ABSTRACT

Glassy-winged Sharpshooter (GWSS) egg masses, deposited on *Euonymus japonica* cuttings, were stored 1d after oviposition at either a constant temperature of 12ºC or under a regime that cycled daily, stepwise, (10, 11, 12, 13ºC @ 6h intervals) under an 8L:16D photoperiod. After storage under the cycled temperature regime for 15 and 20d, the hatch was 74 and 63%, respectively. Control hatch at 20d was about 80% and 50% after storage at a constant 12ºC. The survival to adulthood, length of the nymphal stage, and the fecundity of the adult females were all affected by cold storage during the egg stage, regardless whether the temperature was held constant or cycled. Survival to adulthood was reduced 30 to 40% and the time required to complete the nymphal stages was significantly longer than the control. The number of eggs oviposited by females and length of the ovipositional period after being held at 12ºC during the egg stage was about one-half that of the control group, while the values for the 20d cycled group are yet to be determined. The rates of parasitism and emergence by *Gonatocerus ashmeadi* decreased with the length of time that 1-d-old unparasitized GWSS eggs were stored under the cycled regime. When held up to 25d in storage, parasitism by wasps and emergence of their progeny remained statistically similar. After 50d of storage, parasitism and progeny emergence dropped 30% and 20%, respectively. After a storage period of 25d, parasitoid emergence from parasitized eggs stored at a constant 4.5ºC was significantly higher than those stored similarly at 4ºC. The cycled stepwise-increasing temperature regime of 4.5, 6.0, and 7.5ºC changing at 8h intervals yielded a significantly higher parasitoid emergence than a cycled regime of 4, 6, and 8ºC. When stored under the regime starting at 4.5ºC, for 10, 20 and 25d, the emergence of wasps was 66%, 59% and 59%, respectively. Parasitized eggs stored under this regime for 80d produced no wasps.

INTRODUCTION

Studies on cold storage of insects and their eggs have shown that developmental age, storage temperature, time in storage, and inherent species tolerance are the factors which influence survival after a cold storage period (Leopold 1998). The most effective temperature for storage of GWSS eggs was determined to be 12ºC (Leopold et al. 2003). Storage of 1-d-old GWSS eggs at 10ºC resulted in no survival after only 8d period. Storage at 13 and 14ºC resulted in high survival and parasitism by *Gonatocerus ashmeadi* and *G. triguttatus* at 20d, but in-storage hatching of the GWSS eggs occurs after 30d and successful parasitism by the wasps decreases under these constant temperature regimes. The within-host cold tolerance of the *Gonatocerus spp*. is significantly greater than that of the unparasitized GWSS eggs. Emergence of the wasps occurs at temperatures $\geq$ 5ºC when the parasitized eggs are stored < 20d. Since certain conditions, such as temperature variation and fluctuation and high or low humidities have been reported to enhance survival of insects and their parasites during cold storage (Iacob and Iacob 1972, Gautum 1986, Liu and Tian 1987, Leopold et al. 1998), the present study was initiated to determine whether we could lengthen the survival time of GWSS eggs and the egg parasitoid by varying the temperature while in storage. We were especially interested in determining whether any latent damaging effects of chilling would be expressed, beyond diminished emergence, that might affect the quality of previously cold-stored insects.

OBJECTIVES

1. Compare the cold tolerance of GWSS eggs stored at a constant temperature with eggs stored under a cycled stepwise temperature regime and evaluate the post storage developmental time of nymphs and reproduction of adults.
2. Compare the effects of cold storage of unparasitized GWSS eggs under constant and cycled stepwise low temperatures regimes on the subsequent parasitism and emergence of *G. ashmeadi*.
3. Determine whether a cycled stepwise cold temperature regime enhances the shelf-life of parasitoids while in host eggs.

RESULTS AND CONCLUSIONS

Cold storage of Unparasitized GWSS Eggs

GWSS egg masses deposited on *Euonymus* cuttings were stored in incubators set at constant (12ºC) and cycling stepwise-increasing temperatures (10, 11, 12, and 13 ºC @ 6h intervals) under an 8L:16D photoperiod for varying lengths of time. After removal from storage, the cuttings bearing GWSS egg masses were incubated at room temperature (ca. 22 ºC) to record egg hatch. After storage at 12ºC for 30d, 52.7 ± 10.2% of 1-d-old eggs (n = 102), 50.7 ± 7.1% of 3-d-old eggs (n = 87) and 44.7 ± 5.1% of 5-d-old eggs (n =61) hatched. However, no hatching was observed after 30d storage. When stored at the stepwise cycling temperature (10-13 ºC) for 15, 20, and 25d, the hatch of 1-d-old eggs was 73.9 ± 11.1% (n = 142), 62.6 ± 9.1% (n = 98) and 44.6 ± 9.1% (n = 104), respectively. There was a significant difference in percentage hatch of 1-d-old GWSS eggs between the control eggs (83.0 ± 7.4%, n = 317) and those eggs stored for 25d ($F = 3.939$, $df = 3,45$, $P = 0.014$), but no significant differences were found between those groups stored in the cold for 15 or 20 days and the control. After storage for 80d under the daily cycled regime, no hatching was observed.

To determine effect of cold storage during GWSS egg stage on nymphal development and adult reproduction, newly hatched nymphs from eggs stored at 12 ºC for 20 days, and at the daily cycled temperature regime for 15 and 20 days were reared on sunflower plants until they emerged as adults. When the characteristic patch of brochosomes was observed on the forewings of the adult females (brochosomes were considered as the sign that females had mated), they were then individually maintained on sunflower plants and their egg mass output recorded until death occurred. Our preliminary data (Table 1) shows that 50% of nymphs from eggs stored at 12ºC for 20 d and 50% and 40% of nymphs held under the stepwise temperature regime for 15 days and 20 days, respectively, successfully developed into adults. In comparison with the control groups, GWSS males and females from those eggs that had been exposed to either cold storage regime took significantly longer to complete their nymphal stages (Table 1). There were no differences in male and female developmental times among the nymphs that hatched from GWSS eggs that had undergone cold storage. The number of eggs produced/female and the ovipositional period was considerably greater for the control groups and approached 2-fold differences.

Effects of Cold Storage of GWSS Eggs on Parasitism and Emergence by G. ashmeadi
Following storage in incubators set at a constant 12 or 12.5ºC and also at the stepwise cycled regime as described above, GWSS egg masses were exposed to caged *G. ashmeadi* colonies for 2 days at room temperature (ca. 22 ºC) and under an 10L:14D photoperiod. Before statistical analysis, the data recorded for parasitism and emergence were square-root transformed to correct non-normality because the number of eggs/mass was not constant.

After storage at 12ºC for 30d, 69.6 ± 11.7% of the 3-d-old GWSS eggs (n = 90) and 47.7 ± 11.7% (n = 106) of the 1-d-old eggs were successfully parasitized by *G. ashmeadi*. The percentage wasp emergence was 68.5 ± 11.3 for 3 day-old eggs and 35.3 ± 10.0% for the 1 day-old eggs. There were no significant differences in the incidence of parasitism, as determined by egg dissection, ($F = 4.034$, $df = 1,14$, $P = 0.066$) and emergence ($F = 1.728$, $df = 1,14$, $P = 0.211$). Further, *G. ashmeadi* successfully parasitized about 77% of the 4-d-old , 52% of the 5-d-old, and 45% of the 3-d-old GWSS eggs stored at 12.5ºC for 30d, and 46% of 3-d-old eggs stored for 50d. As above, there were no significant differences between parasitism and emergence in any of the comparable groups (data not shown).

When stored under the cycled stepwise temperature regime (10-13 ºC), the parasitism ($F = 14.934$, $df = 8,137$, $P < 0.001$) and emergence ($F = 13.661$, $df = 8,137$, $P < 0.001$) of 1-d-old GWSS eggs by *G. ashmeadi* varied significantly with storage time (Table 2). More than 75% of GWSS eggs stored up to 25d were successfully parasitized and there was no significant difference in the incidence of parasitism between the control (92.1 ± 9.9%, n = 172) and the eggs stored for 15, 20 or 25d ($F = 1.764$, $df = 3,35$, $P = 0.172$). However, percentage emergence for the eggs stored for 25d was significantly lower than that for the control (91.7 ± 2.7%, n =172) ($F = 3.250$, $df = 3,35$, $P < 0.033$). Further, there were no significant differences in percentage emergence between the control eggs and the eggs stored for 15 or 20d ($P = 0.099$). After storage for 65d, < 44% eggs were parasitized by *G. ashmeadi*, and about 23% of wasps emerged, which was significantly lower than for eggs for stored for 25d or less. When stored for over 80d, the percentage parasitism and emergence were less than 12% and 7%, respectively. When these data were analyzed via a regression analysis, the percentage parasitism and emergence vs. storage time was found to be inversely correlated (Figures 1 and 2).

Cold Storage of GWSS Eggs Parasitized by G. ashmeadi
The experimental conditions for this study consisted of a constant 4 or 4.5ºC storage temperature and 2 daily cycled stepwise-increasing regimes (4, 6, and 8ºC or 4.5, 6, and 7.5ºC - each temp. changing at 8h intervals) under an 8 L: 16 D photoperiod. After the parasitized eggs were stored at 4 ºC for 10d, only 7.2 ± 5.0% (n = 85) of the wasps emerged, which was significantly lower than those parasitoids similarly stored at 4.5ºC (33.5 ± 7.2%, n = 280), 20 days (33.9 ± 6.9%, n = 114) or 25 days (21.7 ± 5.2%, n = 125) ($F = 11.962$, $df = 4,66$, $P < 0.001$). No parasitoids (n = 164) emerged from host eggs stored at 4ºC for 20d (Figure 3). When parasitoids were stored within hosts under the cycled stepwise temperature regime starting at 4 ºC, percentage emergence was 42% (n =126) at 10 d, 8 % (n = 420) at 20d and 0% (n = 184) at 25d (Figure 4). However, for parasitized eggs stored at the other cycled regime starting at 4.5ºC, the wasp emergence was at or above 60% throughout the 25d of storage. Thus, the percentage emergence for the parasitoids stored under the stepwise regime starting at 4.5ºC for 10-25d was significantly higher than that for the eggs stored for 15d under the regime starting at 4ºC ($F = 48.237$, $df = 5, 114$, $P < 0.0001$). Parasitoids within GWSS eggs did not emerge after storage for 80 days, but further research is needed to ascertain if maintenance of the *Euonymus* cuttings that bear the egg masses during the storage period is causing a problem.

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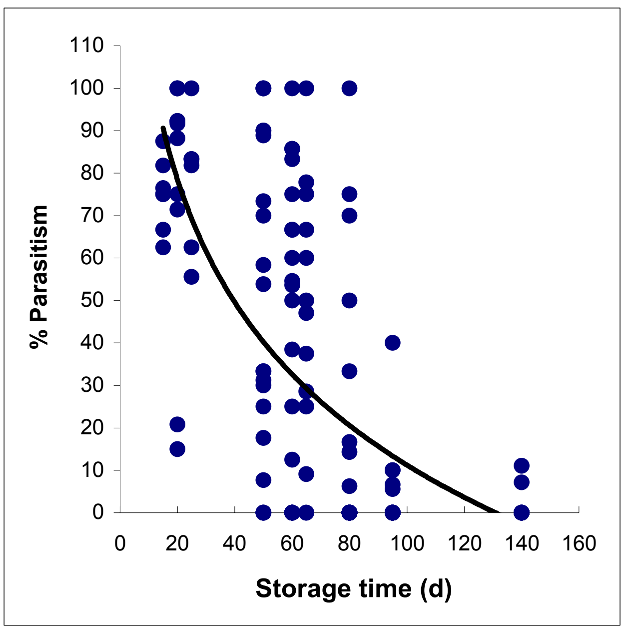

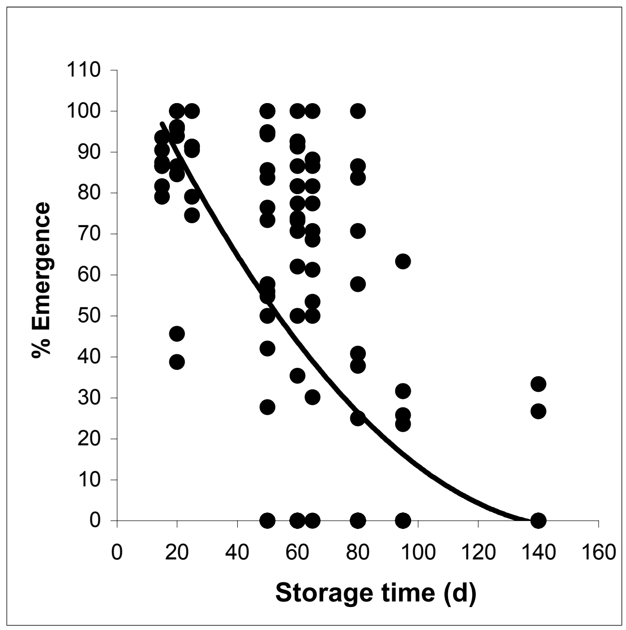

Figure 1. Relationship of the % parasitism (y) of *G. ashmeadi* to storage time (x) of the GWSS eggs at stepwise temperatures (10~13ºC)($y = 5.10 + 1393.18/x$, $r = 0.58$) ($F = 68.24$, $df = 136$, $P < 0.001$)

Figure 2. Relationship of the % emergence (y) of *G. ashmeadi* to storage time (x) of the GWSS eggs at stepwise temperatures (10~13ºC) ($y = -0.35 + 1286.50/x$, $r = 0.59$) ($F = 79.01$, $df = 136$, $P < 0.001$).

Table 1. Egg hatch, development time of nymphs and reproduction of adults for GWSS eggs stored under different temperature conditions (mean ± SE).

Storage conditions	Egg hatch (%)	Development time of nymphal stage			Adult reproduction	
		% survival	Male (d)	Female (d)	No. eggs/ female	Ovipositional period (d)
Control (25ºC)	82.9 ± 7.4	80.2	35.9 ± 0.5 a	35.3 ± 0.6 a	1068.8 ± 187.7	113.4 ± 49.6
12ºC for 20 d	52.7 ±10.2	50.0*	43.9 ± 0.9 b	42.5 ± 0.7 b	589.3 ± 81.9	65.7 ± 26.0
10-13ºC for 15 d	73.9 ± 5.4	50.0*	43.0 ± 0.7 b	41.0 ± 1.2 b	662.7 ± 111.1	65.0 ± 11.2
10-13ºC for 20 d	62.6 ±10.3	40.0*	43.0 ± 3.5 b	41.9 ± 0.4 b	In progress	In progress

Only 1 replicate. Means within a column followed by different letters were significantly different at the significant level of 0.05 (SAS Proc GLM with LSD). Data for egg hatch were square-root transformed before analysis.

Table 2. Parasitism and emergence by *G. ashmeadi* on the GWSS eggs exposed to the daily stepwise temperature regime (10, 11, 12, 13ºC - changing at 6h intervals) for 15 to 140 d.

Storage time	No. egg masses	No. eggs	Parasitism (mean % ±SE)	Emergence (mean % ± SE)
15 d	7	88	74.99 ± 3.20 a	68.25 ± 3.11 a
20 d	11	106	76.98 ± 9.26 a	67.18 ± 9.23 ab
25 d	6	69	77.76 ± 6.58 a	57.15 ±13.49 ab
50 d	21	226	47.75 ± 8.15 b	41.89 ± 8.05 bc
60 d	23	208	37.27 ± 7.49 b	28.69 ± 6.61 c
65 d	13	126	44.36 ± 8.69 b	22.58 ± 7.11 c
80 d	31	253	11.79 ± 4.68 c	7.31 ± 3.84 d
95 d	17	193	4.25 ± 2.40 c	1.90 ± 0.89 d
140 d	9	96	2.02 ± 1.38 c	2.02 ± 1.38 d
			$F = 14.934$ $df = 8{,}137$ $P < 0.001$	$F = 13.661$ $df = 8{,}137$ $P < 0.001$

Means within a column followed by different letters were significantly different at the significant level of 0.05 (SAS Proc GLM with LSD). Data were square-root transformed before analysis.

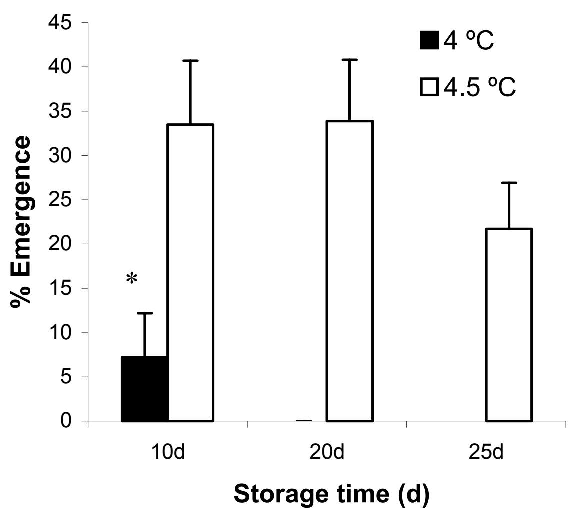

Figure 3. Percentage emergence of *G. ashmeadi* from GWSS eggs stored at constant temperatures for 10-25 d. Bar marked by an asterisk represents a significant difference (P < 0.05).

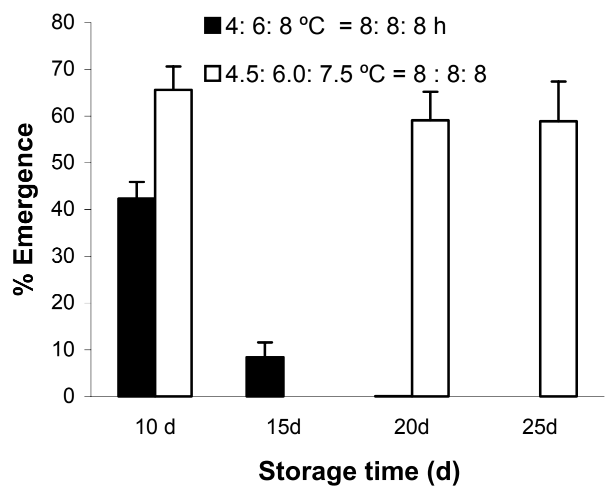

Figure 4. Percentage emergence of *G. ashmeadi* from the GWSS eggs stored at stepwise temperatures for 10-25 d. Bar marked by an asterisk represents a significant difference (P < 0.05).