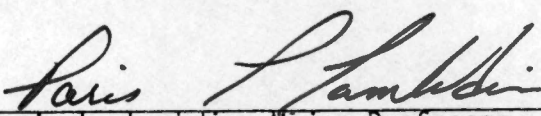
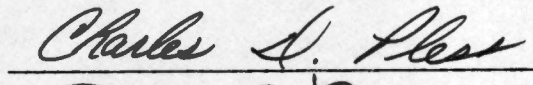
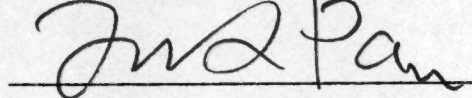


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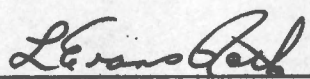
I am submitting herewith a thesis written by Ione Irene Maddox entitled "Cold Storage of Podisus maculiventris (Say): Effect on Survival, Longevity, Fecundity and Egg Viability." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.


Paris L. Lambdin, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


Vice Chancellor
Graduate Studies and Research

COLD STORAGE OF PODISUS MACULIVENTRIS (SAY): EFFECT ON
SURVIVAL, LONGEVITY, FECUNDITY AND EGG VIABILITY

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Ione Irene Maddox

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ABSTRACT

Adults of Podisus maculiventris (Say) were stored at 5, 10, 15 and 27°C from one to eight weeks, and three-day-old eggs were stored for seven days. The number of females surviving cold storage was significantly higher than the number of males, although both were lower than survival at 27°C. No males or females survived storage after six or seven weeks, respectively. For each week, mean longevity for males and females was significantly lower for those individuals removed from cold storage when compared with the control. The mean number of eggs laid by females exposed to cold storage and the resulting eclosion and number of second instars was significantly lower than that of the control. Percent eclosion and the percent reaching second instar were comparable to the control.

Eclosion was significantly lower for those eggs placed in cold storage when compared with the control. Eggs placed in cold storage hatched three to five days after removal from storage compared with the control in which eclosion occurred four to six days after oviposition.

Cold storage could be used in order to stockpile adults and eggs to facilitate laboratory rearing and maintenance, or in field releases to augment or establish a population of P. maculiventris. Larger numbers of cold-stored adults would simply be needed than if non-stored individuals were used.

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CHAPTER I

INTRODUCTION

The spined soldier bug, Podisus maculiventris (Say), is an effective predator of many lepidopteran and coleopteran larvae (Mukerji and LeRoux, 1965). Its range includes all of the continental United States, southeastern Canada and northern Mexico.

Host ranges and nymphal dispersal are known for the species (Waddill and Shepard, 1974; McPherson, 1980b). P. maculiventris has been shown to be effective in reducing field populations of such pests as the Mexican bean beetle, soybean looper and cabbage looper (Waddill and Shepard, 1974; Ignoffo et al., 1978; Marston et al., 1978). This predator currently is used in biocontrol or integrated pest management programs on such crops as soybeans because of its wide host preference and the relative ease with which it can be reared in the laboratory.

Laboratory studies on biology, life history, food preference and prey consumption rates of the species have been conducted (Mukerji and LeRoux, 1965; Mukerji and LeRoux, 1969a,b,c; Warren and Wallis, 1971; Marston et al., 1978; Richman and Whitcomb, 1978). Bullock (1955) described the development of the egg from oviposition to hatch. However, no work has been conducted on a method to store the adults or their eggs for field releases.

Cold storage has been utilized as a means to stockpile adults and eggs of several insect species for future use in the laboratory or in field releases (Stewart and Walton, 1965; Cline, 1970; Gordon,

1974; Archer et al., 1976; Hamalainen, 1977; Branson, 1978). The incorporation of cold storage techniques in a mass rearing program would facilitate the development of more effective integrated pest management programs by increasing the number of P. maculiventris available for release without a proportionate increase of laboratory maintenance.

This study was undertaken to determine if it was possible to maintain P. maculiventris adults and eggs at low temperatures over extended periods of time and to ascertain the effects of cold storage on adult longevity, fecundity and egg viability.



CHAPTER II

LITERATURE REVIEW

Prior to 1899, Podisus maculiventris (Say) was known as P. spinosis Dallas. At that time, a paper written by Thomas Say in 1835 was discovered which contained a prior description of the insect (Esselbaugh, 1948).

Early field and laboratory investigations on this species consisted of egg, immature and adult descriptions and type of prey attacked (Kirkland, 1897; Morrill, 1906; Prebble, 1933). Whitmarsh (1916) published notes on a partial life history of the insect.

Essig (1926) stated that the insect's range included all of the western states. The distribution range of the species was updated by McPherson (1980a) to include the northeast from Minnesota, south to Missouri and east to Virginia. Mukerji and LeRoux (1965) listed 53 prey records, compiled from 1906 to 1964, consisting of 7 orders and 33 families. Later, McPherson (1980b) expanded the list to 96 prey representing 8 orders and 41 families.

Mukerji and LeRoux (1969a,b,c) investigated food consumption, prey size and energetics of the species under laboratory conditions. They found both nymphs and adults survived and matured on small prey, but needed larger prey to accelerate development, increase body size and maintain reproduction. The predator killed higher numbers of smaller lepidopterous larvae than larger ones, possibly because of the greater defense and survival techniques of the larger larvae.

Marston et al. (1978) suggested that the predator's efficiency was affected by its prey preference based on the prey species which reacted most vigorously to attack suffered the least mortality. Conversely, those which offered the least resistance were killed most frequently.

Various temperatures and photoperiods were evaluated in the laboratory for rearing P. maculiventris. Richman and Whitcomb (1978) found the average duration from egg to adult was 27 (24-29) days at fluctuating temperatures of 18-30°C with a 14:10 hour photoperiod. Warren and Wallis (1971) reported no development of first instar nymphs at 10°C, and the average duration from egg to adult was 35.6, 26.2 and 22.8 days at 21.0, 26.8 and 32.0°C respectively, when maintained at a 12:12 hour photoperiod.

Cold storage of adults. Studies on cold storage of insects and their eggs have dealt with one of two main objectives. The first was to obtain insect mortality with the use of cold temperatures, usually below freezing, in combination with time (Salt, 1950). The second objective was to maintain insects at low temperatures over an extended time period with low mortality to aid in mass production and release programs (Tsiropoulos, 1972).

Early experiments with stored grain and dried fruit products utilized cold temperatures to obtain insect mortality of the Indian meal moth, Plodia interpunctella (Hubner); driedfruit beetle, Carpophilus hemipterus (L.); sawtoothed grain beetle, Orzaephilus surinamensis (L.); cadelle, Tenebroides mauritanicus (L.); and the

driedfruit mite, Carpoglypus lactis (L.) (De Ong, 1921). Constant exposure at 2.3°C for three months was fatal to both adults and immatures. This procedure gave growers "insurance" against product loss by insects by placing the product in refrigerated railroad cars over the summer months.

Another aspect of cold storage research involved transport of live insects on aircraft. Knipling and Sullivan (1957) evaluated the survival of seven species which were exposed at temperatures from -30 to 0°C for periods up to 60 minutes. They found the Japanese beetle, Popillia japonica Newman, had 32 to 90% mortality at -5 to -30°C respectively.

Salt (1950) theorized that if a species was adequately protected by its hibernacula and had a high degree of coldhardiness, mortality from freezing would be low. In places where an insect had been expanding its range, one severe cold spell could eradicate it from the area. This was not the case with the alfalfa weevil, Hypera postica (Gyllenhal). The weevil was first recorded from Utah in 1904. By 1949, the weevil was established in southern Montana and was discovered four years later in the northeastern United States and southern Alberta, Canada (Peterson, 1960). Researchers believed that the weevil was becoming adapted to lower temperatures. Peterson concluded that establishment of the species in colder temperatures was due to natural selection of those insects which possessed slower responses to rising temperatures, not through development of increased cold resistance.

Salt (1953) found that the presence of food in the digestive tract reduced the coldhardiness of an insect. Food in the alimentary tract was considered foreign to the insect tissue and froze at a higher temperature than did surrounding tissues. He found insects that had been starved for a short time before cold exposure had a lower mortality than did insects which had been allowed to feed before cold exposure.

Cold storage has been utilized as an effective tool for reducing colony maintenance and insuring the availability of a ready supply of experimental insects (Byrne, 1965; Tsiropoulos, 1972; Gordon, 1974). Gordon (1974) reported long-term storage of adults was effective for insects which overwinter as adults. He found that 200 adult large milkweed bugs could be maintained in a cage at 14°C for five months when provided milkweed or sunflower seed and a sucrose/yeast solution. Byrne (1965) stored 1000 alfalfa weevils at 0°C in a small ice cream carton and found survival was higher for those provided with a 2% sucrose solution than for those provided with fresh alfalfa shoots.

Cold storage has been used to accumulate and maintain large numbers of predators and parasitoids for later use (Archer et al., 1976; Hamalainen, 1977). From field collections, Coccinella septempunctata (L.) or Adalia bipunctata (L.) were placed in plastic cages, 50 individuals per container, and maintained in total darkness at 6°C and 70-90% R.H. (Hamalainen, 1977). After one month, mortality for C. septempunctata was 55%, and none of the specimens survived beyond three months of storage.

Parasitoids stored in prepupae and pupae stages in the parasitized aphid "mummies" were found less susceptible to the effects of cold storage than the adults (Archer and Eikenbary, 1973). Three and six-day-old Lysiphlebus testaceipes (Cresson) were stored at 1.7 to 10.0°C at 70-80% R.R. for 15 to 90 days. Overall survival was higher in the three-day-old prepupae than the six-day-old-pupae. Optimum storage temperatures were 4.4 and 7.2°C. In storage, no emergence of adults occurred at 1.7 or 4.4°C (Archer et al., 1973). Another parasitoid, Aphelinus asychis (Walker), was stored in the same manner as described above. Time in storage was extended when adults were provided with honey (Archer et al., 1976). Optimum storage temperature was 4.4°C, where there was 5% survival after 120 days of storage. Females survived longer storage and lived longer than males by a ratio of 1.5:1.0.

Gordon (1974) suggested that cold storage artificially selects a different strain than the normal laboratory strain. He stated that the strain exposed to cold might be more like the wild strains which annually undergo selection for overwintering resistance. Exposure to cold temperatures has been used to artificially select cold-tolerant biotypes of the predator, Cryptolaemus montrouzieri Mulsant, originally imported from Australia for control of the citrus mealybug in California (Bartlett, 1974).

Cold storage of eggs. Various methods of egg storage have been utilized in the laboratory to supplement current production and facilitate regulation of rearing schedules. The most common method of stockpiling eggs for future use has been by cold storage.

Eggs most capable of withstanding extended periods of cold storage were reported from insect species which overwinter in the egg stage (George and Ortman, 1965; Patel and Apple, 1967; Gross et al., 1972; Goonewardene and Townsend, 1974; Branson, 1978). For insects which did not overwinter in the egg stage, shorter exposures to sub-optimum temperatures have extended the duration of the egg stage in the laboratory (Stewart and Walton, 1965; Cothran and Gyrisco, 1966; Kishaba and Henneberry, 1966; Tsiropoulos, 1972; Gordon, 1974; Press et al., 1976; Hamalainen, 1977).

Effects of cold storage on egg mortality of stored product pests was studied as an alternative means of disinfecting food. Adler (1960) subjected eggs of the Angournois grain moth, Sitotroga cerealella (Olivier), Indian meal moth, P. interpunctella (Hubner) and confused flour beetle, Tribolium confusum Duval to temperatures of -17.0 and 3.8°C for 1 to 8 hour periods. No eggs hatched after exposure at -17.0°C for one hour, and 5 to 8 hours of exposure were needed to obtain 100% mortality at 3.8°C. Watters (1966) exposed T. confusum eggs of different ages to 5 or 10°C for 1 to 15 days. Eggs were then removed and maintained at 22 or 28°C until hatch. He reported eggs 1 to 4 hours old were more susceptible to injury than eggs 1 to 4 days old, and larval mortality was highest in eggs which had been stored for 10 or 15 days.

Cline (1970) incubated Indian meal moth eggs at 27°C and 60% R.H. for 0.5 to 71.0 hours, then placed them into storage at 2.4°C and 85% R.H. for 1 to 192 hours. Eggs less than 4.5 hours old prior to exposure were most susceptible. Average hatch was 1% after 90 hours

of exposure. Eggs older than 17.5 hours had a 23% hatch after 144 hours of exposure. Cline (1970) hypothesized that the differences between his findings and those of Adler (1960) were because of such factors as relative humidity, age of eggs, laboratory strain differences, diet and population density of adults.

Mullen and Arbogast (1979) tested time-temperature-mortality relationships for eggs of five insect species that infest stored food. Eggs of the sawtoothed grain beetle, O. surinamensis (L.), red flour beetle, T. castaneum (Herbst) and the almond moth, Ephestia cautella (Walker), required exposures of 7, 8 and 9 hours respectively at -10°C to obtain 95% mortality. Eggs of the cowpea weevil, Callosobruchus maculatus (F.), and cigarette beetle, Lasioderma serricorne (F.), required exposures of 28 to 62 hours respectively at -10°C to obtain 95% mortality.

Cold storage studies have served as a means to study the effects of low winter temperatures in relation to insect biology. Soil samples containing eggs of the northern corn rootworm, Diabrotica longicornis (Say), were subjected to temperatures ranging from -23.0 to 3.5°C for periods of 2-10 weeks. There was no hatch at -10 and -23°C after two weeks of storage and reduced hatch at 3.5 and -2.0°C (Patel and Apple, 1967). Similar tests conducted by Morrison and Pass (1974) and Crain and Armbrust (1978) verified Patel and Apple's conclusion that alfalfa weevil eggs became less susceptible to cold temperatures as the egg developed.

Alfalfa weevil eggs were found to be capable of storage for extended periods of time. Cothran and Gryisco (1966) stored eggs at

4°C for 245 days to observe time-percent viability relationships. Egg hatch was greater than 50% after 147 days of storage, but only 24% of the larvae were able to initiate feeding. After 105 days in storage, there was a marked decrease in larval viability even though there was a 70% hatch.

Cold storage provided a means to explain the northward movement of the European pine sawfly, Neodiprion sertifer (Geoffroy), into northern Ontario. Sullivan (1965) theorized that natural selection occurred favoring those eggs which were supercooled (cooling below 0°C without ice crystal formation) at increasingly lower temperatures.

The use of cold storage to extend the egg stage has allowed for greater flexibility in rearing and mass release programs. Gross et al. (1972) found that 22-28 day old eggs of whitefringed beetles, Graphognathus spp., stored at 18.3°C and 95% R.H. resulted in a 63.2% hatch after eight months. Younger eggs, 1-14 days old, were most susceptible, and complete mortality occurred after the third month in storage.

Eggs of the Japanese beetle, P. japonica, did not hatch at temperatures below 15°C, and the rate of egg development increased in proportion to temperature (Ludwig, 1928). Goonewardene and Townshend (1974) stored 1-7 day old eggs for 10 to 30 days at 1.5 to 7.0°C. Eggs removed from storage and allowed to incubate at 26.5°C generally hatched in 10-16 days, regardless of age, duration and temperature of storage. The period between egg collection and hatch was extended from 13 to as many as 27 days, a 100% increase.

Hamalainen and Markkula (1977) stored eggs of the coccinellids C. septempunctata and A. bipunctata at 3.0 to 10.0°C for periods of 1 to 3 weeks. C. septempunctata eggs were able to withstand storage for 1 week and A. bipunctata eggs for 2 weeks at 10°C without significant decrease in mortality for use in greenhouse releases.

Stewart and Walton (1965) exposed eggs of different ages of the southwestern corn borer, Diatraea grandiosella (Dyar) to temperatures of 7 to 13°C for 4 to 11 days. Eggs 3 to 10 hours old did not hatch at 7°C, but a 12% hatch was obtained at 13°C. Eggs in the "blackhead" stage (99-106 hours old) were cooled from 1 to 3 days and resulted in a 94 to 96% hatch. Longer periods of storage resulted in eggs hatching while in storage. Eggs of the cabbage looper, Trichoplusia ni (Hubner) exposed to temperatures of 5 and 12°C for 7 to 21 days had no decrease in viability after 7 days at 5°C, but 100% mortality had occurred after 21 days (Kishaba and Henneberry, 1966). At 12°C, eggs hatched during the last 3 of 4 days of storage. Egg viability was decreased after 14 and 21 days of storage.

Gordon (1974) and Press et al. (1976) found hemipteran eggs to be susceptible to cold exposure, with nymphal survival to the second instar severely decreased. Eggs of the large milkweed bug stored at 14°C for more than 2 weeks had reduced viability. Nymphs from these eggs did not develop as quickly as eggs which had been maintained at laboratory temperature (Gordon, 1974). All eggs of the predaceous bug, Xylocoris flavipes (Reuter), stored at 5°C for 4 days or at 10 or 15°C for 16 days did not hatch. Nymphs from these cold

stored eggs did not survive as long as those from eggs maintained in the laboratory (Press et al., 1976).

CHAPTER III

MATERIALS AND METHODS

All experiments were conducted using specimens taken from a stock colony that had been maintained in the laboratory at $27\pm4^{\circ}\text{C}$, and a 16 hour photophase. A 1.2 m fluorescent light fixture fitted with two Sylvania SupersaverTM 35 watt tubes was placed 45 cm above each rearing area and wired to a ParagonTM automatic timer set for the desired photoperiod.

The insects were reared in inverted 100 X 15 mm plastic petri dishes. The bottom of each dish was lined with a 9.0 cm disc of filter paper. A 12 X 35 mm shell vial of distilled, sterilized water with a 10 mm cotton plug was provided for each dish. Larvae of the greater wax moth, Galleria mellonella (L.), were reared in the laboratory on Bio-ServTM wax moth diet to serve as a food source.

General rearing procedures. After oviposition in the rearing dish, each female was removed and placed in another petri dish. Female designation, date of oviposition and hatch, number hatched and number successfully reaching the second instar were recorded for each egg mass. First instars were provided only water, as they are not predaceous (Warren and Wallis, 1971). Immediately upon reaching the second instar, the nymphs were offered third-stage wax moth larvae to ascertain if they were capable of initiating and maintaining feeding activities. Nymphs from the same egg mass, ranging in number from 2 to 46, could be maintained together until the third instar with

minimal loss to cannibalism, if provided with an ample food supply. After reaching the third instar, the nymphs were placed in individual dishes. They were maintained separately through the remaining fourth and fifth instars, and as adults. Wax moth larvae were offered immediately upon completion of each moult and twice weekly thereafter.

Adults were first paired 2 to 6 days after ecdysis for approximately 12 hours. Females were paired approximately once a month to maintain fertile egg production (Warren and Wallis, 1971).

Cold storage of adults. *P. maculiventris* were reared as described previously, but separated after adult emergence into groups of 40 males or 40 females. These adults were fed immediately with wax moth larvae after moult, this being the only feeding before placement into storage 4 to 6 days later. In preliminary tests, there was a marked decrease in survival for those insects which had been fed within 1 to 3 days prior to placement in cold storage and for specimens where humidity levels were inadequate. After feeding, they were acclimated at 12:12 hour photoperiod away from the rearing lights. To retard movement and facilitate placement in storage containers, the specimens were initially chilled for 20 to 30 minutes at 18.5°C. Groups of 40 insects were placed in half-liter ice cream cartons that had been covered with foil and lined with rolled paper toweling. The toweling was thoroughly moistened with sterilized, distilled water prior to placement in storage and moistened every third day thereafter. No food was placed in storage with the adults. Two containers of adults, one with males and one with females, were placed into incubators at each of three temperatures. The temperatures were 5, 10 and 15°C.

A group of 40 males and 40 females 4 to 6 days old was maintained at 27°C to serve as a control. This group was fed wax moth larvae two times weekly. The experiment consisted of four replications over time.

Every 7 days for a period of 8 weeks, a sample of 5 individuals was taken from each carton and the control group. After removal from storage, adults were immediately placed into individual dishes to avoid cannibalism. Approximately 1 hour later wax moth larvae and vials of water were provided for those adults that were alive. After 3 days, surviving adults which had been stored at the same temperature were paired. Because females survived storage significantly longer than males, some males had to be used twice. Mating was observed and recorded for each pair over a period of 10 to 14 hours. The pairs were disturbed by tapping the dish and then placed in individual dishes and maintained as described previously in the general rearing procedures. Surviving females were remated approximately 30 days later. Data were recorded for longevity of each male and female after storage. Data were also recorded for the number of eggs laid, number hatched and number of nymphs successfully reaching second instar. Upon oviposition, the eggs were handled as described previously in the general rearing procedures, except that data were recorded on nymphs only through the first successful feeding of the second instar. None of the progeny were used in any future cold storage experiments.

Cold storage of eggs. Because of the female's ovipositional behavior to lay her eggs on a vertical surface, it was decided that the placement of a substrate inside the vertical edge of the petri dish

would facilitate egg collection and manipulation. To obtain eggs for this experiment, each gravid female was placed in a petri dish provided with a hard plastic strip, 1 X 28 cm, fitted around the periphery of the dish. Date and number of eggs were recorded on each strip having 20 or more eggs. Only strips containing at least 20 eggs were used in this experiment.

In preliminary studies, 1 to 3-day-old eggs did not hatch after 7 days in storage. Therefore, eggs were held in the laboratory at 27°C for 3 days before placement in cold at 5, 10 or 15°C. After the 3 day incubation period, fertile eggs had turned dark charcoal gray as opposed to infertile eggs which were light green. Ten strips containing at least 20 fertile eggs each were then placed into an opaque plastic container at each temperature. The bottom of each container was lined with moistened filter paper to maintain humidity. The containers were then sealed with a thin strip of ParafilmTM. Eggs were stored at 5, 10 or 15°C for 7 days, then returned to 27°C to observe hatch. Ten egg-strips were maintained at 27°C throughout the storage period until hatch as a control. The experiment consisted of four replications over time.

As with the eggs from the stored females, data were taken to the first successful feedings of the second instar nymphs. None of these insects were used in future cold storage experiments.

Analysis of data. Mean survival of adults and eggs during storage, at each week and temperature, was analyzed by the Freeman-Tukey transformations for the binomial and Poisson distributions (Mosteller and Youtz, 1961). An analysis of variance and Duncan's

multiple range test at the 5% level were conducted on the transformed means.

Mean longevity of adults at each week and temperature was transformed before analysis by square root transformations. Mean number of eggs laid, number of eggs hatched and number of nymphs reaching second instar were transformed before analysis by natural logarithm transformation. An analysis of variance and Duncan's multiple range test at the 5% level were conducted on the transformed means.

CHAPTER IV

RESULTS AND DISCUSSION

Cold storage of adults. The number of males surviving storage was significantly higher for those stored through 4 weeks at 5°C and 3 weeks at 10°C compared with males kept in cold storage for longer periods (Table 1). At 15°C, there was no significant difference in male survival from 1 to 3 weeks; however, there were no significant differences between those stored for 4 weeks and those stored 2, 3, or 5 weeks. No males survived after 6 weeks of storage except those of the control maintained at 27°C.

Female survival was significantly higher for those stored through 5 weeks at 5°C compared with females kept in storage for longer periods. At 10°C, there was no significant difference in the number that survived from 1 to 4 weeks, and there was no significant difference between weeks 4 and 5. At 15°C, female survival in the first and second weeks was not significantly different than week 4, while week 4 did not differ significantly from week 5. Week 3 was not significantly different than weeks 1 and 2. No females survived after 7 weeks of cold storage.

At each week, mean longevity for males and females was significantly lower for those individuals removed from cold storage when compared to the control (Table 2). There was a decrease in longevity as the exposure time increased. No significant differences in male and female longevity were noted within each week and storage

Table 1. Mean Survival of Podisus maculiventris (Say) Adults in Storage from 1 to 8 Weeks at Various Temperatures^{ab}

Weeks	Temperature							
	5°C		10°C		15°C		27°C	
	Male	Female	Male	Female	Male	Female	Male	Female
1	4.5 abc	5.0 a	4.0 abcde	5.0 a	4.5 abc	4.5 abc	5.0 a	5.0 a
2	4.5 abc	4.8 ab	5.0 a	4.8 ab	3.8 bcdef	4.8 ab	5.0 a	5.0 a
3	4.3 abcd	5.0 a	4.0 abcde	5.0 a	3.8 bcdef	5.0 a	4.8 ab	4.8 ab
4	4.5 abc	4.5 abc	2.8 fghij	4.0 abcde	3.3 defgh	3.8 bcdef	4.0 abcde	4.8 ab
5	3.0 efghi	4.3 abcd	1.8 jk	3.0 efghi	2.3 hijk	3.0 efghi	4.0 abcde	4.0 abcde
6	1.3 k	2.5 ghij	0.3 l	2.5 ghij	2.0 ijk	2.3 hijk	4.0 abcde	4.0 abcde
7	0.0 l	0.5 l	0.0 l	0.3 l	0.0 l	0.3 l	3.5 cdefg	3.5 cdefg
8	0.0 l	0.0 l	0.0 l	0.0 l	0.0 l	0.0 l	3.0 efghi	3.0 efghi

^aFour replications of 5 females and 5 males at each temperature.

^bAny two means not followed by the same letter are significantly different at $P \leq 0.05$ on transformed data, Duncan's Multiple Range Test.

Table 2. Mean Longevity of Podisus maculiventris (Say) Males and Females after 1 to 7 Weeks of Exposure at Various Temperatures^a

Weeks	male $\bar{x}$ longevity in days				female $\bar{x}$ longevity in days			
	5°C	10°C	15°C	27°C	5°C	10°C	15°C	27°C
1	47.3 de	45.1 def	45.8 de	77.9 a	41.1 gh	47.0 ef	48.3 ef	77.6 a
2	49.8 cd	44.3 def	46.4 de	66.4 b	43.3 fg	50.3 e	47.3 ef	68.4 b
3	39.8 efg	38.3 fg	35.9 g	63.2 b	38.9 gh	43.2 fg	42.1 fg	60.6 bc
4	26.9 h	29.8 h	25.5 h	54.6 c	37.6 ghi	32.6 i	34.5 hi	57.7 cd
5	12.3 i	13.6 l	12.6 i	44.7 def	21.7 jk	24.2 j	22.8 jk	51.3 de
6	8.4 ij	2.0 j	9.5 j	38.9 fg	14.9 lm	18.1 kl	15.6 lm	32.3 i
7	---	---	---	13.1 i	9.0 mn	5.0 n	7.0 mn	26.1 j

^aAny two means within each sex followed by the same letter are significant different at $P \leq 0.05$ on transformed data, Duncan's Multiple Range Test. Untransformed means given.

temperature, except for reduced female longevity at 5°C after 1 week in storage, and between 5°C and 10°C after 2 weeks in storage. Male and female longevity dropped dramatically after 4 weeks in storage and was less than half that of individuals maintained at 27°C for weeks 5, 6 and 7. The decline in female longevity was less severe than that for males.

The mean number of eggs laid by females exposed to cold storage was significantly lower than for those of the control except for females stored at 5 and 10°C for 6 weeks (Table 3). A decline in the number of eggs laid occurred for females at all temperatures over the 7 week storage period except for weeks 3 and 4 at 5°C and weeks 2 and 3 at 27°C. These data support the conclusions of Mukerji and LeRoux (1969b) who indicated that peak oviposition period occurred during the first 4 to 5 weeks after adulthood followed by a decline until death.

Because fewer eggs were laid by females exposed to cold storage, eclosion was significantly lower compared with the control (Table 4). However, no significant differences were noted between treatment temperatures in the number of nymphs hatched from eggs laid by cold stored females. Cold storage did not appear to have an adverse effect on egg viability as 89% (69-95%) successfully hatched compared to 93% (92-95%) for the control.

Because of the low egg numbers laid by females exposed to cold storage, the number of nymphs that successfully developed to second instar was significantly lower compared to the control (Table 5). However, no significant differences were noted between cold treatment temperatures except for the low number of nymphs obtained at 5°C after

Table 3. Number of Eggs Laid by Podisus maculiventris (Say) Females after Exposure to Various Temperatures from 1 to 7 Weeks^a

Weeks	$\bar{x}$ no. eggs laid/female			
	Temperature			
	5°C	10°C	15°C	27°C
1	104.2 fghi	119.3 cde	117.7 def	307.7 a
2	98.5 fghi	101.8 efgh	104.7 efg	246.7 b
3	84.9 ghijk	95.3 ghijk	86.6 ghijk	249.3 ab
4	84.5 ghijk	76.5 hijklm	78.1 hijkl	192.0 c
5	68.0 ijklm	66.1 klmn	57.4 mn	162.3 cd
6	55.4 lmn	63.0 jklmn	37.7 o	68.9 ijklm
7	23.5 p	14.0 p	13.0 p	47.1 n

^aAny two means not followed by the same letter are significantly different at $P \leq 0.05$ on transformed data, Duncan's Multiple Range Test, untransformed means given.

Table 4. Mean Number of First Instars that Hatched from Eggs Laid by Females Exposed to Various Temperatures from 1 to 7 Weeks^a

Weeks	$\bar{x}$ no. hatched eggs/female			
	Temperature			
	5°C	10°C	15°C	27°C
1	83.6 ghij	103.9 e	100.2 ef	296.0 a
2	88.1 efghi	90.4 efgh	96.4 efg	230.1 b
3	78.0 fghi	87.5 efghi	78.1 fghij	229.6 b
4	78.0 efghi	71.7 hijk	74.1 ghijk	149.9 d
5	64.9 hijk	61.8 ijkl	53.5 kl	149.9 d
6	51.5 jkl	60.0 hijkl	34.2 l	63.9 hijk
7	21.0 mn	11.0 mn	9.0 n	43.4 l

^aAny two means not followed by the same letter are significantly different at $P \leq 0.05$ on transformed data, Duncan's Multiple Range Test, untransformed data given.

Table 5. Second Instars That Developed from Eggs Laid by *Podisus maculiventris* (Say) Females after 1 to 7 Weeks of Exposure at Various Temperatures^a

Weeks	$\bar{x}$ no. second instars/female			
	Temperature			
	5°C	10°C	15°C	27°C
1	74.7 fghi	92.8 d	93.1 d	231.3 a
2	81.1 defg	83.9 def	91.1 de	215.2 b
3	73.6 defgh	81.8 defg	74.3 defgh	212.5 b
4	71.1 defgh	67.8 fghi	69.9 fghi	170.5 bc
5	61.5 fghi	55.6 ghij	49.7 ij	140.4 c
6	48.4 hij	57.4 fghij	32.1 k	59.9 fghi
7	18.0 kl	10.0 kl	8.0 l	40.4 j

^aAny two means not followed by the same letter are significantly different at $P \leq 0.05$ on transformed data, Duncan's Multiple Range Test, untransformed means given.

1 week and 15°C after 6 weeks. Cold storage did not appear to have an adverse effect on the nymphs that successfully developed to second instar as 82% (62-90%) developed to second instar compared to 85% (75-89%) for the control.

Cold storage of eggs. Eclosion was significantly lower for those eggs placed in cold storage compared with the control (Table 6). There were no significant differences among the cold storage treatments. Eggs placed in cold storage hatched 3 to 5 days after removal from storage compared with the control in which eclosion occurred 4 to 6 days after oviposition.

Because the use of cold storage lengthens the incubation period, it would be possible to significantly increase the number of eggs available at one time by placing them into cold storage as they become available (after a 3-day incubation period) and removing them simultaneously. Eggs laid on day 1 could be removed from cold storage at the same time as those eggs laid on days 2 through 7. The number of eggs available at any one time after the tenth day would be seven times greater using cold storage than would be available using current laboratory methods.

In preliminary studies, it was determined that 1 to 3 day old eggs would not hatch after 7 days in cold storage. Bullock (1955) found that development of the P. maculiventris egg occurred in nearly two equal periods, each of approximately 60 hour duration. The embryo revolves to the ventral surface of the yolk mass in the second stage of development. This development to the second stage before storage

Table 6. Eclosion and Subsequent Development to Second Instar of *Podisus maculiventris* (Say) Eggs after Exposure for 7 Days at Various Temperatures^a

Temp ^o C	first instar x no.	second instar x no.
5	17.9 a	16.2 a
10	17.8 a	16.6 a
15	17.6 a	16.0 a
27	19.0 b	18.6 b

^aNumber determined from 40 samples of 20 eggs/temperature.

^bMeans not followed by the same letter are significantly different at $P \leq 0.05$ on transformed data, Duncan's Multiple Range Test. Untransformed means given.

appears to be a crucial factor for eclosion, because eggs had to incubate for greater than 60 hours before placement in storage to obtain successful hatch.

Number of first instars that successfully reached the second instar was significantly lower for those eggs placed in cold storage as compared with the control. There were no significant differences among the cold storage treatments. A 2 to 3 day duration from eclosion to second instar occurred for all storage temperatures. No morphological deformities were found in nymphs from stored eggs.

CHAPTER V

SUMMARY AND CONCLUSION

Adults and eggs of Podisus maculiventris (Say) were stored at 5, 10 and 15°C to determine the feasibility of cold storage over various periods of time, compared with maintenance at the control temperature of 27°C. The number of females surviving cold storage was significantly higher than the number of males. There was no significant decrease in survival at 5°C for males stored 1 to 4 weeks and females stored 1 to 5 weeks.

For each week, mean longevity for males and females was significantly lower for those individuals removed from cold storage when compared with the control. No significant differences were noted in longevity within each week and temperature, except for the reduced female longevity after 1 week in storage at 5°C, and between 5 and 10°C after 2 weeks in storage.

The mean number of eggs laid by females exposed to cold storage and the resulting eclosion and number of second instars was significantly lower than that of the control. Percent eclosion and the percentage reaching second instar were comparable with that of the control.

Adults could be stockpiled for short durations of time for later use in the laboratory or in field releases. Removal of females from cold storage could be used to increase daily egg production when needed. Adults that had been stored for short periods of time could be utilized for augmentation or establishment of a field population despite the

reduced longevity and fecundity. Higher numbers of cold-stored adults would be needed than non-stored adults to compensate for the reduced fecundity.

There were significant differences in eclosion and resulting number of second instars from P. maculiventris eggs that had been stored for 7 days at 5, 10 and 15°C compared with the control temperature of 27°C. There were no significant differences among the cold storage temperatures. Three-day-old eggs placed in storage for 7 days hatched 3 to 5 days after removal from storage, compared to the control which hatched 4 to 6 days after oviposition.

By delaying hatch for up to 7 days, these techniques could facilitate stockpiling large numbers of eggs for use in the laboratory or field. Egg hatch could be synchronized by placing them in storage as they become available (after a 3-day incubation period) and removing them simultaneously. Hatch would occur 3 to 5 days after removal from storage. Eggs laid on plastic strips in the laboratory could be easily transported to the field for use in biocontrol or integrated pest management programs.

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