Selection of Cold Injury Treatments to Facilitate Release of the Parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) Reared on the Rice Weevil (Coleoptera: Curculionidae)

C. S. BURKS,[1] D. W. HAGSTRUM, AND J. E. BAKER

Grain Marketing and Production Research Center, USDA, 1515 College Avenue, Manhattan, KS 66502

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ABSTRACT The use of cold treatments to kill the rice weevil, *Sitophilus oryzae* (L.), before eclosion but allow the parasitoid *Anisopteromalus calandrae* (Howard) to complete development and eclosion was examined. Cold tolerance, crystallization temperature, and developmental stage of rice weevils were examined at 2-d intervals between 18 and 30 d after rice weevil oviposition. The median crystallization temperature dropped from −16 to −24°C over this time, and cold tolerance was lowest between day 20 and 24. Based on these data, rice weevil immatures were exposed on days 18, 19, 21, 22, 23, and 24 after oviposition for 2 h to various subzero temperatures. Hosts were exposed to parasitoids for oviposition on day 20. Before parasitization, rice weevils were either frozen internally by exposure to −25°C, or were subjected to nonfreezing chilling injury by exposure to −12°C. Rice weevils exposed to cold after parasitization were subjected to chilling injury by exposure to either −12 or −10°C. Freezing rice weevil larvae before parasitization resulted in poor production of parasitoids and a sex ratio favoring males. Exposure to −12°C for 2 h, 2 or 3 d after parasitization resulted in production of parasitoids similar to unchilled controls, and a slightly higher female-to-male ratio than the controls. The production of *A. calandrae* was better after exposure to −10°C, but at this temperature some rice weevils eclosed. The treatment of 2 h at −12°C, 3 d after parasitization gave the best parasitoid survival and ensured rice weevil mortality.

KEY WORDS *Anisopteromalus calandrae*, *Sitophilus oryzae*, biological control, cold injury

THE PARASITOID *Anisopteromalus calandrae* (Howard) is one of several wasps that parasitize insects feeding internally in stored grain and other seeds (Brower et al. 1996). It is an ectoparasitoid that oviposits on the host inside the grain and shows little specificity in host selection. Both larvae and pupae are parasitized, and the relative frequency of parasitization of these stages apparently depends on the host species and age and the grain or seed inhabited by the host (Chatterji 1955, Smith 1993, Ahmed 1996). Recent studies have shown that augmentative releases of parasitoids are a viable means of suppressing populations of grain pests (Brower et al. 1996, Flinn et al. 1996, Schöller et al. 1997), and that biological control and aeration to cool grain can be used synergistically in an integrated pest management (IPM) program (Flinn 1998, Reed and Harner 1998).

Cold injury of hosts has been used to improve parasitoid rearing programs for an egg parasite (Drooze and Weems 1982), and in rearing, release, and monitoring the release program's performance for pupal parasites (Pickens and Miller 1978, Klunker 1982, Petersen and Matthews 1984). For the egg parasitoid *Ooencyrtus ennomophagus*, host eggs exposed to −10°C supported parasitoid survival at later stages of egg development than fresh eggs (Drooze and Weems 1982). Also, eggs could be stored for long periods of time at −10°C, and this temperature was sufficient to ensure that no unparasitized hosts survived. The pupal parasites *Pachycrepoideus vindemiae* (Rondani) and *Muscidifurax zaraptor* Girault & Sanders parasitize living and dead pupae of *Musca domestica* L. and other muscids (Pickens and Miller 1978, Klunker 1982, Petersen and Matthews 1984). Fly pupae continue to be suitable for *M. zaraptor* development after prolonged storage at −20°C (Klunker 1982, Petersen and Matthews 1984), although their ability to support parasitoid development deteriorates more rapidly when the pupae are stored at −7°C (Klunker 1982).

These studies have not addressed the mechanism of host cold injury. Mechanisms can vary with the insect species, stage, and the temperature (Lee 1991). For instance, most insects have crystallization temperatures (i.e., supercooling points) distinctly below 0°C. Body fluids exist as supercooled liquid until an ice nucleation event occurs and their body fluids crystallize as ice. Although a few insects are able to tolerate internal freezing, most cannot and are killed by internal freezing. Many insects are killed by nonfreezing injury, referred to as chilling injury, at temperatures above their crystallization temperature. This distinc-

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[1] Current address: Horticultural Crops Research Laboratory, 2021 South Peach Avenue, Fresno, CA 93727.

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tion between freezing and chilling injury is potentially important because freezing injury generally causes greater mechanical damage to cells than chilling injury (Lee 1991). One might, therefore, predict that freezing injury will degrade host quality more than chilling injury.

The objective of the current study was to develop methods for using hosts killed by cold exposure to rear the parasitoid *A. calandrae* on the rice weevil *Sitophilus oryzae* L. Such methods could be used to release parasitoids into bulk grain storage as infested, parasitized, grain kernels. A strategy for using cold injury in parasitoid production was devised using the following 5 steps: (1) determination of host suitability at various ages; (2) determination of host crystallization temperatures and tolerance for chilling injury at various suitable ages; (3) determination of whether wasps successfully parasitized hosts killed by internal freezing; (4) determination of time-temperature exposure combinations providing acceptable host mortality at various suitable ages; and (5) selection of a cold treatment providing optimal parasitoid production with acceptable host mortality.

Materials and Methods

Insects. Rice weevils were from a colony that originated in Kansas and had been maintained in the laboratory for several years. *A. calandrae* were from a laboratory colony that originated in Savannah, GA. Rice weevils were reared at 27°C. Rice weevil adults (500 adults per 500 ml of wheat) were allowed to oviposit on hard red winter wheat *Triticum aestivum* L. with a moisture content of 13%, and they were sieved from grain after 48 h. To obtain infested kernels, the wheat was attached to a cellulose sheet and x-rayed using the materials and procedures described by Throne (1994), except that the exposure time was 3 min. The resulting X-ray was used as a template to pick infested kernels off the taped cellulose sheet. While taped to cellulose sheets or held in glass tubes, the wheat was held at 75% RH, maintained by saturated sodium chloride solution (Winston and Bates 1960). The X-ray procedure was performed 12–14 d from the beginning of the oviposition period. *A. calandrae* were reared on weevil-infested wheat at 27°C as described by Baker and Throne (1995). Adult females aspirated from the culture jars after 18 d were 2 d posteclosion. The females, presumably mated before being isolated, were given access to a food source (honey) 12–24 h before introduction to infested wheat kernels.

Rice Weevil Cold Tolerance. To measure relative cold tolerance of rice weevils, 20 infested wheat kernels were exposed to −9°C for 2 h on days 14, 18, 20, 22, 24, and 28 after oviposition. After exposure, the wheat kernels were kept overnight at 27°C and 75% RH to allow the rice weevils to recover before they were dissected out of the kernels. Rice weevils that moved when probed with the tip of a pair of forceps were considered alive. Crystallization temperature was determined by dissecting 15 rice weevil immatures from wheat kernels, placing them against a 30-gauge copper-constantan thermocouple (Omega Engineering, Stamford, CT) in a 0.5-ml microcentrifuge tube capped with foam rubber, and using the apparatus and methods previously described (Burks et al. 1997). For these experiments the cooling rate was −0.5°C/min. Frequency distribution of developmental stages was examined using rice weevils dissected for crystallization temperature determinations.

Preparasitization Cold Treatment. Effects of exposure of hosts to subzero temperatures before parasitization were examined by exposing groups of 5 infested kernels to individual *A. calandrae* females. Sixty infested kernels and 12 *A. calandrae* females were used for each treatment. Infested kernels (300) were isolated as described previously, and 240 kernels were exposed to −25 or −12°C for 2 h, 18 or 19 d after oviposition, whereas 60 kernels served as controls not exposed to cold. To expose the infested kernels to subzero temperatures, groups of 5 kernels were placed in 13 by 100-mm glass tubes. The 13 by 100-mm tubes were held in a cold bath using 25 by 150-mm glass tubes with beaded rims suspended in the bath through a stainless steel cover with 25-mm holes. A 36-gauge copper-constantan thermocouple was placed among 5 uninfested wheat kernels in a "dummy" 13 by 100-mm tube in a 25 by 150-mm tube to confirm that the infested wheat kernels were actually exposed to the test temperatures. After cold exposure, the tubes containing infested wheat kernels were held at 27°C and 75% RH until day 20 after oviposition. At that time, an *A. calandrae* female was placed in each of the tubes for 24 h, after which the parasitoid females were removed and the infested wheat kernels redistributed into individual tubes. The potentially parasitized wheat kernels were held at 27°C and 75% RH until 40 d after rice weevil oviposition. During this time, the tubes were monitored at 2-d intervals for emergence of weevils and parasitoids. After 40 d, all wheat kernels that showed no signs of host or parasitoid emergence were dissected to confirm initial infestation.

Postparasitization Cold Injury. To examine the effects of chilling rice weevil immatures after they had been parasitized, 540 infested wheat kernels were exposed to 108 parasitoid females for 24 h starting 20 d after rice weevil oviposition. This procedure was performed as described for the previous experiment, except that infested kernels were exposed to *A. calandrae* females before cold exposure. After 24 h the *A. calandrae* females were removed and the glass tubes containing the 5 kernels were held at 27°C and 75% RH. At 21, 22, 23, or 24 d after rice weevil oviposition, the tubes containing the wheat kernels were placed in glass holding tubes and exposed to −10 or −12°C for 2 h as described previously. A control group of 60 infested kernels, representing potential progeny from 12 *A. calandrae* females, was not exposed to cold. After cold treatment, the kernels were put in individual 13 by 100-mm tubes and monitored as described previously. Forty days after rice weevil oviposition, all kernels that showed no signs of host or parasitoid emergence were dissected to verify that the kernel had been infested and to ascertain the species, stage, and viability.

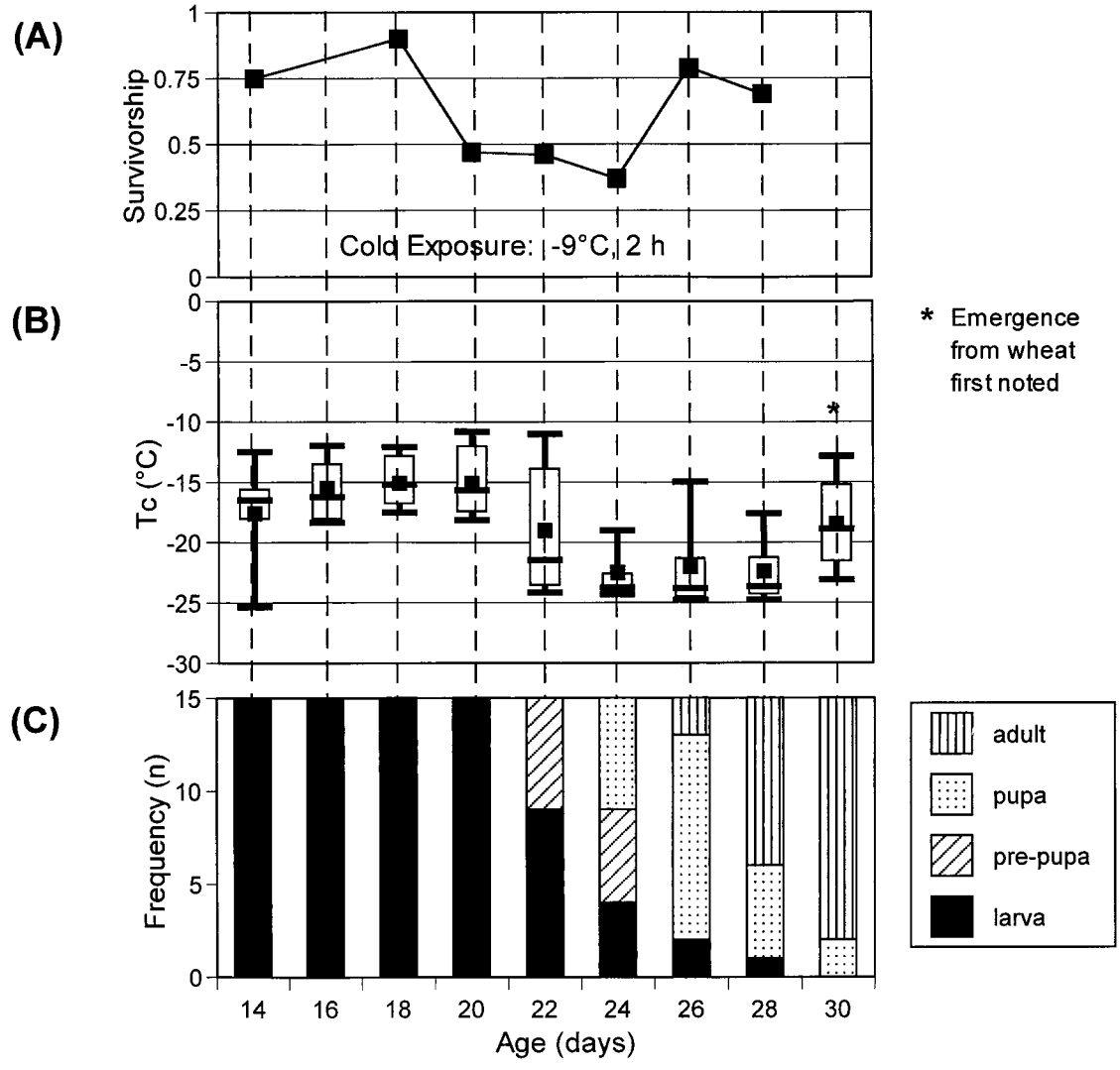

Fig. 1. Cold tolerance and developmental profile for the rice weevil. (A) Cold tolerance, as indicated by the proportion of weevils surviving 24 h after a 2-h cold exposure (n = 20). (B) Box plots showing the distribution of crystallization temperatures (n = 15). The whiskers represent the 10th and 90th percentile; the ends of the box represent the 25th and 75th percentile, the center cross bar is the median, and the square indicates the mean. (C) Stage frequencies for each age group (n = 15).

Results

Rice weevil survivorship after a 2-h exposure to -9°C was lowest between days 20 and 24 (Fig. 1A), coinciding with the late larval, prepupal, and early pupal stages (Fig. 1C). Crystallization temperatures were lowest between days 24 and 28 (Fig. 1B), coinciding with the prepupal, pupal, and teneral adult stages (Fig. 1C).

In the preparasitization cold treatments, there was a lower rate of weevil emergence compared with the control (27°C) (Fig. 2). No weevils or parasitoids emerged from 11% of the infested controls and from 50 to 90% of the infested cold-treated kernels. Infestation of these kernels was confirmed by dissection, but the insects inside were too deteriorated to identify the stage or species. There was a significant difference in survivorship between treatments when comparing all preparisitization treatments (Fisher exact test, P = 0.001). Of the 4 cold treatments in the preparasitization experiment, only 1 rice weevil emerged from the 2 h at −12°C treatment 18 d after rice-weevil oviposition. There was a significant difference in production between days on which the kernels were exposed and between −12 and the −25°C treatments (Fisher exact test, $P < 0.001$ for both comparisons), with more *A. calandrae* emerging on day 19 than on day 18. There

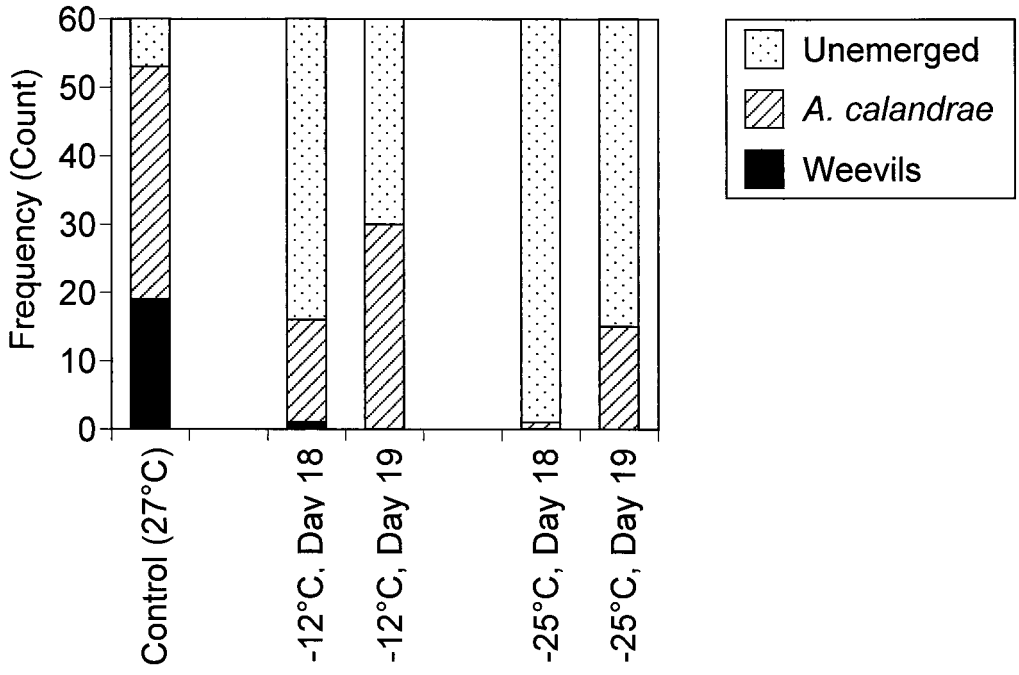

Fig. 2. Emergence of rice weevils and parasitoids from infested wheat kernels subjected to cold exposure and then parasitization.

was also a significant difference in production between temperatures for wasp emergence on day 19 (Fisher exact test, $P = 0.009$). Emergence of *A. calandrae* from the −12°C treatment was 88% of that from the unchilled control.

The ratio of *A. calandrae* progeny emerging from wheat kernels in the preparasitization experiment was 1.43 female-to-male for the controls, but was <1 for all of the cold treatments (Table 1). Sex ratios of *A. calandrae* progeny emerging from rice weevils exposed to −12°C were not significantly different from those of the controls (Fisher exact test, $P = 0.538$ on day 18 and 0.316 on day 19). The sex ratios of wasps emerging from the −25°C treatments were significantly different from that of the controls (Fisher exact test, $P = 0.09$ and 0.001, respectively, for kernels exposed on days 18 and 19).

There was generally a higher rate of *A. calandrae* emergence with the postparasitization cold exposure compared with the preparasitization, and a lower incidence of infested kernels from which no insect emerged within a 40-d period (Fig. 3). *A. calandrae* emerged from 51 of the 60 kernels not exposed to cold, and rice weevil adults successfully emerged from 9 kernels. For all cold treatments, there were some infested wheat kernels from which neither *A. calandrae* nor rice weevils successfully emerged. Some rice weevil adults successfully emerged from −10°C treatments, but not from −12°C treatments (Fig. 3). Of the 126 infested kernels from which no emergence was observed, 10 kernels contained *A. calandrae.* One parasitoid and 1 rice weevil were alive at the time of dissection; the remainder were dead. There was no clear difference between the stage distribution of rice weevils dissected from wheat kernels exposed to −12 and −10°C (unpublished data).

Of *A. calandrae* that emerged after exposure to cold, there was an apparent trend toward an increase in the

Table I. Female-to-male ratio (*n*) of *A. calandrae* that emerged or were dissected from wheat kernels exposed to cold before or after parasitization on day 20

Temp of 2-h cold exposure, °C	Day after rice weevil oviposition of 2-h cold exposure							
	Preparasitization cold treatments			Postparasitization cold treatments				
	Control[a]	18	19	Control[a]	21	22	23	24
27	1.43 (34)[b]	–	–	1.22 (51)	–	–	–	–
−10	–	–	–	–	1.54 (33)	1.64 (50)	2.00 (54)	2.47 (52)
−12	–	0.88 (15)	0.93 (30)	–	1.18 (24)	1.79 (39)	1.42 (46)	2.15 (41)
−25	–	0.20 (6)	0.00 (10)	–	–	–	–	–

[a] Controls are for separate cohorts.
[b] Females:males (*n*).

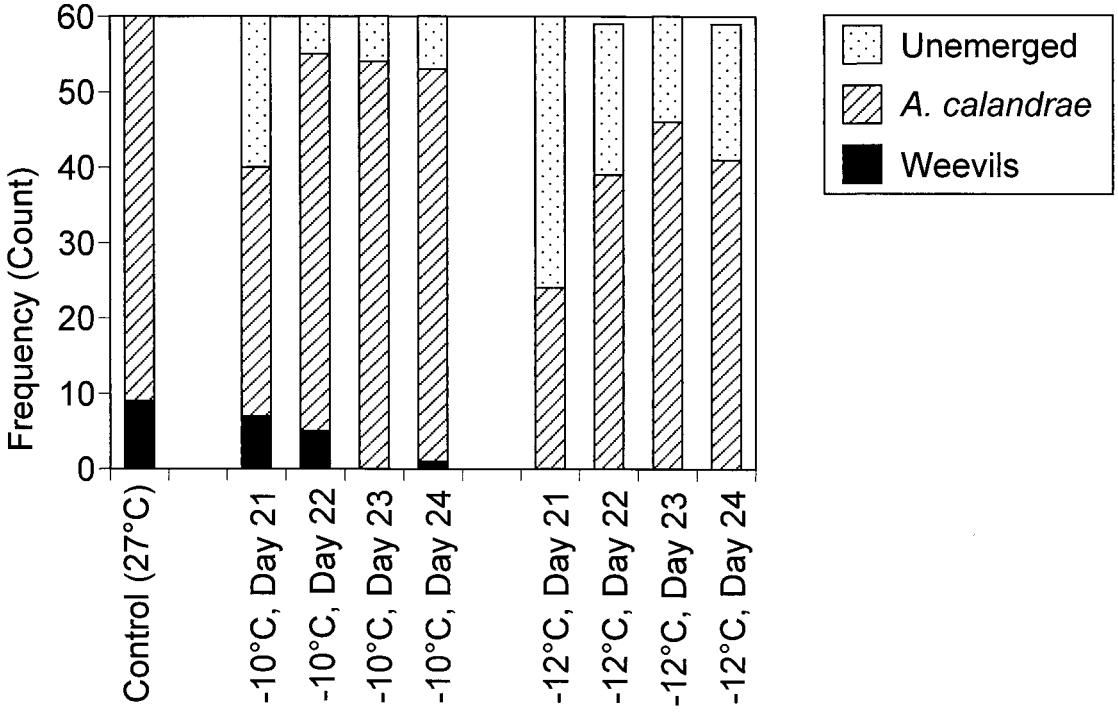

Fig. 3. Emergence of rice weevils and parasitoids from infested wheat kernels subjected to parasitization and then cold exposure.

female-to-male ratio with later cold treatments, and with −10°C compared with −12°C (Table 1). Among controls, the sex ratio in this experiment on postparasitization cold treatments (1.22) was similar to that for preparasitization cold treatments (1.43). The sex ratios on day 21 were more like that of the controls than were most of the sex ratios from other treatments (Table 1).

Discussion

This study differs from previous descriptions of the use of cold injury for rearing parasitoids. Exposure temperatures were systematically examined for a range of host and parasitoid ages. Previous studies have generally not provided reasons for choosing an exposure temperature (Pickens and Miller 1978, Klunker 1982, Petersen and Matthews 1984). Moreover, the use of cold injury in biological control mass rearing is limited compared with the number of parasitoid species that have been mass reared.

The host suitability of various ages was determined primarily from previous studies. Both crystallization temperature and susceptibility to cold injury often change markedly with age and stage, as demonstrated by data from the house fly *Musca domestica* L. (Strong-Gunderson and Leopold 1989). It is therefore desirable to examine crystallization temperature and cold injury in groups of hosts of the narrowest practical age range. For the rice weevil, high levels of infestation could be obtained after a 2-d oviposition period, but not with only 1 d (data not shown). We therefore examined crystallization temperature and cold tolerance at 2-d intervals.

Obtaining data on crystallization temperature for various ages allowed us to distinguish between freezing and chilling injury. For cold treatment before parasitization, −25 and −12°C were selected because the crystallization temperature data indicated that most rice weevil larvae or pupae exposed to −25°C would freeze internally, whereas most of those exposed to −12°C would not. We were able to obtain eclosion of *A. calandrae* of up to 44% of that of control by using internally frozen hosts (day 19, −25°C).

Effects of chilling injury on rice weevils were examined both before and after parasitization. If cold treatment was applied 23 or 24 d after rice weevil oviposition, we were able to get up to 90% emergence of *A. calandrae* compared with the control using −12°C. Greater production of *A. calandrae* was obtained on days 22–24 than on day 21 at both −12 and −10°C, indicating that later larval instars were more cold tolerant. Up to 100% parasitoid emergence was obtained using −10°C exposure.

Selecting an optimum cold treatment to support a program of parasitoid rearing or augmentative release is highly dependent on the context. Among the chilling injury treatments in this study, better parasitoid production was obtained with −10°C than with −12°C. However, for the purpose of releasing infested,

parasitized kernels into grain storage, any host survival is unacceptable. Therefore rice weevil survival after −10°C exposure on days 22 and 24 and the rice weevil cold tolerance data suggesting no change in cold tolerance over this time contraindicate −10°C as a cold treatment. Among chilling injury treatments in this study, postparasitization exposure to −12°C for 2 h on day 23 after rice weevil oviposition best optimizes parasitoid production and host mortality.

The use of freezing injury resulted in poorer parasitoid production (44% at −25 versus 90% at −12°C) and very unfavorable sex ratios. Pre- and postparasitization chilling injury treatments also seemed to affect the sex ratio of *A. calandrae* progeny. Cold treatments before parasitization reduced the proportion of females that successfully eclosed, whereas proportionately fewer males emerged from rice weevils chilled after parasitization. *A. calandrae* and other parasitoids select the sex of their progeny and oviposit selectively based on host size and quality (van den Assem et al. 1984). The highly male-biased sex ratio and poor production of males on frozen weevils compared with those exposed to chilling injury suggest that freezing greatly reduced host quality. The differences between sex ratios in the postparasitization experiments were not statistically significant, but, more importantly, a slight increase in the female-to-male ratio would probably not affect a biological control program as much as a drastic decrease.

Where it is feasible, the use of freezing injury could have advantages, even with poorer parasitoid production. If very low host survival is desired (e.g., probit 9 or quarantine-level), then freezing injury is more likely to provide that level of mortality. Also, it may be possible to stockpile frozen hosts. This approach has been reported to work well in the house fly/*M. zaraptor* system (Klunker 1982, Petersen and Matthews 1984).

Practitioners who need to scale up parasitoid cold treatments such as those described here should be aware of the need to measure temperatures actually experienced by the host rather than that of their surrounding medium. For instance, if the glass tubes used in this study were placed in a −25°C laboratory freezer instead of a refrigerated bath, individual wheat kernels and rice weevils would take longer to actually cool to −25°C. Grain is a good insulator, and this problem would be exacerbated if larger amounts of grain are used. Scale-up of cold treatments for large-scale augmentative release might require the use of a forced-air refrigeration (i.e., a small-scale version of the units commercially available for chilled aeration of grain [Maier et al. 1997]).

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References Cited

Ahmed, K. S. 1996. Studies on the ectoparasitoid, *Anisopteromalus calandrae* How. (Hymenoptera: Pteromalidae) as a biocontrol agent against the lesser grain borer, *Rhyzopertha dominica* (Fab.) in Saudi Arabia. J. Stored Prod. Res. 32: 137–140.

Baker, J. E., and J. E. Throne. 1995. Evaluation of a resistant parasitoid for biological control of weevils in insecticide-treated wheat. J. Econ. Entomol. 88: 1570–1579.

Brower, J. H., L. Smith, P. V. Vail, and P. W. Flinn. 1996. Biological control, pp. 223–268. *In* B. Subramanyam and D. W. Hagstrum [eds.], Integrated management of insects in stored products. Marcel Dekker, New York.

Burks, C. S., D. W. Hagstrum, K. E. Hampton, and A. B. Broce. 1997. Crystallization temperature and chilling injury during overwintering in a feral face fly (Diptera: Muscidae) population. Environ. Entomol. 26: 1124–1130.

Chatterji, S. 1955. Studies on the biology of *Aplastomorpha calandrae* Howard (Insecta: Hymenoptera: Calcidae) parasitic on some storage pests. Proc. Zool. Soc. 8: 11–23.

Drooze, A. T., and M. L. Weems. 1982. Cooling eggs of *Eutrapela clemataria* (Lepidoptera: Geometridae) to −10°C forestalls decline in parasite production with *Ooencyrtus ennomophagus* (Hymenoptera: Formicidae). Can. Entomol. 114: 1195–1196.

Flinn, P. W. 1998. Temperature effects on efficacy of *Choetospila elegans* (Hymenoptera: Pteromalidae) to suppress *Rhyzopertha dominica* (Coleoptera: Bostrichidae) in stored wheat. J. Econ. Entomol. 91: 320–323.

Flinn, P. W., D. W. Hagstrum, and W. H. McGaughey. 1996. Suppression of beetles in stored wheat by augmentative releases of parasitic wasps. Environ. Entomol. 25: 505–511.

Klunker, V. R. 1982. Die wirtsergnung kaltekonservierter *Musca domestica*—puparien fur die massensucht der schlupfwespe *Muscidifurax rapter* (Hymenoptera: Pteromalidae). Angew. Parasitol. 23: 32–42.

Lee, R. E., Jr. 1991. Principles of insect low temperature tolerance, pp. 17–46. *In* R. E. Lee, Jr., and D. L. Denlinger [eds.], Insects at low temperature. Chapman & Hall, New York.

Maier, D. E., R. A. Rulon, and L. J. Mason. 1997. Chilled versus ambient aeration and fumigation of stored popcorn. Part 1: temperature management. J. Stored Prod. Res. 33: 39–49.

Petersen, J. J., and J. R. Matthews. 1984. Effects of freezing of host pupae on the production of progeny by the filth fly parasite *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). J. Kans. Entomol. Soc. 57: 387–393.

Pickens, L. G., and R. W. Miller. 1978. Using frozen host pupae to increase the efficiency of a parasite-release program. Fla. Entomol. 61: 153–158.

Reed, C., and J. Harner, III. 1998. Thermostatically controlled aeration for insect control in stored hard red winter wheat. Appl. Eng. Agric. 14: 501–505.

Schöller, M., S. Prozell, A.-G. Al-Kirshi, and C. Reichmuth. 1997. Towards biological control as a major component of integrated pest management in stored product protection. J. Stored Prod. Res. 33: 81–97.

Smith, L. 1993. Host-size preference of the parasitoid *Anisopteromalus calandrae* (Hymenoptera: Pteromalidae) on *Sitophilus zeamais* (Coleoptera: Curculoinidae) larvae with a uniform age distribution. Entomophaga 38: 225–233.

Strong-Gunderson, J. M., and R. A. Leopold. 1989. Cryobiology of *Musca domestica:* supercooling capacity and low-temperature tolerance. Environ. Entomol. 18: 756–762.

Throne, J. E. 1994. Life history of immature maize weevils (Coleoptera: Curculionidae) on corn stored at constant

temperatures and relative humidities in the laboratory. Environ. Entomol. 23: 1459–1471.

van den Assem, J., F. A. Putters, and Th. C. Prins. 1984. Host quality effects on sex ratio of the parasitic wasp *Anisopteromalus calandrae* (Chalcidoidea, Pteromalidae). Neth. J. Zool. 34: 33–62.

Winston, P. W., and D. H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41: 232–237.

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