manently injured if not preconditioned at a low nonfreezing temperature. Emergence records from pupae after 48 h at -2°C are variable, may depend upon the stage at which pupae encounter the subzero temperature, and imagoes from cold-treated pupae are often weak, fail to unfold their wings and their appearance would offer no support to an hypothesis that the pupal stage is the overwintering stage for C. amoena.

However, the ability of the larvae to survive 72 h at mild subzero temperatures without injury indicates that the freeze resistance factor(s) is(are) present despite maintaining cultures at room temperature. Zachariassen and Hammel (1976) found that freeze tolerant tenebroid beetles lost this capacity upon warm acclimation; Duman (1977a,b) discovered that the thermal hysteresis factor in a tenebroid larva disappeared only after a 16 h photoperiod and short daylength, low relative humidity or low temperature could all induce production of the thermal hysteresis factor. These insects within the continental United States are not relying on the accumulation of glycerol or other polyols for freeze resistance but on proteins. Thermal hysteresis factors lower the supercooling point in a freeze susceptible insect; cryoprotectants enable an insect to freeze without injury; some contain both (Duman 1979) although diptera investigated (Duman 1979) did not show evidence of thermal hysteresis. Further work on the effects of photoperiod and on the mechanism by which C. amoena larvae survive subzero conditions in winter is planned.

References: Duman, J.G. 1977a, J. Exp. Zool. 201:85-92; ____ 1977b, J. Exp. Zool. 201: 333-337; ____ 1979, J. Insect Physiol. 25:805-810; Zachariassen, K.E. and H.T. Hammel 1976, Nature 262:285-287.

Band, H.T. Michigan State University, East Lansing, Michigan. Duplication of the delay in emergence by C. amoena larvae after subzero treatment.

Chymomyza amoena is a cold-hardy drosophilid that now can be grown in the laboratory. Emergence time from field-collected apples in summer versus spring indicates that the overwintering stage is the late larval stage. Experiments subjecting larvae and pupae to -2°C have shown that all stages can be stored at that temperature for 24 h and recover, but only the late instar stage can withstand prolonged storage at this mild subzero temperature provided the larvae are preconditioned first at a low nonfreezing temperature. Timed emergence tests after storage at -2°C and -5/6°C have now been carried out. -2°C is achieved in the freezing compartment of a refrigerator, -5/6°C in a Labline incubator in which the temperature fluctuated between -5°C and -6°C during the time cultures were kept in it. For -5/6°C preconditioning intervals at 10°C and -2°C varied, and larvae were transferred from room temperature appropriately to fresh media so that all were subjected to -5/6°C and postconditioning temperatures simultaneously.

Table 1. Emergence data on C. amoena, field collections + laboratory.

Source	No. Collections	Emergence period after maintenance at 22°C
Field collected apples		
Spring (March,April,May)	4	15-23 days
Summer-Fall	7	22-49 days
Laboratory		
apples	Oct. '78 F_2s	26-52 days
media	3	20 days minimum
oviposition to pupa		10 days
pupa to imago		10 days

Table 1 summarizes the emergence data on C. amoena obtained from field-collected fallen apples, from apples in the laboratory and from C. amoena media. Duration of larval and pupal stages on media have been the same for C. amoena populations from Northern Lower (East Jordan) and mid-Michigan (Lansing, Grand Rapids).

In the larval stage, the last 3 days the larvae are equivalent in size to D. m. 3rd instars, i.e., 1 mg or greater in weight. This is the size which has been stored successfully 28-33 days at -2°C and which was used in the experiments reported here. Table 2 gives the days stored at the specified temperatures, the number of larvae recovered from the media and transferred again to fresh media, number pupating and number of imagoes as well as duration of the larval and pupal stages after subzero treatment.

Table 2. Emergence data on C. amoena larvae after subzero treatment.

	Days at specified t°					Numbers			Days		
Source	10°C	-2°C	-5/6°C	-2°C	10°C	l	p	i	l-p	p-i	T
E.J.	7	7				2	2	1	5	10	15
E.J.	7	8	5	8	1	7	6	4	5-10	11-15	16-20
G.R.	-	-	22h	-	-	5	1	0	no emergence		
G.R.	3	-	5	8	1	6	0	0	reactive		
G.R.	7	1	5	8	1	5	0	0	reactive		

As shown in Table 2, successful emergence after -5/6°C requires at least several days preconditioning at 10°C and -2°C beforehand. This is similar to the previous discovery that storage at -2°C a week or longer requires preconditioning at 10°C and is therefore equivalent to what botanists call "hardening". Whatever changes are occurring are necessary to withstand successive lower temperatures, which in Michigan may be well below -6°C when there is no snow cover.

After both -2°C and -5/6°C larvae require at least 5 days to reach the pupal stage again. Emergence data are comparable, 15-16 days, and compare favorably to emergence data for C. amoena from spring-collected apples kept in the laboratory at 22°C.

As a check that the 3rd or late instar is the overwintering stage for C. amoena, apples were collected from a nearby orchard in early March after a period of very cold weather in a winter of little snow cover. They were held at 10°C overnight, then inspected for C. amoena larvae. Five were found; one 2nd instar was dead, three of those actively mobile and feeding were late instars, and one was borderline late instar in size. The apples were quite soggy after defrosting, which suggests that C. amoena larvae may be freeze tolerant rather than freeze-susceptible with a very low supercooling point (Zachariassen and Hammel 1976a,b).

References: Zachariassen, K.E. and H.T. Hammel 1976a, Norw. J. Zool. 24:349-352; ____ and ____ 1976b, Nature 262:285-287.

Beck, A.K., R.R. Racine and F.E. Würgler.
Institute of Toxicology, Swiss Federal Institute of Technology, and University of Zürich, Switzerland. Primary nondisjunction frequencies in 7 chromosome substitution stocks of D. melanogaster.

In continuation of the work by Racine, Beck and Würgler (1979) a number of chromosome substitution stocks were studied for the frequency of primary nondisjunction in females. The balancer stock contained the following chromosomes: Cy = In(2LR)SM5, al^2 Cy lt^v cn^2 sp^2; Pm = In(2LR)bw^{V1}; Ubx = In(3LR)Ubx^{130}; Sb = In(3R),Sb; pol = spa^{pol}. H indicates unmarked chromosomes from a Hikone-R stock, P unmarked autosomes from a stock containing the attached-XY chromosome Parker 110-8 and A the autosomes with inversions (balancer stock). Since all chromosome substitution stocks contain identical X-chromosomes (from the Hikone-R stock) and identical 4th chromosomes (pol from the A stock) the abbreviations used indicate only the stock constitution with respect to chromosomes 2 and 3 (see the table).

In the nondisjunction tests we studied the meiotic segregation of the sex-chromosomes in the females of the chromosome substitution stocks. We crossed 1-2 day old males of the genotype $Y^SX.Y^L$, In(1)EN, y B to 1 day old females. Three pairs were used per vial. After 3 days the parents were discarded. The progeny were classified according to sex and phenotype. Two types of males could not be distinguished by phenotype: $Y^SX.Y^L$, In(1)EN,y B/0 (resulting from primary nondisjunction in XX females) and $Y^SX.Y^L$, In(1)EN, y B/Y (resulting from secondary nondisjunction in XXY females). These males were crossed to C(1)DX, y f / y^+ Y B^S; bw; st pP females and surviving C(1)DX progeny indicated the presence of a free Y chromosome in the male tested, because C(1)DX, y f contains a Y-suppressed lethal.

Female	Total	Normal progeny		Nondisjunctional progeny		
stock	progeny	F	M	F	M	ND %
AA	48577	18994	29575	4	4	0.02
HA	20383	9645	10722	9	7	0.08
AH	22787	9591	13183	8	5	0.06
HH	24139	10007	14073	3	56	0.24
PA	24056	9573	14456	12	15	0.11
AP	25207	10339	14834	17	17	0.13
PP	22022	8434	13573	9	6	0.07

F = females, M = males, ND % = nondisjunctional progeny in percent of total progeny.