$r \geq 2.00$, and highest for $0.80 \leq r \leq 2.00$ m, and therefore SED should be estimated for the circular ring area, as follows;

$$SED_{cra} = n/\pi\ (r^2_{max} - r^2_{min}) \quad (2)$$

The difference between a complete circular area i.e. $0.00 \leq r \leq 2.00$ m and the cra $0.80 \leq r \leq 2.00$ m is not very large. Assuming $n = 100$ eggs (i.e. a normal egg-production during a 2-week period) the difference is 1.5 eggs/m^2, which is a large numerical difference but a small biological difference during a 2 week period.

The explanation given previously[1], based on the SED theory, only stated that the flicking-away of eggs could have advantages in making it more difficult for a predator to locate the eggs deposited by a more-or-less immobile female. Now further explanations can be given. In *E. tiaratum* (and probably in other Phasmida as well) the feces are just dropped, and not flicked away like the eggs. Furthermore, the 'bad' feeding habits of phasmids often results in the dropping of large fragments of leaves on to the ground below[10]. It is very likely that these somewhat moist residues can act as olfactory attractants to predators. Therefore the eggs lying in a circular ring outside the centre of feces and food-plant residues, will be safe from predators locating these residues, especially since SED_{cra} is extremely low. Furthermore, the adult female living high up in the canopy will also be safe from this type of predation. The extremely low value of SED_{cra} in centre of the cra is shown in figure B.

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A mild winter delays supercooling point elevation in freeze tolerant *Chymomyza amoena* larvae (Diptera: Drosophilidae)

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Summary. Supercooling point elevation to -3.2 ± 1.3 °C among 17 freeze tolerant *Chymomyza amoena* larvae was delayed until January, 1983 in the mild winter of 1982–83 and occurred despite particulate matter in the gut. The larval population in mid-Michigan is polymorphic for both freeze tolerant and freeze sensitive larvae. One substrate, *Malus coronaria* fruits, appears to be a neutral niche supporting both phenotypes in the population.

Insects have 2 basic mechanisms for overwintering survival: they may become tolerant to freezing or they may remain freeze intolerant and rely on supercooling to maintain body fluids in the liquid state. Freeze tolerant (FT) insects may actually elevate the supercooling point (SCP) in winter to hasten freezing[2–8]. Insects which remain freeze sensitive (FS) need to reduce the chance of innoculative freezing and so may lower the SCP or adopt means to mask or eliminate nucleators[9–16]. These insects remain susceptible to innoculative freezing induced by food in the gut[10,14–16], so usually evacuate the gut prior to entering diapause[10]. Insects which are freeze tolerant are able to survive freezing despite particulate matter in the gut[8,9,16]. One species was found to show a shifting polymorphism between FT and 50% FS types in a severe winter but in mild ones many larvae remain FS[11,17].

Chymomyza in the family Drosophilidae overwinter as larvae[18–22]. *C. costata* larvae diapause and evacuate the gut, as expected[20]. *C. amoena* larvae appear to be among the dipteran species that have no obligate diapause[21,23].

C. amoena is the first drosophilid overwintering in the larval stage to be studied in the natural environment. In mid-Michigan this species has now been found to breed successively in endemic crabapples, *Malus coronaria,* in spring, apples in summer, and pears, apples, ornamental crabapples, black walnuts and endemic crabapples in autumn. The latter 4 are overwintering niches. It also manifests polymorphism for freeze tolerant and freeze sensitive types.

A feeding polymorphism seemed indicated[3]. Larvae in frass (insect excreta) or around the apple seed were found to be potentially FT, those in apple flesh were FS and were eliminated by November while SCPs among the FT group elevated to −4 °C. Numbers were low. Larvae found in walnuts *(Juglans nigra)* in January continued to be FS with an average SCP of −14 °C[22].

The ability to sample larvae from a variety of substrates from spring through winter of 1982–83 has provided more data on larval cold hardiness. Additional contrasts with *C. costata* and other species emerge. Among 17 FT larvae SCPs elevated to -3.2 ± 1.3 °C in all substrates and despite the presence of food in the gut. This was delayed until January, 1983 during the mild 1982–83 winter[24]. However, the population is polymorphic for both FT and FS larvae. *Malus coronaria* fruits appear to act as a neutral niche for both phenotypes which can manifest differential survival in other substrates as apples and walnuts. The probable genetic mechanism underlying the polymorphism remains unknown.

Materials and methods. Larvae were sampled from endemic crabapples, *Malus coronaria;* apples; ornamental crabapples and black walnut husks, *Juglans nigra.* In late May the potential cold hardiness characteristics of larvae 3 mm, 3.5 mm and 4 mm in *M. coronaria* fruits were determined to compare 2nd and 3rd instar larvae. By late June females oviposit in apples. Later in summer this niche is shared with *Drosophila melanogaster* and *D. simulans* larvae as are ornamental crabapples[25,26]. *C. amoena* can readily be distinguished from other drosophilids or pest larvae as *Rhagoletis pomonella.*

In May, 27 softened overwintered *M. coronaria* fruits and in June, 21 small green apples were collected, inspected for *C. amoena* eggs, kept at 22 °C and 3 each were dissected for larvae several weeks later. All other cold hardiness determinations were ideally made on larvae taken from substrates either the same day substrates were collected (September, Oc-

tober) or 1 to 2 days after substrates were defrosted overnight and held at 4 °C.

Beginning in September, 3 to 5 apples were dissected monthly for larvae. October through January, 25 ornamental crabapples were dissected monthly. In December and January, 25 endemic crabapples were dissected each month. In January, 10 walnuts were gathered. Infestation rate is low for the ornamental crabapples, the nut-like *M. coronaria* fruits in autumn and winter, and for walnuts. When SCPs were observed to elevate in January among FT larvae, second collections of apples and ornamental crabapples were made and larval cold hardiness again determined.

Endemic crabapples were collected in February to assess the maintenance of both FT and FS larvae. Then larvae in apples were sampled to verify that SCPs had declined among FT larvae. Walnuts were collected but no larvae were found.

However, available larvae have had to be partitioned among ongoing experiments so not all could be used for cold hardiness work. Laboratory populations from endemic crabapples (May) and from walnuts (January) were established and interfertility with populations from apples verified. Some larvae were also used for hemolymph protein electrophoresis to compare summer and winter larvae.

For larvae used in the cold hardiness work, location in seed areas, frass or pulp was recorded for those taken from endemic and ornamental crabapple and apples. Larvae were also scored for particulate matter in the gut, as determined by intestinal discoloration. In December, 2 larvae were dissected for gut contents.

SCP determinations were made as before[22]. For potentially FT larvae, the freezing point (FP) = the supercooling point (SCP) and a period of chilling is required to convert potential into recoverability at the FP. The lower lethal temperature remains unknown but can be determined by experimental means. For FS larvae, the SCP represents the latent heat of fusion and becomes the lower lethal temperature to the organism. Larvae were affixed by vaseline to the probe of a Model YS142SC telethermometer connected to a Sargent Recorder Model SR and cooled at the rate of 2 °C min in a freezing chamber cooling to −28 °C. In October, a larva cooled to −20 °C registered neither FS nor FT, recovered but formed an abnormal pupa. Thereafter, larvae initially cooled to −12 to −15 °C were rewarmed and tested again. All chi-square determinations have been made according to formulae given in Strickberger[27]. Weather data comparisons for the past 3 years, 1980–83, have also been included.

Results and discussion. C. amoena is an endemic North American species that can now be found in Europe[28]. *M. coronaria* and *Juglans nigra* are also endemic North American species. *C. amoena* was known to breed in the latter and to have invaded domestic (imported) apples,[29] but had not been reported in endemic crabapples. Only 2 adults emerged from 7 nut-like *M. coronaria* fruits on which *C. amoena* eggs were present out of 263 inspected in October, 1981. Ten fruits under 1.1 m snow cover contained 2 small larvae in January, 1982, a time when this size is eliminated in apples and walnuts. The softened overwintered fruits collected in May, 1982 had newly laid *C. amoena* eggs and some larvae; 48 adults emerged. In January 17 adults emerged from walnuts; however, females deposited 277 eggs on softened overwintered *M. coronaria* fruits but only 8 on walnut husks over 8 days. Crabapple, apple and walnut populations have been interfertile.

This broad niched species[30] can have multiple generations a year. Summer and autumn feeding versus winter non-feeding stages and the possibility that different instars have different cold-hardiness characteristics can influence results.

Larvae in endemic crabapples in May provided data on 2nd and 3rd instar sized larvae. Cold-hardiness characteristics did not differ. In all substrates between May, 1982 and January, 1983 15 2nd instar larvae were potentially FT (SCP = −7.8 ± 1.3 °C), 11 were FS (SCP = −11.4 ± 2.8 °C). All have been included in the data in table 1. These results contrast with other species: *C. costata* larvae are FS but all stages have low SCPs[20]. *D. melanogaster* 2nd instars have low SCPs, 3rd instars elevate to −15 °C but are not cold hardy[31]. *Eurosta solidaginis* larvae have low SCPs as 2nd instars, then elevate to −6 °C in the FT 3rd instar stage[3–5]. This suggests that different genes can function in different stages.

Larvae in endemic crabapples in May were found to be both potentially FT and FS. Also larvae in pulp and in seed or frassy parts of these fruits were in both categories and in equal numbers. Migration between the 2 areas cannot be excluded in this or other substrates in 1982–83. Consequently larvae in table 1 are grouped only into FT and FS categories. Overall 8 larvae were potentially FT, 22 were FS in this niche.

In apples 69 larvae were measured between June and January:

Table 1. Freezing points (FP) and supercooling points (SCP) of freeze tolerant (FT) and freeze sensitive (FS) *C. amoena* larvae in different freeding/overwintering substrates in 1982–83

	Freeze tolerant FP = SCP	n	Freeze sensitive FP	SCP	n
Malus coronaria					
May	− 8.3 ± 1.3	8	− 10.3 ± 2.1	− 11.1 ± 2.2	8
Dec	(−15;1) − 7.5	1	− 11.3 ± 4.2	− 13.0 ± 4.4	3
Jan	(−10;1) − 7.2 ± 2.9	5	− 7.4 ± 3.2	− 7.7 ± 3.2	3
Feb	− 11.3 ± 3.4	4	− 9.4 ± 3.1	− 11.1 ± 3.2	8
Apples					
Jul	− 5.3 ± 0.7	7	− 5	− 6	1
Sept	− 6.7 ± 0.7	5	− 9.4 ± 2.0	− 10.4 ± 2.5	4
Oct	− 7.6 ± 1.6	9	− 8.0 ± 0.8	− 9.3 ± 1.0	4
Nov	− 6.7 ± 0.6	6	− 8.6 ± 1.5	− 10.5 ± 2.2	8
Dec	(−15;2) − 7.4 ± 1.2	6	− 10.4 ± 2.2	− 12.4 ± 2.8	7
Jan	(−12.5;3) − 2.6 ± 1.3	9	(−12;2) − 9.0 ± 1.7	− 9.6 ± 1.9	3
Feb	− 12.1 ± 0.9	7	− 13.2 ± 1.9	− 14.2 ± 1.0	3
Ornamental crabapples					
Oct	(−20;1) − 9.0 ± 1.4	3	− 10.4 ± 1.4	− 12.0 ± 1.5	9
Nov	− 7.0 ± 1.0	3			
Dec	− 8.0 ± 1.4	2	− 8.6 ± 1.5	− 10.0 ± 1.4	12
Jan	− 3.9 ± 1.2	6	− 7.7 ± 2.9	− 9.2 ± 3.0	7
Juglans nigra					
Jan	− 4	1	− 8.6 ± 1.5	− 9.8 ± 1.6	6

n = number of larvae measured; number in parenthesis = a prior chilling; the number following indicates how many such larvae were cooled to the temperature indicated. Temperatures in °C.

38 of 42 potentially FT larvae were in seed/frass areas; 22 of 27 FS larvae were in pulp ($\chi^2_1 = 33.2$; $p < 0.001$). Apples in February were in pieces. Nevertheless, most larvae in apples express potential or actual freeze tolerance although none completed development after recovery until February, a month after SCP elevation. Rate of cooling may be critical as well as post-cooling recovery temperatures. None of the FT type were in pulp in ornamental crabapples, but fruit size is small.

Larvae were still feeding in December as determined by moulds and yeast in the gut or other particulate matter, as shown in table 2. This may explain why there is little difference in seasonal cold-hardiness during 1982. Climate plays a role in the phenotypic adjustment of a population[3]. Table 3 confirms that in autumn and winter in the Lansing area 1982–83 was milder than the 2 previous years.

In January, SCPs elevated among FT larvae to -3.2 ± 1.3 °C. Numbers include 1 in the seed area of *M. coronaria*, 1 in walnuts and 15 in double collections from apples and ornamental crabapples in January. Also it occurred despite particulate matter in the gut, as shown in table 2. SCP elevation has been found in other dipteran larvae: *Belgica antarctica*,[6] *E. solidaginis*,[3–5] and a *Xylophagus* sp. in Canada[7].

Most larvae in walnuts remain FS in 1983 but SCPs are not as low as in 1982. If we compare numbers of larvae in apples and walnuts in January, 1983, there is a significant probability that the 2 contain contrasting types of larval overwinterers ($\chi^2_1 = 4.32$; $p < 0.05$). None had 2nd instar sized larvae in contrast to 4 in *M. coronaria* fruits in January, 1983.

Both FT and FS larvae were present in *M. coronaria* fruits in February. SCPs declined among FT larvae, which recovered at the SCP (−11.3 °C) as expected; 2 from apples completed development, although −12 °C is well below the mean winter temperature; recovered larvae were kept at 15 °C for 2 weeks before returning them to room temperature. FT larvae in both niches indicate that SCPs elevate, then decline as observed in Texas *E. solidagensis*[3]. They also indicate that the entire population may not become FT, as observed for *Dendroides canadensis* larvae. Both species suggest that larvae may continue winter feeding in mild periods in temperate climates. In particular, the decline in SCPs among FT and FS *C. amoena* larvae parallels an increase in temperature in February. Table 4 shows that only January maxima averaged nearly 0 °C. This increases the importance of the fat body and fat body products, like lipids[32] and the larval hemolymph proteins[11,25,33], in the maintenance of cold hardiness. One protein in *C. amoena* appears to accumulate in the hemolymph. *C. amoena* is among dipterans which seem not to store glycerol or other polyols[7,15,22,34].

The role of environmental heterogeneity in the maintenance of polymorphisms has long been recognized in genetics[35,36]; polymorphism as the outcome of disruptive selection has been demonstrated[37]. Variation must exist in the population for differential survival to occur in apples and walnuts in this species. The existence of both FS and potentially FT types in *M. coronaria* fruits in May, repeated among 15 potentially FT and 14 FS frass feeding larvae in early fallen apples in 1983, and among *D. canadensis* larvae under tree bark suggests there is a genetic base. Baust has pointed to the importance of gene regulation and hormonal interactions in overwintering[3]. Substrate interactions may also be important. For different species some substrates may function as neutral niches, supporting contrasting phenotypes in the population.

Table 2. Freezing points (FP) and supercooling points (SCP) of freeze tolerant (FT) and freeze sensitive (FS) larvae with particulate matter in the gut as revealed by intestinal discoloration

	Freeze tolerant			Freeze sensitive			
	S	FP = SCP	n	S	FP	SCP	n
Dec	A	−7	1	A	-6.7 ± 0.6	-8.3 ± 0.6	3
Jan	A,OC	-3.0 ± 0	2	W	−7.5	−9	1

S, source; A, apples; OC, ornamental crabapples; W, walnuts; n, number of larvae. Temperatures in °C.

Table 3. Mean seasonal temperatures in °C from 1980 to 1983 in Lansing, MI, USA to demonstrate the milder autumn and winter of 1982–83

Year	Spring	Summer	Autumn	Winter
1980–81	6.94	20.73	8.83	−4.90
1981–82	7.83	20.73	8.78	−6.29
1982–83	7.50	19.23	10.44	−0.73

Table 4. Average maximum and minimum temperatures in °C from November, 1982 to February, 1983 in Lansing, MI

	Nov	Dec	Jan	Feb
Maximum	8.50	5.33	0.33	3.44
Minimum	0.44	−2.22	−6.46	−4.70

1 Discussions with K.E. Zachariassen and J. Duman before beginning the work on *C. amoena* cold-hardiness were invaluable. Visits of both at Michigan State University were jointly sponsored by the Depts. of Zoology and Entomology. H. Carson suggested that *C. ameona* might be breeding in endemic crabapples. G. Parmalee at MSU showed one of us (HTB) a long established thicket of *Malus coronaria*. The MSU Climatology Center supplied all Lansing area weather data.

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38 This paper is dedicated to E.R. Dempster on the occasion of his 80th birthday.

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