Overwintering of *Chymomyza amoena* larvae in apples in Michigan and preliminary studies on the mechanism of cold hardiness

Henretta T. Band and R.N. Band

Department of Zoology, Michigan State University, East Lansing (MI 48824, USA), 21 April 1980

Summary. Chymomyza amoena, a member of the family Drosophilidae, is now breeding in fallen apples in the state of Michigan. *C. amoena* larvae can overwinter in fallen apples, completing development and emerging as adults in the summer. Cold resistance during the overwintering phase may not depend upon the accumulation of glycerol or other sugar alcohols.

Chymomyza amoena is a species endemic to North America. Sturtevant[1] reported that it bred in walnut and butternut husks and in acorns. Chymomyzids in general are considered to be sap feeders[2]. Steyskal[3] collected them around the cut ends of trees in the 1950s in Michigan.

C. amoena, a member of the family Drosophilidae, now seems to be breeding and feeding in fallen apples in Michigan. The larval stage can overwinter in them. Table 1 presents emergence data on *C. amoena* from apples collected at sites outside East Jordan, Michigan, on Lake Charlevoix in spring and autumn, 1978, and again in spring, 1979. After collection all apples were transported to the laboratory and kept at 22 °C until 1 week after the last *C. amoena* emerged. The 2 sites are about 200 m apart, S-site consists of 2 trees, an early and a late variety. N-site consists of 1 tree, a late variety. All are old but still produce apples; the late variety comes into production in alternate years. Adults do not normally appear until mid-July when the current season's apple crop has begun to fall at S-site. Numbers emerging from apples collected during spring, 1978, may have been reduced due to competition with *D. melanogaster* in summer, 1977. This species did not appear in 1978; numbers of *C. amoena* increased and remained elevated despite the severe winter conditions intervening between autumn, 1978 and spring, 1979. The area is snow-covered from November to April; Lake Charlevoix freezes solid enough for snowmobiling. Winter 1978/79 Lake Michigan froze.

Table 1 also includes emergence data on *C. amoena* from apples collected in June, 1979, in 2 different abandoned orchards near different cities in mid-Michigan, in late 1979 in 2 commercial orchards and again in March, 1980, at one orchard to search for larvae and pupae in the rotting apples. Apples in the last collection were still frozen so were thawed overnight in a refrigerator, then inspected. 8 larvae, mid- to late instar in size, were found, presumably *C. amoena* from the posterior spiracular area. Only 3 adults emerged.

The data in table 1 indicate the widespread occurrence of *C. amoena* in fallen apples now in Michigan. In all localities except the Grand Rapids orchard apples collected were randomly scattered on the ground. At the Grand Rapids site they were in a pit and may have been better protected from direct winter conditions if all emergees from the June apple collection represent an overwintering group. 4 *Drosophila* species also emerged; in August only 2 of the 4 were present, *D. melanogaster* and *D. immigrans,* in addition to *C. amoena.* Emergence of *C. amoena* from apples collected late in the year at the commercial orchards may represent an invasion, not a year-round habitat. Coddling moth larvae were also present in 1 collection; spraying for these pests ceases in August.

Adults and larvae can feed on apples; however adults require a break in the skin to do so. Females do not oviposit through the skin as does *Rhagoletis pomonella,* the apple maggot fly. Although *C. amoena* has been found in

Table 1. Emergence of *Chymomyza amoena* from fallen apples collected in spring 1978, October 1978 and April 1979 in northern lower Michigan, in June 1979 from 2 areas in mid-Michigan, in late 1979 from 2 commercial orchards and in March 1980 to search for larvae and pupae

Collecting date	Locality and site	No. apples	No. emerging	Date first and last *C. amoena* emerged at 22 °C
A) Abandoned or remnants of abandoned orchards				
24 March 1978	East Jordan, S	3	4	14–16 days
16 April 1978	East Jordan, S	4	4	23 days
7 May 1978	East Jordan, S	4	5	15–21 days
8 October 1978	East Jordan, S	3	9	22–38 days
22 October 1978	East Jordan, N	3[a]	10	27–49 days
15 April 1979	East Jordan, N (1)	3	15	15–22 days
	East Jordan, N (2)	3	6	16–28 days
12 June 1979	Lansing	3	6	16–27 days
25 June 1979	Grand Rapids	many[b]	13	15–17 days
B) Commercial orchards				
18 November 1979	St. Johns-1	20	56[c]	17–33 days
11 December 1979	St. Johns-1	16	3	17–22 days
11 December 1979	St. Johns-2	10	8	17–22 days
C) Spring 1980				
3 March 1980	St. Johns-1	7	3[d]	18 days

[a] 1 *Drosophila melanogaster* emerged. [b] Other drosophilids emerging included *D. melanogaster, D. immigrans, D. robusta* and *D. athabasca*[18]. [c] 8 *D. melanogaster* emerged. [d] 8 larvae were found, mid to late instar in size. Pupal duration periods are 10 and 14 days for larvae grown on apples. Egg-eclosion time can vary from 26 to 52 days.

Table 2. Generalized sugar alcohol tests on frozen and unfrozen *C. amoena* larvae and pupae and on *D. melanogaster* controls

Locality	Species	Preadult stage	Treatment 10 °C (days)	−2 °C (days)	10 °C (days)	No.	x̄ wt	Activity	Amount*
E. Jordan	*C. a.*, 1978	Larvae	12	33	1	10	1 mg	Motile	++
		Pupae	12	33	1	7	1 mg		+++
	C. a., 79 (2)	Larvae	11	33	1	2	too few	Motile	-
	C. a., 79 (1)	Larvae	1	33	1	10	1 mg	Dead	+++
Lansing	*C. a.*,	Larvae	0	1	0	9	1 mg	Motile	++
		Pupae	0	1	0	10	2 mg		++
Grand Rapids	*D. m.*	Larvae	0	1	0	8	1 mg	Dead	++
	C. a.,	Larvae	0	1	0	10	1 mg	Motile	+
E. Jordan	*C. a.*, 79 (1)	Larvae	0	0	0	10	1 mg	Motile	+
	C. a., 1978	Larvae	0	0	0	10	1 mg	Motile	+
Grand Rapids	*C. a.*,	Larvae	0	0	0	9	1 mg	Motile	+

*+++ Symbolizes approximately 4 µg glycerol equivalents of sugar alcohol per mg larval or pupal weight; ++ symbolizes approximately 2 µg glycerol equivalents of sugar alcohol per mg larval or pupal weight; + indicates a trace amount (< 2 µg).

the trees at the East Jordan sites, it is not classified as a pest. Drosophilid cold-temperature resistance is a relatively new area of research[4–7]. To date, both the genetics and the mechanisms of cold-hardiness are more readily studied on laboratory-reared populations. *C. amoena* is among the chymomyzids of the Drosophilidae that can be grown in the laboratory[2]. An applesauce-protein-cream-of-wheat media, a modification of the Wheeler-Clayton formula used for the Hawaiian Drosophila[8] has proved successful for Michigan *C. amoena.*

Glycerol has been found to be present in many overwintering insects in the egg, larva, pupa or adult, whichever is the overwintering stage[9,10]. Sorbitol and threitol have been identified as the substances conferring cold-hardiness in others[11,12], while proteins may be used as cryoprotectants[13,14] or thermal hysteresis factors[15,16] in insects within the continental United States. Late instar larvae transferred to fresh media, chilled at 10 °C 1 week then stored at −2 °C 1, 2, 4 weeks, and 33 days have been able to resume development, pupate, and eclose when returned to room temperature (22 °C). *C. amoena* larvae also survive 24 h at −2 °C when transferred from room temperature. Michigan *D. melanogaster* 3rd instar larvae do not survive −2 °C for 24 h whether preconditioned 1 week at 10 °C or placed directly from room temperature into this mild subzero environment.

Species that rely on glycerol for cold hardiness can accumulate it at 0 °C in 1 week[17]. Preliminary studies for the detection of glycerol were done on East Jordan *C. amoena* larvae only. Larvae to be used were transferred to fresh media, kept at 10 °C for 1 week, then placed in −2 °C for 2 weeks. *D. melanogaster* controls were treated similarly. Larvae were then removed, weighed and extracted with cold 5% perchloric acid. Following KOH neutralization the perchloric acid extract was analyzed for glycerol content using the enzyme portion of the Triglyceride 320-UV kit produced by Sigma Chemical Co. This assay is specific for glycerol. Results indicated that only trace quantities of glycerol were present (< 0.5 µg/mg larval wt) both in the *D. melanogaster* (dead) and the *C. amoena* larvae, motile by the time 10 per strain were separated from the media.

Periodate oxidation of the neutralized perchlorate extract was used to assay for sugar alcohols as a group with the colorimetric portion of the triglyceride-405 kit produced by Sigma Chemical Co. Pupae were also included; all larvae were of the 1 mg wt size. Preconditioning intervals varied between 12 days, 1 day or 0 days at 10 °C; storage at −2 °C was for 33 days, 1 day or 0 days. Larvae in the latter group were included since *C. amoena* shows immediate cold resistance which *D. melanogaster* lacks. Cultures stored over 30 days had to be defrosted overnight in order to separate larvae and pupae from the media without injury. Results are given in table 2. Storage at −2 °C enhances the detectable sugar alcohol content in dead *D. melanogaster* larvae as well as *C. amoena* larvae and pupae whether the larvae become motile or not. However the sugar alcohol level is not noticeably greater for larvae stored for 33 days vs 1 day at −2 °C.

Failure to detect significant amounts of glycerol or sugar alcohols makes it likely that *C. amoena* is using proteins to achieve cold hardiness. Whether they function as cryoprotectants or as thermal hysteresis factors is as yet unknown. Thermal hysteresis factors have not been found in Dipterans[16]. Although warm acclimation does not eliminate cold resistance in *C. amoena,* preconditioning for about 1 week at a low nonfreezing temperature has been found to be necessary for larval survival at minimal subzero temperatures for prolonged periods. What changes may be occurring are unknown. Dry weight determinations reveal that the body water content of late instar larvae reared at 22 °C differs little from that of late instars maintained at 10 °C for 1 week. The watery condition of the rotting apples collected in March, 1980, after thawing and the large size of the late instars within them support the laboratory findings that cold hardiness may not depend on a reduction of body water content.

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18 *D. robusta* and *D. athabasca* were identified by Dr P.T. Ives at Amherst College, Amherst, Mass. *C. amoena* was identified by Dr M.R. Wheeler at the University of Texas, Austin in August, 1978. Our thanks to them both.