5'-Nucleotidase activity in liver homogenate of rats treated with CCl_4 and ethionine

	Control group		Experimental group	
	Female	Male	Female	Male
CCl_4	74.5± 8.59 (15)	78.6±10.39 (6)	73.5±13.30 (16)	70.1±13.68 (6)
Ethionine	92.9±12.45 (18)	104.5±11.52 (9)	160.5±11.52 (18)*	87.9±11.13 (11)

Values are given as μmoles substrate utilized/100 g b.wt and are the mean±SE of the number of determinations given in parentheses. * $p<0.01$ from the appropriate control.

Ethanol was administered as 1:1 water solution, by stomach tube, in 2 different doses. The first one (700 mg/100 g b.wt), described as inducing a well-defined fatty liver[1], produces in all the animals signs of strong intoxication, such as ataxia, somnolence and in some rats coma or even death before the 6th h following administration. Experiments were therefore also carried out using a lower dosage corresponding to 200 mg ethanol/100 g b.wt. The animals were killed by decapitation 6 h after the 1st injection of ethionine and 6 h after the administration of other drugs. About 50 mg (wet wt) of each liver was homogenized in 0.5 ml of 0.3 M sucrose (pH 7.4 with $NaHCO_3$) and 5-N determined at 37 °C, for 15 min, pH 7.4[9]. After the addition of trichloroacetic acid, the inorganic phosphate was estimated[10] in the supernatants. Each value was corrected for the inorganic phosphate present at zero time and for the possible hydrolysis of the substrate. All incubations were carried our between 16.00 and 17.00 h. The statistical significance was evaluated with Student's t-test[11]. No statistical significance has been attached to differences with a probability value $p > 0.05$. Since most of the experiments were performed at different times, a set of control rats was included with the experimental groups.

Results and discussion. The results for CCl_4 and ethionine are summarized in the table. Despite the well-controlled conditions of feeding and housing there is a certain degree of variability among the control values taken at different times. The variability does not appear to be linked to circadian rhythms since the incubations were carried out at about the same time of day. In view of the differences between the individual control groups, the experiments with ethionine were repeated several times, with slightly variable results. All these data have been collected in the table. As compared with corresponding controls, liver homogenates of female rats treated with ethionine show an increase of 5-N of about 73% while no significant differences in livers of male rats treated with the same drug were found. This result is in accordance with several findings showing that most morphological and biochemical changes in the liver caused by ethionine are much slighter or altogether absent in the livers of male as compared with female rats[12]. No significant changes in 5-N of liver homogenates either of female or male rats treated with CCl_4 or with the other compounds used in this research have been observed.

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Effect of daminozide on tomato fruit ripening

F.H. Karam and D.P. Murr[1]

Horticultural Science Department, University of Guelph, Guelph (Ontario, N1G 2W1, Canada), 22 October 1979

Summary. Fruits of tomato (*Lycopersicon esculentum* Mill. cv. Fireball) were harvested 32 and 37 days after anthesis and dipped for 30 min in 10,000 ppm daminozide. Ripening of treated fruits, as manifested by an advancement in ethylene production and the respiratory climacteric, was accelerated by up to 7 days over control fruit.

Preharvest application of the growth retardant daminozide (succinic acid −2, 2-dimethylhydrazide, SADH, Alar, B-9) prevents premature fruit drop but delays the onset of both C_2H_4 production and the respiratory climacteric of apples after harvest[2,3]. However, this inhibition can be reversed by application of C_2H_4 gas[2,3]. In contrast to its effect on apples, preharvest daminozide treatment of peaches hastened ripening by accelerating the onset of the climacteric, by increasing internal flesh colour and external skin colour, and by decreasing flesh firmness[4]. Daminozide also significantly increased C_2H_4 production associated with peach fruit ripening[5]. An effect similar to ethephon (ethrel) treatment was induced by dipping mature-green tomato fruits in daminozide for 30 min[6]. This treatment enhanced cellulase activity, colour formation and softening of the fruits. Because daminozide is considered to be a ripening inhibitor and because the tomato appears to differ from other climacteric-type fruits in response to exogenous regulators such as C_2H_4[7,8], we were interested in studying the effect of daminozide on C_2H_4 production and the climacteric of tomato fruit.

Materials and methods. Seeds of 'Fireball' tomatoes (*Lyco-*

persicon esculentum Mill.) were sown directly into 30-cm pots containing Nova Mix 400, a soilless mix. The plants were grown in the greenhouse under ambient temperature and light conditions prevailing in southern Ontario during the summer and were watered when required. Fertilizer (20:20:20) was applied once a week and was supplemented with calcium nitrate and magnesium sulfate when the plants reached 40 cm in height. In order to obtain fruit of uniform physiological age, flowers of a cluster were pollinated when the petals were fully reflexed, each flower was identified with a dated tag[8], and the clusters were thinned to 3 flowers/cluster to minimize competition. It took an average of 45 days from anthesis for fruit to develop to the breaker stage of maturity. Fruit was harvested at 2 stages of development, 32 days (approximately 70% of growth period elapsed) and 37 days (80% of growth period elapsed) after anthesis and dipped for 30 min in 10,000 ppm daminozide prepared by dissolving 20 g of Alar-85 in 2 l of distilled water containing 2 ml Plyac as surfactant. Control fruit were dipped in water plus surfactant. The fruits were air-dried at 20 °C and then placed into 120 ml respiration jars at 20 °C with a continuous humidified (90–95% relative humidity) air flow of 96 ml/min. Carbon dioxide and C_2H_4 in the effluent air stream were measured daily using a Beckman IR 15A analyzer for CO_2 and a Beckman GC-5 gas chromatograph equipped with a hydrogen flame for C_2H_4.

Results and discussion. Daminozide treatment significantly advanced the onset of C_2H_4 production of tomato fruits harvested 32 and 37 days after anthesis (figure 1). A rise in C_2H_4 production occurred within 1 day following daminozide application which peaked then dropped 3–4 days later. This initial C_2H_4 peak was then followed by a peak similar to that produced by control fruit, except that peak C_2H_4 production by daminozide-treated fruit was 20–25% greater and occurred 2 or 7 days earlier than in control fruit (figure 1). The initial small C_2H_4 peak by treated fruit may represent stress C_2H_4 production in response to the high level of daminozide. Since control fruit did not exhibit such a peak, it could not have been due to physiological stress induced by merely dipping the fruit. Nevertheless, the subsequent rate and intensity of the C_2H_4 peak indicates that daminozide stimulated C_2H_4 synthesis. It is possible that a daminozide stress resulted in ethylene levels within the fruit above the threshold required for induction of autocatalytic C_2H_4 production and subsequent ripening. It should be noted that 37-day-old control fruit exhibited an C_2H_4 peak 2 days sooner than 32-day-old control fruit which conforms with other C_2H_4 data for tomato fruit harvested at similar development stages[8]. The respiratory response of fruit treated with daminozide suggests that these fruit indeed experienced stress injury (figure 2). The lack of a clearly discernable climacteric in these fruit is probably due to the high amounts of CO_2 released after injury. Although there is a slight CO_2 peak at 42 days for treated fruit, it is not as evident as the climacteric exhibited by the controls (figure 2). However, if this peak truly represents a climacteric in treated fruit it occurred 6–7 days sooner than in the controls. When colour formation was monitored visually it began 6 days earlier in treated than in control fruit. Therefore, it appears that daminozide promotes certain parameters often associated with tomato fruit ripening.

Because daminozide delays the ripening process of apples[2,3], but enhances ripening of peaches[4,5] and apparently tomatoes, daminozide has different effects on different fruits which probably reflects different modes of action. The contrasting effects of daminozide on different fruits makes any speculation about its mode of action extremely difficult, especially since daminozide may participate in a variety of reactions at the cellular level. It has been suggested that daminozide may induce ripening of peach

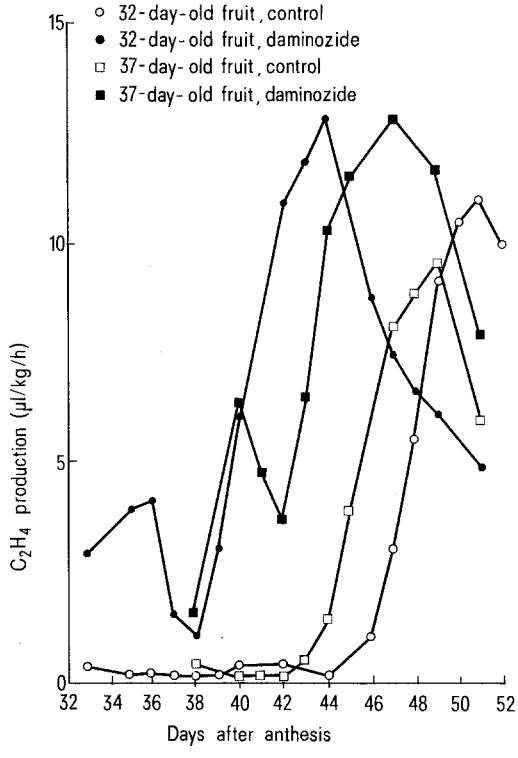

Fig. 1. Effect of daminozide on C_2H_4 production of tomato fruit harvested 32 and 37 days after anthesis.

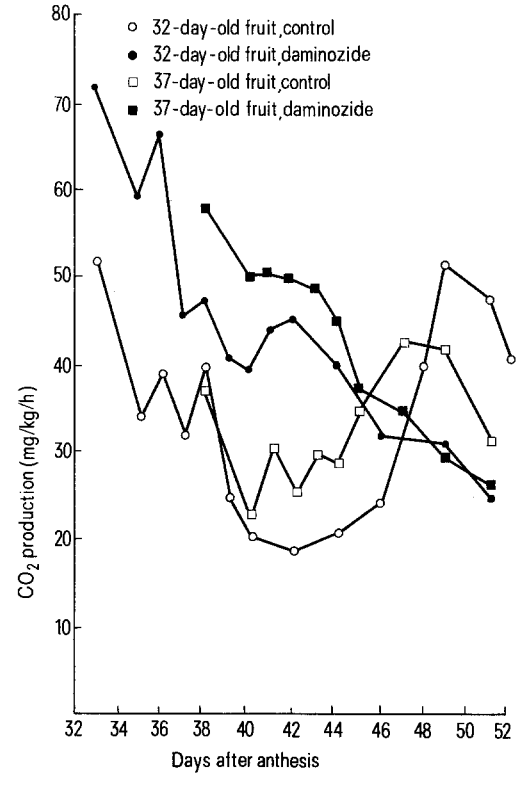

Fig. 2. Respiratory behaviour of tomato fruits harvested 32 and 37 days after anthesis and treated with daminozide.

fruits by suppressing the gibberellin level in the fruit[4]. This idea could be valid for daminozide-treated tomatoes[6]. Daminozide may also exert its effect on ripening by inhibiting tryptamine oxidation[9] or by increasing peroxidase and IAA oxidase activities[10] thus, leading to a lower level of auxin. Recently, the onset of ripening has been attributed to oxidative turnover of auxins in the fruit[11]. In tomatoes daminozide may also reduce auxin synthesis and stimulate auxin breakdown which may, in turn, enhance the ripening process. Nevertheless, the indication is that daminozide may function in the regulation of fruit ripening by affecting more than one of the naturally occurring plant hormones.

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Responses of *Drosophila* to environmental ethanol from ecologically optimal and extreme habitats

P.A. Parsons[1]

Australian Drosophila Research Unit, Department of Genetics and Human Variation, La Trobe University, Bundoora (Victoria 3083, Australia), 30 November 1979

Summary. Adult tolerances and larval behaviour in the presence of ethanol is more variable in populations derived from optimal habitats than those from extreme habitats. Since the habitat types are defined ecologically in terms of climatic and biotic factors, this result has biological significance for defining the properties of populations from extreme habitats.

Recent studies provide comparative data on resource utilization of *Drosophila* species attracted to fermented-fruit baits[2,3]. For example, ethanol is a fermentation product utilized as a resource to a threshold where it becomes toxic in all such species so far tested[4-7]. *D. melanogaster* shows variability for ethanol tolerance inside and outside a winery, as well as before, during and after vintage, which can be directly attributable to ethanol as a selective factor[8,9]. At the broad biogeographic level, the threshold between ethanol as a benefit and cost tends to fall towards the tropics in the northern hemisphere[10]. Since resources vary among localities these variations are not surprising.

Comparing 3 Australian east-coast localities, Melbourne, Brisbane and Townsville, LT_{50} values on 12% ethanol fit the northern hemisphere trend of falling ethanol thresholds towards the tropics (table). On biogeographic grounds, a greater diversity of resources would be expected in tropical compared with temperate zones[12], so that the threshold fall may be expected to be associated with increasing variability. Comparisons among isofemale strains within populations, which is a quantitative genetic technique of value in comparing natural populations[13], confirm this prediction (table).

Larvae, at the stage of maximum feeding, are additionally good indicators of resource utilization[14]. As expected the mean number of newly-hatched larvae out of 10 choosing ethanol in 15 min falls towards the tropics for the same 3 localities, and variability among isofemale strains increases especially for Townsville.

Townsville has a non-stressful humid tropical climate, as has Brisbane to a lesser extent, when compared with the temperate climate of Melbourne where temperature extremes are greater. The finding of parallel climatic races in *D. melanogaster* and its sibling species, *D. simulans*, whereby Melbourne populations are more resistant to both desiccation and cold stresses than Townsville populations show the importance of direct climatic selection upon *Drosophila* populations[15]. The number of sympatric *Drosophila* species commonly attracted to fermented-fruit baits

Adult ethanol tolerances and larval preferences for ethanol in *D. melanogaster*

	Latitude (°S)	No. of isofemale strains	LT_{50} for adults on 12% ethanol (h)	F-values for variability among isofemale strains	No. of larvae out of 10 choosing 6% ethanol	F-values for variability among isofemale strains
Melbourne	37	8	60	8.3[b]	8.2	1.6
Brisbane	28	9	29	12.0[b]	6.8	3.1[a]
Townsville	19	9	9	70.8[b]	6.4	4.7[b]
Darwin	12	10	41	8.2[b]	5.4	2.2

Adult tolerances are expressed as mean LT_{50}'s (number of h at which 50% of flies had died) exposed to 12% ethanol using techniques described by Parsons et al.[11], based upon 5 replicates of 25 flies per sex per isofemale strain. Larval preferences are expressed as means of the number of newly-hatched larvae out of 10 choosing agar containing 6% ethanol after 15 min when given a choice of plain agar and ethanol containing agar (8 replicates per isofemale strain). [a] $p<0.05$; [b] $p<0.01$.