In mitochondria, the LFP content was almost the same on the 3rd post-irradiation day as it was 1 h after irradiation. Nonsignificant tendencies to a decrease (92%) and to an increase (135%) followed. However, a significant decrease of mitochondrial LFP was recorded on day 18 (54% of initial value).

Discussion. LFP formation is associated with lipid peroxide formation. As a stable product, LFP exert a deleterious effect for a long time and their accumulation can lead to cell death[7]. Thus the mechanisms which prevent their accumulation are of vital importance for the cell. Up to now, centrophenoxine is the only drug known to eliminate the LFP from cells[8], but the mechanism of this effect has not been elucidated yet.

Mitochondria are well protected against lipid peroxidation by gluthathione peroxidase which inhibits malondialdehyde formation[9,10]. This action of glutathione peroxidase could explain why after the initial burst of LFP formation a further increase of LFP was observed in the homogenate, but not in mitochondria. A diffusion of malondialdehyde from cytoplasm[11] might participate in the slight LFP increase observed in mitochondria from the 7th to the 14th post-irradiation day. It is suggested that the terminal LFP decrease in the mitochondria of irradiated rats is due to an enhanced elimination of LFP from mitochondria and/or the cessation of malondialdehyde production in the cytoplasm. The observed decrease of LFP during the time after irradiation deserves further study, as it could bring more understanding of the mechanisms leading to LFP elimination.

LFP content in rat liver homogenate and mitochondria during the time after irradiation

Time after irradiation	LFP ($\bar{x} \pm$ SEM), relative units		Homogenate / Mitochondria
	Homogenate	Mitochondria	
Control	5.76±0.33	2.83±0.41	2.04
1 h	8.57±0.27**	4.30±0.10**	2.00
3rd day	9.84±0.30**	4.23±0.15*	2.33
7th day	11.81±0.72**	2.61±0.18	4.52
14th day	9.99±0.53**	3.81±0.32	2.62
18th day	9.19±0.82**	1.52±0.04**	6.05

$\bar{x}$, Mean value of 6 animals. *$p<0.05$; **$p<0.01$ (related to the control group).

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Inhibition of insect larval growth by phenolics in glandular trichomes of tomato leaves

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Summary. We have found that the foliar tetracellular glandular trichomes (tetrads) of the tomato plant, *Lycopersicon esculentum* Mill., contribute significantly to the antibiotic effect of the leaf against the fruitworm *Heliothis zea* (Boddie), as measured by reduction in larval growth. This effect is attributable to phenolic compounds localized within the tetrads. We have found that the cellular fluid of the tetrads is particularly rich in the flavonol glycoside rutin, accompanied by lesser amounts of other phenolics.

In both natural and agro-ecosystems, the trichomes of several plant species are known to contribute to defense against herbivorous insects[2]. It is known that high densities of 4-lobed glandular trichomes on certain commercial cultivars of tomatoes and related wild species of *Lycopersicon*, can enhance resistance to several pests[3]. Often leaf hairs function as a physical barrier to insects' movement, feeding, and/or oviposition, or as an enmeshing trap in which small insects die from dessication or starvation[4]. The physical trapping of small insects on tomato foliage (whiteflies, flea beetles, aphids, or mites) is often accomplished via a sticky exudate released on breakage of the fragile trichomes[5]. Glandular trichomes of a similar type have been shown to protect commercial cultivars and related wild species of the potato, *Solanum tuberosum*[6] against some insect pests. However, many plants' glandular trichomes contain secondary compounds[7] that are potentially toxic or deterrent to phytophagous insects. Our analyses of tomato leaf tetrads quantify the toxic effects of phenolics on a larval insect pest.

The chemical composition of the tomato leaf tetrads was determined by rubbing (without crushing) leaves and stems of field-grown *L. esculentum* (vars. VF 145, Ace 55, VF 198, UC 134, VF 315, VFN Bush, Royal Flush) with tissue papers. The fluid from the shattered tetrads was subsequently eluted with 70% methanol, and evaporated in vacuo, yielding a bright yellow precipitate. TLC on cellulose or polyamide[8] or column chromatography with Sephadex LH-20[9] revealed a mixture of about 10 phenolics. Rutin (quercetin-3-rutinoside), by comparison with TLC mobilites and UV-spectra of a standard, was identified as the major phenolic (80–90%) admixed with a lesser amount of an unidentified catechin (5%) and, traces of chlorogenic acid, other conjugates of caffeic acid, and several uncharacterized flavonoid glycosides.

We estimated the density of tetrads on the upper and lower (adaxial and abaxial) surfaces of tomato leaves of several cultivars by replicated direct counting of tetrads using an ocular guide on a dissecting microscope. Amongst the 6 cultivars, the density of adaxial tetrads ranged from 1200/

cm^2 to 3300/cm^2, and the abaxial from 450 to 1000/cm^2 (fig. 1). The phenolic content in the adaxial tetrads/cm^2 was estimated by firmly pressing a filter paper disc (0.75 cm diameter) onto the leaf surface to absorb the fluid from the crushed tetrads, and then placing the disc into an ammonium molybdate reagent[10] for direct colorimetric determination.

By this method, we estimated that the phenolics in the adaxial tetrads, dependent upon cultivar, range from about 6 to 32% of the total phenolic content of the leaf. TLC analyses of leaf extracts with tetrads removed, showed that rutin and other phenolics are not just localized within the tetrads, but are also present in the leaf tissue at significant levels. There is no correlation between the total foliar phenolic content of any cultivar and its densitiy of tetrads. Interestingly, the glycoalkaloid α-tomatine, a major constituent of tomato foliage and green fruits[11], is absent from the tetrad fluid, as determined by a blood hemolysis assay[12]. Furthermore, we could not detect phenolase in the tetrad fluid, hence the 'sticky exudate' to which small insect adhere in tetrad fluid is not the result of oxidative polymerization, but probably merely gumming or hardening on drying.

Since both rutin and its aglycone quercetin are known to reduce larval growth of *Heliothis zea*[13], we sought to determine whether or not the tetrad fluid, rich in rutin and other phenolics, would also inhibit growth of *H. zea*. Purified rutin, or manually collected tetrad phenolics were mixed into semi-defined artificial diets at 4 different concentrations representing the biological range of total phenolics in tomato foliage (0.1–0.4% wet wt). Newly hatched 1st instar larvae were allowed to feed on the artificial diets for 8 days and then weighed. From this, we generated dose-responses for both rutin and the tetrad fluid (fig. 2), indicating that the tetrad phenolics are essentially equitoxic to technical rutin. Hence, the tetrads in planta could give rise to significant levels of antibiosis. To test this hypothesis, larvae of *H. zea* were provided either intact tomato leaflets (var. Royal Flush), or leaflets from which the tetrads had been removed with a cotton swab. Over a 9-day feeding period, larval growth (weight gain) was significantly poorer on the intact leaflets (over 60% reduction), compared to that on the leaflets from which tetrads had been removed (table). We do not as yet understand the mode(s) of action of rutin or other phenolics on larval *H. zea*. Our results rule out inhibition of feeding behavior because there was no measureable reduction in consumption rates of diets by larvae, even in diets containing as much as 1.0% (wet wt) rutin (unpublished results). The mode(s) of action of rutin appear then to be the result of interference with some internal physiological process involving larval growth.

Although these findings suggest that the presence of high densities of glandular trichomes on tomato plants may constitute a source of resistance in the field, there is no correlation between relative tetrad density and relative antibiosis (ability to support larval growth of *H. zea*) amongst the cultivars we have studied (unpublished data). Therefore, in determining relative resistance to *H. zea*, other chemical and/or physical factors in the foliage obscure the contribution of tetrad fluid to resistance.

Thurston[14] documented the toxicity of trichome exudates from species of *Nicotiana* and *Petunia* to larvae of the tobacco hornworm *Manduca sexta*, but the toxicity which he observed represented acute toxicosis from direct body contact with the exudates. Similarly, leaf trichome exudates from the tomato have been shown to have been topically toxic to the spider mite *Tetranychus urticae*[15]. A morphologically different type of glandular trichome on the wild tomato *L. hirsutum* contains 2-tridecanone, which is topically acutely toxic to *H. zea* and *M. sexta*[16]. However, only

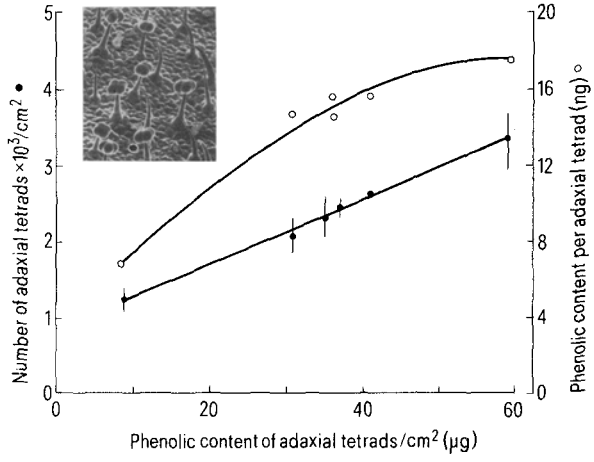

Fig. 1. Relationship between tetrad density (●) and phenolic content per tetrad (○) and phenolic content of tetrads per unit area of tomato leaf. Each point represents a different cultivar; error bars indicate the 95% confidence interval about the mean. Varieties from left to right of X-axis are VF 198, Royal Flush, VF 315, Ace 55, VFN Bush and UC 134 for both ○ and ●. Tetrad density was measured by direct counting, phenolic content per unit area by the paper disc method (see text), and phenolic content per tetrad estimated by dividing phenolic content per unit area by the tetrad density of that area. Inset: scanning electron micrograph of upper surface of tomato leaf (var. Ace 55) with tetrads (≈ approximately 50 µm in diameter) clearly visible (magnification × 100).

Larval growth of *H. zea* on intact tomato leaflets (var. Royal Flush) and on leaflets from which tetrads had been removed

	Intact leaflets	Leaflets without tetrads
Larval weight (mg)*	26.7 ± 4.2**	73.1 ± 13.2**

* Values are means ± SE for 14 and 16 larvae, respectively.
** Significant difference, $p < 0.05$. Larvae were reared individually in plastic petri dishes in an environmental cabinet at 30 °C, approximately 90% relative humidity and 16L:8D. Fresh plant material was provided daily; duration of experiment = 9 days. In this variety of *L. esculentum*, phenolic content in the tetrads constitutes approximately 15% of the total phenolic content of the leaf.

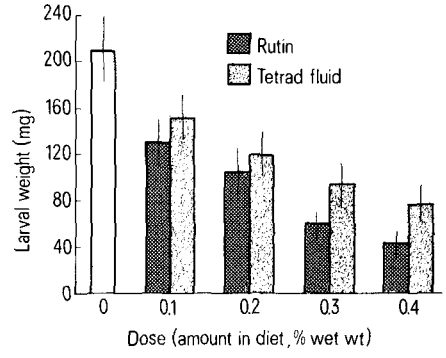

Fig. 2. Inhibition of larval growth of *H. zea* by addition of technical rutin or tomato tetrad fluid to artificial diets. Histograms represent means of 26–30 larvae; error bars indicate the 95% confidence interval about the mean. Unshaded bar represents control diet (no additives). Rearing conditions as described in the table, duration of experiment = 8 days. Concentrations of approximately 0.25% wet wt reduce growth by 50% relative to control.

minute quantities of this compound occur in commercial tomatoes, such as those in our study. In contrast, our findings constitute an unambiguous case of chronic toxicity to an insect (inhibition of larval growth) by ingestion of 'secondary compounds' from trichomes without a concomitant inhibition of feeding. Thus, in *L. esculentum*, trichomes on leaves and other organs may provide multiple defensive functions (physical and/or chemical) against different insect herbivores attacking this plant species.

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Acetaldehyde: a low-concentration resource and larval attractant in 3 *Drosophila* species

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Summary. Acetaldehyde is used as an energy source and attracts larvae up to low concentrations where it becomes a stress and a larval repellant, in *D. melanogaster*, *D. simulans*, and *D. immigrans*. This result is expected, since acetaldehyde is an intermediary compound between ethanol and acetic acid, both of which are utilized as resources and attract larvae to varying thresholds according to species and genotype.

Ethanol and acetic acid are normal energy sources in *Drosophila* species attracted to fermented-fruits in nature[2-5] to thresholds where they cease to be resources and become stresses. The threshold ranking for 3 sympatric *Drosophila* species from Melbourne, Australia is *D. melanogaster* > *D. simulans* > *D. immigrans* for both metabolites[6], which is expected because of their close metabolic association[7]. Indeed, the concentrations of the 2 metabolites tend to be correlated in nature[8], so that parallel utilization patterns would be predicted to occur through natural selection.

The threshold ranking between larval attraction and avoidance follows the same sequence. In addition, an alcohol dehydrogenase-null mutant[9] of *D. melanogaster*, Adh^{n2}, utilizes ethanol to an extremely low threshold, while acetic acid is utilized to a threshold close to that of the *D. melanogaster* population[10]; this predictable result is paralleled by larval attraction to acetic acid but not ethanol[10].

Acetic acid is normally formed from ethanol via acetaldehyde, and thence to products producing energy. Even though acetaldehyde is often regarded as highly toxic[11], low concentrations presumably occur in nature. Here we show

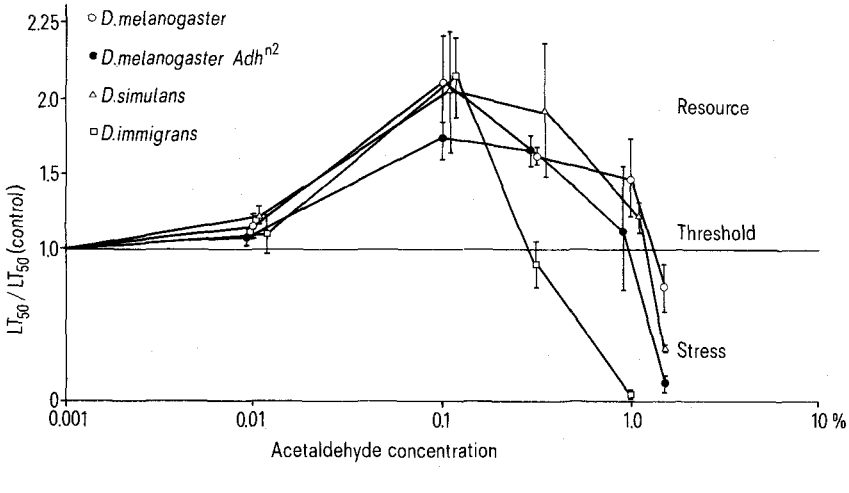

Fig. 1. Adult survivorship expressed as the ratio LT_{50}/LT_{50} control for 5 replicates of 20 flies (10 per sex) per acetaldehyde concentration tested for each species and genotype. The vertical bars indicate 95% confidence limits. The intersection of the plots with the horizontal straight line gives the threshold concentration between acetaldehyde as a resource and as a stress. Mean LT_{50} control life spans were *D. melanogaster* 52 h, *D. melanogaster*, Adh^{n2} 49 h, *D. simulans* 34 h, and *D. immigrans* 47 h.