flies by topical application. The results of our toxicity studies are presented in this paper.

All insecticides used were of technical grade having purity above 90%. Solutions of these insecticides of desired strength were prepared in acetone and applied topically to 3–4-days-old female houseflies using an aglamicrometer syringe as described earlier[4]. The housefly culture which we are rearing in our laboratory for the past 30 years was used for the toxicity studies. The normal houseflies were fed citric acid through diet (0.1%) from the day of emergence right up to F_2 generation. In each generation the toxicity of the four insecticides permethrin, bromophos, malathion and lindane was tested against 3–4-days-old female flies. The results were subjected to probit analysis and from the regression equations obtained, LD_{50} values were calculated. These are presented in Table 1. For citric acid determination the flies were homogenized with 5% trichloroacetic acid and citric acid was determined by colorimetric method of Natelson *et al.*[5]

Table 1 indicates a gradual fall in LD_{50} values of all the four insecticides right from the present up to F_2 generation. This fall in LD_{50} values of the insecticides was associated with a simultaneous increase in the citric acid content of the flies. The citric acid content of the normal houseflies was 0.68 μM/g. This quantity increased gradually, and in F_2 generation the citric acid content was 1.976 μM/g (Table 2), almost 3 times that of normal flies. The increase in citric acid content was presumed to be due to citric acid feeding. Interestingly, when citric acid mixed with the insecticides was applied topically to normal flies no significant change in the LD_{50} values of insecticides was observed suggesting that citric acid did not act as a synergist. The other changes which were produced presumably due to citric acid feeding during the experimental period, were delayed egg laying, reduction in the number of eggs laid and a slight prolongation of the life cycle.

The mechanism by which citrate feeding reduces insect resistance to insecticides is difficult to explain. Zahavi and Tahori[3] reported that an age-associated citrate increase in houseflies and ceratitis in their work was probably due to discrepancy in the rate of citrate production and utilization at different stages. Since citrate is not acting as a synergist in the present case, the decrease in resistance must be due to some metabolic changes or hindrance. It is possible that the formation of normal metabolic citrate may be suppressed due to higher citrate concentration produced by citric acid feeding. This will naturally create a sort of hindrance or slow down the glycolysis rate thus producing less energy than normal for work. The ultimate result of this low energy production will be the reduced resistance of the insects to insecticides.

The present finding that dietary citrate reduces housefly resistance to insecticides provides useful information for pesticide scientists. This suggests that the development of resistance by insects to insecticides may be reduced possibly by including in the pesticide formulation a chemical like citric acid or a member of citric acid cycle (a citrate former) or some other similar chemical. However, a lot more experimental work has to be done before drawing any conclusion in this matter.

Table 2. Citric acid content of 3-days-old female houseflies.

Housefly	Citric acid μM/g
Normal housefly	0.68
Fed Citric acid for 3 days	1.237
Citric acid-fed-F_1 generation	1.613
Citric acid-fed-F_2 generation	1.976

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C_3-like carbon isotope discrimination in C_3–C_4 intermediate *Alternanthera* and *Parthenium* species

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Carbon isotope discrimination and leaf anatomy in relation to leaf position on the stem were studied for the two recently identified naturally occurring C_3–C_4 intermediate dicot plants, *Alternanthera ficoides* and *Parthenium hysterophorus*. The carbon isotope ratios ($\delta^{13}C$ values) for the two C_3–C_4 intermediate species did not change much with the leaf age but were similar to C_3 plants. Although variation in leaf anatomy with respect to leaf age was noted, the carbon isotope ratios were similar at all stages of leaf growth. These results also confirm that *A. ficoides* and *P. hysterophorus* possess leaf anatomical characteristics intermediate to those of C_3 and C_4 dicot plants but exhibit C_3-like carbon isotope discrimination.

THE existence of naturally occurring plant species showing intermediate characteristics to those of C_3 and

C_4 plants has received considerable attention. Detailed studies on the physiology and biochemistry of photosynthesis and photorespiration in C_3–C_4 intermediate plants are highly useful in understanding the possibility of increasing photosynthetic efficiency of C_3 crops through chemical or genetic modifications of these plants. Further, the study of C_3–C_4 intermediate plants might also provide insight into the precise evolutionary transition from a C_3 to a C_4 plant. Naturally occurring C_3–C_4 intermediate plants have been identified in many genera, *Mollugo*[1], *Panicum*[2–5], *Moricandia*[6,7], *Flaveria*[8–10], *Neurachne*[11,12], *Alternanthera*[13] and *Parthenium*[14,15]. The intermediate nature of these plants includes a Kranz-like leaf anatomy, partially suppressed photorespiration as indicated by reduced CO_2 compensation point and in biochemical process of photosynthesis between those of C_3 and C_4 plants. Although a detailed survey on $\delta^{13}C$ values for a number of C_3 and C_4 plants clearly showed exclusive ranges, -21.1 to -36.7% and -9.2 to -19.3%, respectively[8,16–19], the naturally occurring C_3–C_4 intermediate plants studied till now exhibited these values within the C_3-plant range[6,8,16,17,20–23]. Some C_3–C_4 intermediate *Flaveria*[21,22] and *Neurachne*[17] exhibited higher $\delta^{13}C$ values than the other known C_3–C_4 intermediate plants, but these values were within the range of C_3 plants. Such higher $\delta^{13}C$ values found in some C_3–C_4 plants in comparison to others may be explained on the basis of functionally limited C_4 type of photosynthetic carbon assimilation exhibited by these species[24].

In the present study, the carbon isotope discrimination and the leaf anatomy in relation to leaf age in the newly reported C_3–C_4 intermediate *A. ficoides* and *P. hysterophorus* species were studied in an attempt to understand the mechanism(s) for reduced photorespiration found in these C_3–C_4 intermediate species.

Plants of *Alternanthera ficoides* (L.) R. Br. ex. R. and *Parthenium hysterophorus* L. were raised from vegetative cuttings and seeds, respectively. These were grown on soil supplemented with manure (three parts of soil and one part of farmyard manure) in 30 cm clay pots under 11 h natural photoperiodic conditions. Plants received full sunlight for most of the day during the growth period. The maximum light intensity (PAR, 400–700 nm) available at the top of the canopy on a clear day was about 1800 μmol quanta $m^{-2}s^{-1}$. Daily maximum and minimum air temperatures had ranges 28 to 35°C and 16 to 25°C, respectively. Plants were watered every alternate day to avoid water stress effects throughout the growth of the plant. Other plant species used in the present study for comparison were also grown under similar conditions. Four-to-five weeks old plants were used in the present study.

Leaf segments measuring about 0.5×0.5 cm area were cut from leaves at different positions on the stem and used to study the leaf anatomy. Leaf tissues were initially fixed in formalin, aceticacid and ethanol (70%) mixture (5:5:90, v/v), dehydrated in ethanol series and embedded in paraffin wax (melting point, 58–60°C). Leaf cross-sections with 10 μm thickness were cut on Cambridge rotary microtome, dewaxed in xylol and stained with safranin and fast green. These stained leaf cross-sections were examined under a light microscope.

For determination of $\delta^{13}C$ values, fresh leaves were collected and dried in a forced air draft oven at 50°C and ground into a fine powder. The dry leaf powder was cumbusted at 800°C in excess oxygen. The carbon isotope ratio ($^{13}C/^{12}C$) of CO_2 evolved from cumbustion was measured on an isotope ratio mass spectrometer. Results were expressed as $\delta^{13}C = (R_{sample}/R_{standard} - 1) \times 1000$, where R_{sample} and $R_{standard}$ are mass 45/mass 44 ratios of CO_2 evolved from cumbusted leaf dry powder and standard (PDB), respectively.

Mature leaves of *A. ficoides* and *P. hysterophorus* showed prominent chloroplast-containing bundle sheath cells surrounding the vascular tissue, but these cells were not encircled by a distinct radially arranged mesophyll cells like in C_4 species (*A. pungens*). Instead, the mesophyll tissue of these plant species was differentiated into definite palisade and spongy parenchyma as found in typical C_3 species, *A. sessilis* (Table 1). However, the older leaves of *P. hysterophorus* showed a tendency to radial arrangement of mesophyll cells which can be compared to radial arrangement found in C_4 species, *A. pungens* (Table 1).

Leaf carbon isotope ratios ($\delta^{13}C$ values) for the two species, *A. ficoides* and *P. hysterophorus*, showing Kranz-like leaf anatomy at older stages of leaf growth were similar to those of C_3 species, *A. sessilis* and *E. alba* (Table 1). Although some variation in the leaf anatomy with respect to leaf position on the stem was noted, the $\delta^{13}C$ values were similar at all stages of leaf growth in these plants (Table 1). In the past, a significant difference in the range of $\delta^{13}C$ values between C_3 and

Table 1. Leaf anatomy and carbon isotope ratios ($\delta^{13}C$ values) of leaves at different positions on the stem of two newly reported C_3–C_4 intermediate species *Alternanthera ficoides* and *Parthenium hysterophorus* and a few representative C_3 and C_4 dicot plants.

Plant species	Leaf position on the stem	Leaf anatomy		Leaf carbon isotope ratios, $\delta^{13}C$ (%)
		Type	Mesophyll	
Alternanthera ficoides	Top	Non-Kranz	C_3 type	−25.7
	Middle	Kranz-like	C_3 type	−25.5
	Base	Kranz-like	C_3 type	−25.6
Parthenium hysterophorus	Top	Non-Kranz	C_3 type	−25.8
	Middle	Kranz-like	C_4 type	−27.8
	Base	Kranz-like	C_4 type	−27.4
Alternanthera sessilis	Middle	Non-Kranz	C_3 type	−26.4
Eclipta alba	Middle	Non-Kranz	C_3 type	−26.7
Alternanthera pungens	Middle	Kranz	C_4 type	−11.5

C_4 plants has been reported[8,16–18,21,22]. These differences between C_3 and C_4 plants were mainly attributed to preferential utilization of ^{12}C and partial exclusion of ^{13}C during the initial carboxylation step in photosynthesis of these plants[25,26]. Less discrimination against $^{13}CO_2$ by PEP carboxylase than by RuBP carboxylase is believed to result in the differences in $\delta^{13}C$ values observed between C_3 and C_4 plants[25]. Since PEP carboxylase performs the initial carboxylation step of photosynthesis in C_4 plants, the $\delta^{13}C$ values reported for these plants are far less negative than those of typical C_3 plants which exhibit initial carboxylation step by RuBP carboxylase.

Earlier studies have shown that the PEP carboxylase activity in leaf extracts of *A. ficoides*[13] and *P. hysterophorus*[14,27] was slightly higher than in typical C_3 plants. C_3-like $\delta^{13}C$ values of the two recently identified C_3–C_4 intermediate species, *A. ficoides* and *P. hysterophorus*, reported here have clearly indicated that slightly higher levels of PEP carboxylase reported earlier may contribute less in reducing photorespiration as well as overall growth in these intermediate species. Further, C_3-like $\delta^{13}C$ values obtained for *A. ficoides* and *P. hysterophorus* in the present study clearly indicate that the contribution of C_4 type of photosynthetic carbon assimilation in reducing photorespiratory CO_2 loss is very unlikely in these C_3–C_4 intermediate species.

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Crystal structure of putrescine aspartic acid complex

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Polyamines, putrescine, spermidine and spermine are ubiquitous biogenic cations believed to be important for a variety of cellular processes. In order to obtain structural information on the interaction of these amines with other biomolecules, the structure of a complex of putrescine with aspartic acid was determined using single crystal X-ray diffraction methods. The crystals belong to monoclinic space group C2 with $a=21.504$ Å, $b=4.779$ Å, $c=8.350$ Å and $\beta=97.63°$. The structure was refined to an *R* factor of 8.4% for 664 reflections. The asymmetric unit contains one aspartic acid and half putrescine molecule. The conformation of aspartic acid corresponds to its most favourable extended structure. The putrescine molecule, although on a 2-fold special position, lacks 2-fold symmetry. The putrescine backbone has a *trans-gauche* conformation. The energy required for distorting the putrescine molecule from its most favourable zigzag structure is presumably derived from both hydrogen bonding and electrostatic interactions.

PUTRESCINE, spermine and spermidine are ubiquitous biogenic amines believed to be important for a variety of cellular functions. It has been suggested that they are important for the structural integrity of certain biomolecular assemblies[1,2], protein synthesis[3], cell differentiation[4], flowering in plants[5] and many other biochemical phenomena. Spermidine is known to enhance the efficiency of *in vitro* translation[6]. Usually, inorganic cations either fail to fulfill the role of polyamines, or, are required at much higher concentrations[1]. In order to understand the functional role of polyamines, it is necessary to determine the structure of a large number of complexes of these amines with other ubiquitous biomolecules. These studies will provide information on the conformational flexibility of the polyamine backbone and on their preferred bonding patterns. We have earlier reported the structure of