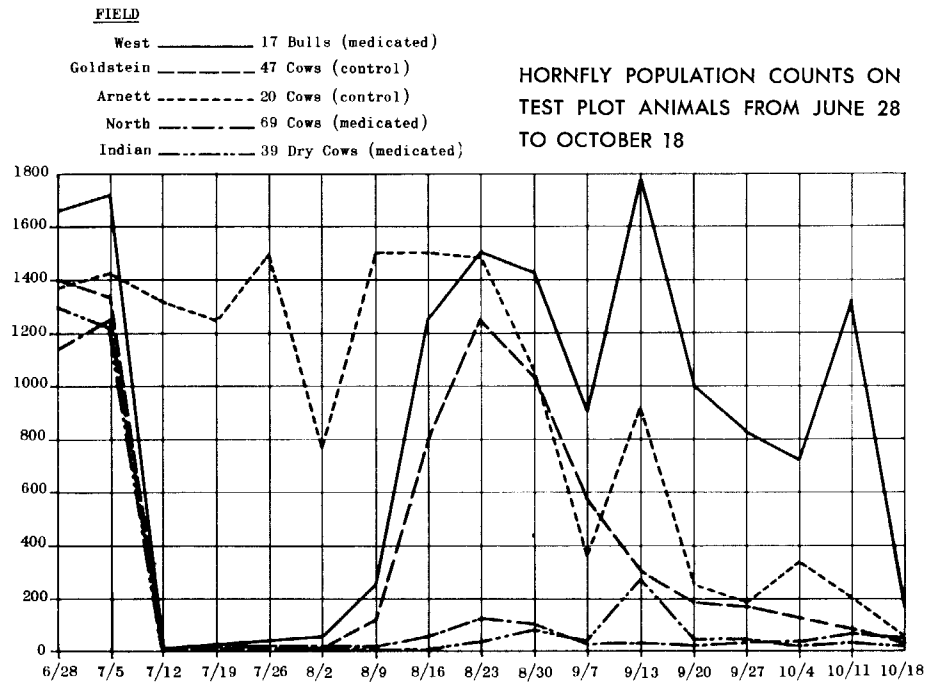


hornfly development. Manure from the Arnett cows had the highest number of immature-stage flies while manure from the North and Indian herds showed much less hornfly development throughout.

On March 1 and 2, 1966, the backs of 55% of the treated cows from the North field, and 48% of the cows from the Goldstein control field, were examined for grubs. In the treated group, four head had one grub each and one head had two grubs for an average of 0.18 grub per head. The 23 head counted in the control group had a total of only 40 grubs or an average of 1.7 grubs per head (range, 0 to 4).

Ronnel is not yet recommended by University of California for systemic control of hornflies or grubs on range cattle.

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# ORGANIC ACID SYNTHESIS IN LEMON FRUITS

E. BOGIN • A. WALLACE

**Q**UANTITY OF LEMONS and of other citrus fruits is affected by the concentrations of acids in the fruit juice. For this reason information concerning the synthesis and accumulation of the acids is important and has been the subject of intensive studies. Until recently organic acids were thought to be synthesized in leaves and then later translocated to the fruit. Sufficient biochemical systems have now been isolated from fruits to warrant the conclusion that lemon and other citrus fruits are quite capable of the acid synthesis.

## Major problem

One of the major problems in the studies involved isolation of the enzyme systems from the highly acid citrus fruits. This was accomplished by the proper use of buffers and special grinding techniques. Mitochondria, which have the ability to oxidize all the tricarboxylic acid cycle intermediates, were prepared from lemon fruits. Soluble enzymes which have the capacity to fix carbon dioxide into organic acids were also isolated from the fruit. Fixation of carbon dioxide is one of two mechanisms that can result in

a net synthesis of organic acids in cells. These particular results demonstrated that the organic acids could be synthesized in fruits.

Many biochemical mechanisms have been explored by the use of mutants, particularly mutants of microorganisms, and this approach was applied to the study of acid synthesis in lemon fruits. Comparative studies were made with Eureka lemon and Tunisian sweet lemon fruits (see table). The latter is generally considered to be a mutant of a sour lemon. In the mutant, some biochemical

DIFFERENCES IN SOUR AND SWEET LEMON FRUITS

Characteristics	Sour Lemon	Sweet Lemon
Organic acid content, % of fresh weight	5-9	less than 1
pH	2.5-3.0	5.0-5.5
Amino acids, mg/ml juice	0.5	1.5
Oxygen uptake by intact vesicles, $\mu$ l O <sub>2</sub> /hr/gram fresh weight	191	282
Oxygen uptake by mitochondria with succinate, $\mu$ l O <sub>2</sub> /hr/mg protein nitrogen	230	318
CO <sub>2</sub> fixation with radioactive C <sup>14</sup> O <sub>2</sub> by phosphoenolpyruvic acid carboxylase, cpm/mg protein nitrogen	36660	66230
by isocitric dehydrogenase, cpm per aliquot	4770	1620
Amination of pyruvate	low	high
Main amino acid	aspartate	alanine
Catalase activity	low	high
Citramalate production	high	low

mechanism which regulates acid accumulation has obviously been lost. By comparing biochemical reactions in the regular lemon with those in the mutant, we hope to learn how acid synthesis is achieved in the regular lemon.

**Organic acids**

The total amount of organic acids in sour lemon juice is far greater than that in the sweet lemon. In contrast, the amino acid level in juice from sweet lemon is about three times as high as in the sour lemon. This could be an important factor, not only in determining the acid content of the juice but also in determining the pH of the juice. The pH of sweet lemon juice is much higher than that of sour lemon (see table). When the juice of both fruits was passed over a cation exchange resin to remove the amino acids, the resulting pH of the filtrate from both fruits was very nearly the same—sour lemon being 0.5 unit lower than the sweet lemon.

**Sweet lemon**

The sweet lemon, which is low in acids, had a greater oxygen uptake (respiration rate) than the sour lemon. An analogy is known with iron-deficient plant leaves where organic acids are increased and oxygen uptake is decreased (relative to green leaves). This provides a clue as to how the organic acid build-up is regulated, and the resulting hypothesis is being studied.

Intact fruit vesicles and biochemically active preparations from the vesicles readily fix carbon dioxide. There are several mechanisms by which this occurs, and one or more are of major importance in the net synthesis of organic acids. Both

malic acid content and the amount of carbon dioxide fixation decrease with age of fruit on both the fresh and dry weight bases. The sour lemon, which has the high acid content, had a higher capacity for carbon dioxide fixation than the sweet lemon, which has the low acid content. Distribution of activity among different carbon dioxide fixing mechanisms, however, is vastly different for the two fruits and one of the mechanisms may be the regulating factor.

A metabolic inhibitor, citramalate, was found to be synthesized in much greater quantities in preparations from sour than in those from sweet lemon. This compound is sufficiently similar to citric acid that it is a competitive inhibitor with citrate for the enzyme aconitase. This is a promising hypothesis to explain why citric acid accumulates in great quantities in the sour lemon.

**Cell storage**

When organic acids accumulate in the fruits, they are stored in a cellular compartment called the vacuole. A membrane separates the vacuole from the protoplasm of the cell. This membrane is extremely efficient because it must maintain a gradient of about 10,000 times for the hydrogen ion concentration between the two sides in sour lemon. Just how the cell can do this is also being studied.

Even though acids are most likely synthesized within the fruits, leaves do have a great effect in regulating the content. This observation was made by varying the number of leaves in proximity to a fruit by girdling the branches leading to a fruit. Whether the determining factor is the supply of photosynthate or of a regulator of some kind is not yet known.

The acid content of lemon and other citrus fruits is influenced by climate, rootstock, several different plant nutrients, and variety. These factors are of considerable importance in determining fruit quality. Continued studies may help to

better control the factors influencing organic acid contents of the fruits.

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