THE PHYSIOLOGY OF GROWTH IN APPLE FRUITS

VII. BETWEEN-TREE VARIATION OF CELL PHYSIOLOGY IN RELATION TO DISORDER INCIDENCE

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Summary

Mean cortical cell size, soluble and protein nitrogen per cell, preclimacteric respiration, mean fruit size, and incidence of disorders have been studied for fruit of each tree in a plot of 35 trees of Jonathan variety. These trees were remarkably uniform with regard to soil, aspect, tree size, and pollinating variety, but provided a range of mean fruit size per tree.

There was a high degree of correlation between the variables. Cortical cell size increased with mean fruit size but more rapidly than would be expected from a proportional increase with size of fruit. Protein nitrogen increased proportionally with cell volume but the ratio of protein nitrogen and cell surface increased with cell size, suggesting that the protoplasm increased in thickness with cell size. Intercorrelation between respiration per cell, protein nitrogen, soluble nitrogen, and cell size were particularly close, remaining highly significant even when mean fruit size per tree was held constant by methods of partial correlation analysis, suggesting that these characteristics are functions of cell growth and are not influenced by between-tree differences due to cropping. Disorder incidence is correlated with the other variables and the implications of these relationships are discussed.

I. INTRODUCTION

The keeping quality of fruit is determined while it is on the tree. Study of the between-tree variation in fruit physiology under uniform cultural conditions may thus be a useful approach to the improvement of keeping quality.

Earlier work (Martin 1954) showed that the best index of susceptibility to disorder in fruits from different trees was the mean fruit size per tree. This was better than any of the other indices associated with ripening (e.g. acid and soluble solids concentration, starch or ground colour change) and it was not improved when combined with any of these indices. Martin and Lewis (1952) showed that between varieties, cell volume, respiration per cell, protein nitrogen per cell, and respiration rate per unit protein (Hulme 1951) were positively correlated.

Robertson and Turner (1951) showed that, in fruit maturing on a single tree, protein synthesis kept pace with cell enlargement and they put forward the hypothesis that higher protein contents made greater demands on the energy distributors of the cells and resulted in higher respiration rates.

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These findings suggested that a study of one variety over a range of crop sizes might help in the elucidation of the remarkably close relation between mean fruit size per tree and breakdown incidence, and might be a useful introduction to attempts to improve keeping quality.

II. Material and Methods

In the 1951-52 season a block of Jonathan trees, 30 yr old and exceptionally uniform with regard to tree size, soil type, and slope became available. It consisted of six adjacent rows of trees planted 16 by 16 ft extending across the orchard with Cleopatra variety for the next six rows adjacent. The mean crop per tree over 4 yr ranged from 600 to 1000 fruits. The trees received no manuring for the season 1950-51 and the 35 trees used for this study were not manured for the 1951-52 season.

On March 19, 1952 a sample of 20 fruits and another of 150-200 fruits were taken by a procedure designed to produce random sampling. The former was used to determine respiration rate at 25°C by a modification of the method of Eaves (1938), taking the mean rate for the 40-48 hr after picking as did Hulme (1951). This respiration was preclimacteric. From 10 of these fruits a transverse section was cut from the mid-cortex region of each fruit midway between stem and calyx, fixed in formalin-acetic-alcohol, stained with ruthenium red, and mounted in "Euparal." Mean cell size was determined on the basis of the work of Bain and Robertson (1951) and by a sampling method of cell ranking devised by McIntyre (1953). This method was about twice as efficient as random sampling for the same number of cells measured. The mid-cortical tissue of the 20 fruits was sliced and dried at 65°F, powdered, and stored in sealed jars at 32°F for protein analysis by the methods used previously (Martin and Lewis 1952).

The larger samples of 150-200 fruits were stored at 33-34°F for 7 months, when they were removed to room temperature. Mean fruit size was then determined and the fruits were examined for disorders immediately, and again after 2 wk at room temperature.

The use of samples of differing sizes introduces difficulties in the mathematical treatment which will be referred to later. Mean fruit size and disorder incidence are easily determined and on a sample of 150-200 fruits involve sampling standard errors relative to the mean of only $\pm$ 1 per cent. in mean fruit size and $\pm$ 3 per cent. in disorder.

Respiration rate and protein and soluble nitrogen contents are determined on samples of only 20 fruits; from a limited number of duplicates it is estimated that the coefficient of variation for bulked material from 20 fruits is of the order of 5·5 per cent.

Determinations of mean cell size from two samples of 10 fruits gave results which differed by 1 per cent. The sampling error in the determination of mean cortical cell size is of the order of $\pm$ 5 per cent. Mean cell size can be regarded in two ways: (*a*) as an estimate of mean cell size of the cortical tissue; and (*b*) as an estimate of mean cell size for the whole fruit. This estimate of mean cell size for the whole fruit is based on the work of Bain and Robertson (1951) and the validity of a comparison between trees requires the assumption that the cell size gradient within fruits did not differ between trees. As the range of mean fruit size between trees in this plot was not great (83-113 g), we consider this assumption justified, and a comparison of mean cell number per fruit to be possible by their methods.

Respiration rate cannot be determined from the cortical tissues only. However, the rate for the whole fruit cannot be very different from that for the cortical tissues only as the cortex is approximately 80 per cent. of the total respiring tissue.

III. Results

The data for the 35 trees are set out in Table 1, and correlation coefficients for a number of variables are given in Table 2. Some of the correlations are examined further by methods of partial correlation in Table 3.

(*a*) *Disorder Incidence and Fruit Attributes*

(i) *Mean Fruit Size.*—The correlation of mean fruit size and percentage breakdown provides further evidence for the accuracy of mean fruit size per tree as an index of breakdown susceptibility in the fruit.

(ii) *Cell Size.*—The correlation of cell size and disorder incidence supports the suggestion in an earlier paper in this series (Martin and Lewis 1952) that variations in disorder level might be related to difficulties of cell organization associated with cell growth.

(iii) *Respiration per Unit Protein.*—This attribute was determined prior to storage, and disorder incidence was the result of 7 months storage, during which respiration proceeded at some function of the prestorage rate. The correlation of these two variables, which are separated by such a long time interval, heightens the possibility that disorder incidence might be determined by difficulties in protein maintenance; the differences in respiration rate per unit protein over the storage period determining the relative depletion of reserves and the extent of breakdown.

(*b*) *Relation of Rots Developing in Store to Mean Fruit Size*

Positive correlation occurred between percentage rots visible when the fruit was removed from store and mean fruit size; with further infections during the period at room temperature, the correlation declined to insignificance. The relationship has not been noted before and while not relevant to the main theme, is reported here as a factor which should be considered in all experiments on rotting where different populations of fruit are compared.

(*c*) *Interrelation of Other Fruit Attributes*

(i) *Mean Fruit Size, Cell Size, and Cell Number per Fruit.*—There was a high positive correlation between mean fruit size and mean cell size but there

TABLE 1

FRUIT ATTRIBUTES OF PLOT OF 35 JONATHAN TREES

Mean Fresh Weight (g)	Disorder (%) = 100 − Sound	Breakdown (%)	Breakdown 2½ in. Fruit (%)	Rots in Store (%)	Jonathan Spot (%)	Cell Volume (cu. mm. × 10^{-5})	Cell Surface (sq. cm. × 10^{-4})	Cell Number per Gram	Respiration per Cell (mg × 10^{-11} CO_2/hr)	Protein Nitrogen per Cell (g × 10^{-14})	Respiration/Unit Protein (mg × 10^{-3} CO_2/g protein N/hr)	Soluble N/Cell (g × 10^{-14})
1	2	3	4	5	6	7	8	9	10	11	12	13
83·7	14·9	10·9	14·0	0·9	17·2	180	9·67	505	463	415	112	177
88·3	34·3	23·7	23·0	4·0	15·2	176	9·60	515	433	389	120	125
88·1	20·3	2·7	3·0	2·2	18·5	148	8·77	622	448	335	134	96
88·0	32·2	13·2	12·0	4·0	19·5	130	8·15	700	380	320	121	94
90·8	45·4	27·6	49·0	5·4	24·9	151	8·94	602	510	357	143	145
91·5	39·5	15·7	29·0	2·3	36·5	183	9·89	496	523	419	125	155
92·0	42·5	26·4	36·0	1·1	25·3	171	9·46	532	500	383	130	98
93·4	39·9	25·8	32·0	1·2	21·5	155	8·99	586	538	365	148	124
93·4	46·8	20·7	34·0	1·8	36·7	171	9·45	582	532	363	147	113
95·0	60·5	59·7	64·0	1·8	14·6	130	8·20	700	381	300	127	112
95·2	37·5	14·7	19·0	7·6	25·9	181	9·73	503	548	403	137	214
95·2	43·0	32·3	43·0	2·5	19·0	164	9·31	553	374	374	118	90
97·5	48·4	38·5	50·0	3·7	14·8	154	8·99	590	485	394	123	128
97·7	53·8	2·8	31·0	3·9	44·9	200	10·27	455	830	475	171	195
97·7	51·5	34·6	41·0	5·0	25·8	180	9·71	505	659	441	150	163
98·0	56·5	39·5	47·0	6·2	18·1	186	9·77	489	694	442	157	228
98·7	58·6	39·4	56·0	2·6	27·7	179	9·68	507	612	392	156	140
100·6	49·0	35·6	41·0	5·0	15·9	187	9·98	485	627	440	143	148
100·7	55·8	32·8	35·0	3·4	44·8	220	10·90	413	852	498	171	309
101·3	62·3	46·3	58·0	3·2	27·8	221	11·07	412	771	520	148	260
101·2	58·1	42·7	48·0	8·0	13·2	216	10·69	420	746	494	151	181
101·9	60·2	24·7	32·0	4·2	46·4	168	9·40	540	501	355	142	165
102·5	74·4	49·7	56·0	4·8	44·9	203	10·44	448	772	460	168	146
102·9	57·4	26·9	29·0	2·4	38·9	174	9·46	522	597	417	143	142
103·5	73·1	44·9	51·0	7·8	41·6	174	9·50	522	672	452	147	191
103·5	51·8	36·0	45·0	10·8	7·9	225	10·44	403	833	552	151	298
105·0	51·9	35·0	43·0	7·3	21·2	196	10·10	463	666	490	136	235
104·3	56·0	40·5	40·0	10·0	13·5	202	10·20	450	765	483	150	195
104·5	68·0	54·6	54·0	5·3	17·3	215	10·85	422	690	485	143	153
105·8	63·6	53·7	58·0	4·6	13·0	231	11·23	394	882	939	164	305
107·7	89·0	78·6	83·0	6·2	29·0	263	12·13	396	980	600	160	291
108·4	75·3	46·6	59·0	6·0	32·7	224	10·93	406	855	482	172	216
109·7	90·1	68·3	71·0	5·0	46·7	256	11·81	355	1085	605	172	287
112·7	83·8	82·3	82·0	6·8	6·2	250	11·60	364	959	531	181	262
112·7	80·0	69·8	66·0	6·8	16·7	217	10·51	418	900	551	163	240

was a significant increase in the residual variation if the fitted regression line of cell size on fruit size was made to pass through the origin. The mean cell size in the cortical tissues increased more rapidly than would have been expected from a proportional increase with the size of the fruit. If the mean cortical cell size is taken as an estimate of the mean cell size of the whole fruit, the converse aspect of this relation is the negative correlation of the total cell number with fruit size (see Table 2). This implies the possibility that the stimulus to cell division may be weaker in light-crop fruitlets; light-crop trees may have not only fewer fruit buds but less cell division in the fruits although they are larger. This point is being investigated further.

TABLE 2

CORRELATIONS OF VARIABLES

Correlation	r	Significance
Mean fruit size and percentage disorder	0·9034	$P<0·001$
Mean fruit size and percentage breakdown (all fruit)	0·7928	$P<0·001$
Mean fruit size and percentage breakdown ($2\frac{1}{2}$ in. fruit)	0·6505	$P<0·001$
Mean fruit size and percentage Jonathan spot	0·1003	N.S.
Mean fruit size and percentage rots developed during cool storage	0·6157	$P<0·001$
Mean fruit size and percentage rots at final examination	0·2505	N.S.
Mean fruit size and mean cell volume	0·7613	$P<0·001$
Mean fruit size and mean cell surface	0·7373	$P<0·001$
Mean fruit size and cell number per fruit	−0·4452	$P<0·01$
Mean fruit size and respiration per cell	0·8320	$P<0·001$
Mean fruit size and protein N per cell	0·7822	$P<0·001$
Mean fruit size and soluble N per cell	0·6677	$P<0·001$
Mean fruit size and respiration/protein	0·7480	$P<0·001$
Mean cell surface and protein N per cell	0·9328	$P<0·001$
Protein N per cell and respiration per cell	0·9371	$P<0·001$
Protein N per cell and R/P ratio	0·6705	$P<0·001$
Protein N per cell and soluble N per cell	0·8661	$P<0·001$
Protein N per cell and percentage breakdown	0·7010	$P<0·001$
Percentage disorder and R/P ratio	0·7513	$P<0·001$
Percentage breakdown and mean cell volume	0·6416	$P<0·001$
Percentage breakdown and R/P ratio	0·5336	$P<0·001$
Percentage breakdown and percentage Jonathan spot	0·5020	$P<0·001$

(ii) *Mean Cell Volume, Cell Surface, and Protein Nitrogen.*—Over the range of mean cell sizes provided by these data the relation of cell volume to cell surface was linear (Fig. 1) owing to the tendency for the cells to increase faster along the major axis than along the minor axis.

The relation of protein nitrogen per cell to cell volume was particularly close, remaining highly significant when mean fruit size per tree was held constant. The protein nitrogen/cell volume line (Fig. 2) passed through the origin or very close to it, showing a proportionality between protein and cell volume.

The ratio of protein nitrogen to cell surface increased as cell surface increased (Fig. 3), suggesting either that the protoplasm of the cortical cells increased in thickness as cell size increased or that the protoplasm became more concentrated.

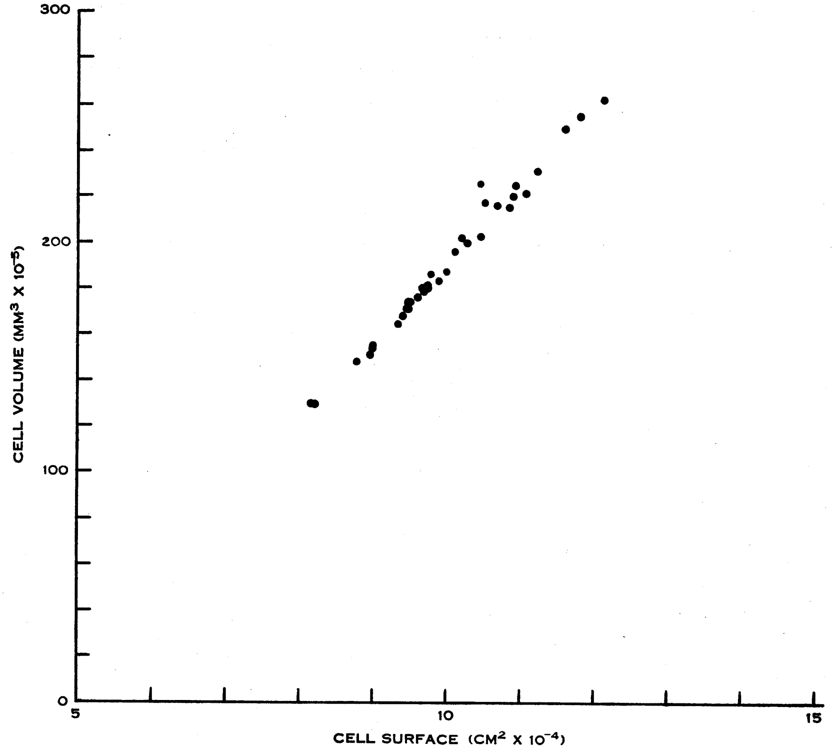

Fig. 1.—Cell surface and cell volume.

(iii) *Protein Nitrogen, Soluble Nitrogen, and Respiration.*—The close relation between protein nitrogen per cell and preclimacteric respiration per cell illustrates the interdependence of these two factors over a range of crop levels. Because the relation between cell volume and cell surface was linear over the range available (Fig. 1), soluble nitrogen was linearly related to both cell volume and cell surface (Figs. 2 and 3). Thus no information was available to suggest whether soluble nitrogen was contained largely in the protoplasm or distributed throughout the cell.

The slope of the regression line for protein nitrogen on cell volume was steeper than that for soluble nitrogen and suggested that the proportion of protein increased with cell size increase.

(*d*) *Jonathan Spot*

There was a complex relationship between Jonathan spot and breakdown which has not been reported before. While there was a negative correlation

($P < 0{\cdot}001$) between the two disorders between trees there was a positive association, i.e. more fruit with joint symptoms than would be expected by chance* ($P < 0{\cdot}001$), but the relation between the two disorders will not be discussed as no theory can be advanced to resolve the apparent paradox.

TABLE 3

PARTIAL CORRELATIONS OF VARIABLES

Correlation	Constant	r	Significance
Protein N per cell and cell surface ..	Mean fruit size	0·8260	$P<0{\cdot}001$
Protein N per cell and respiration per cell	Mean fruit size	0·8283	$P<0{\cdot}001$
Protein N per cell and R/P ratio ..	Mean fruit size	0·2066	N.S.
Protein N per cell and soluble N per cell	Mean fruit size	0·7414	$P<0{\cdot}001$
Protein N per cell and percentage breakdown	Mean fruit size	0·3050	N.S.
Percentage disorder and R/P ratio ..	Mean fruit size	0·2655	N.S.
Percentage breakdown and R/P ratio ..	Mean fruit size	0·1468	N.S.
Percentage disorder and mean fruit size ..	Respiration/protein	0·7795	$P<0{\cdot}001$
Percentage breakdown and mean fruit size	Respiration/protein	0·7007	$P<0{\cdot}001$
Percentage breakdown and mean fruit size	Cell volume	0·6120	$P<0{\cdot}001$
Percentage breakdown and cell volume ..	Mean fruit size	0·0943	N.S.
Respiration per cell and protein N per cell	Cell number/g	0·8225	$P<0{\cdot}001$

IV. DISCUSSION

This paper attempts to test for a wide range of crop size within a variety the indications received from the studies of different varieties at two cropping levels (Martin and Lewis 1952) and to discover something of the physiological connection between mean fruit size per tree and susceptibility to disorder (Martin 1954).

Many of the interrelations of cell attributes which were demonstrated in the first-mentioned of these papers for cell characteristics between varieties are now shown to hold for cell size differences within a variety.

* Calculated as follows: Expected percentage for joint occurrence on chance basis =

$$\frac{\text{Jonathan spot (\%)} \times \text{Breakdown (\%)}}{100}$$

$$\Sigma \text{ individuals}^2 \text{ (actual} - \text{expected)} = 564{\cdot}87$$

$$\text{G.T. (actual} - \text{expected)}^2 30 = 198{\cdot}18$$

Source	D.f.	Sum of Squares	Mean Square	F	Significance
G.T.	1	198·15	198·15	15·67	$P<0{\cdot}001$
Error	29	366·72	12·645		

(*a*) *Mean Cell Volume, Cell Surface, and Protein Nitrogen*

The relation between cell size and protein content has now been demonstrated for within-tree development (Robertson and Turner 1951); between varieties (Martin and Lewis 1952); and now for between-tree variations within a variety. The principle that protein synthesis keeps pace with cell enlargement is thus a characteristic of apple cells under a wide range of conditions.

Kidd *et al.* (1951), by other methods, have demonstrated a decrease in protoplasm thickness as apple cells expand. Unless their conditions were exceptional and cell size increase without accompanying protein synthesis occurred, the two results can be reconciled only by assuming that protein nitrogen becomes more concentrated in the protoplasm as the cells expand.

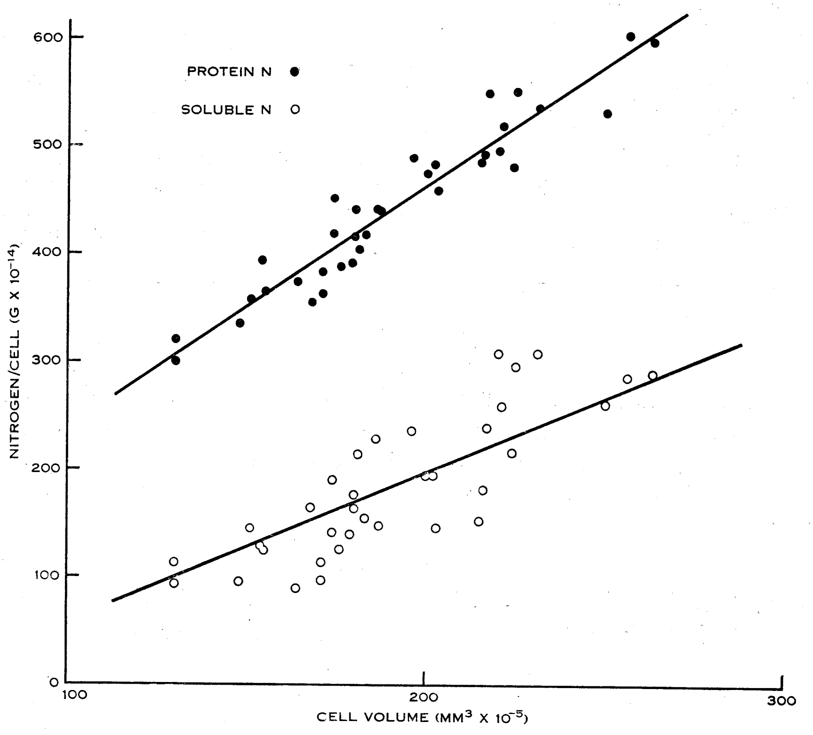

Fig. 2.—Protein and soluble nitrogen and cell volume.

(*b*) *Protein Nitrogen, Soluble Nitrogen, and Respiration*

The close relation between protein nitrogen and respiration is now shown to be a characteristic of cell growth within a variety, as well as between varieties.

Robertson and Turner (1951) suggested a "steady state" relation between protein and soluble nitrogen in their studies of cell enlargement during fruit growth. The very close correlation now shown for these two variables between trees, which remains highly significant ($P < 0{\cdot}001$) when mean fruit size per tree is held constant, supports their suggestion.

The positive correlation of protein nitrogen per cell with respiration per unit protein would be consistent with another of their suggestions, that increased protein content of cell is associated with increased difficulties of protein maintenance and respiration rate. This may be linked with the possibility of an increase in protein concentration suggested in (a) above.

The decline in significance of the relation between respiration per unit protein and protein nitrogen per cell when fruit size per tree is held constant may be due to the difference in level of experimental error of the different terms.

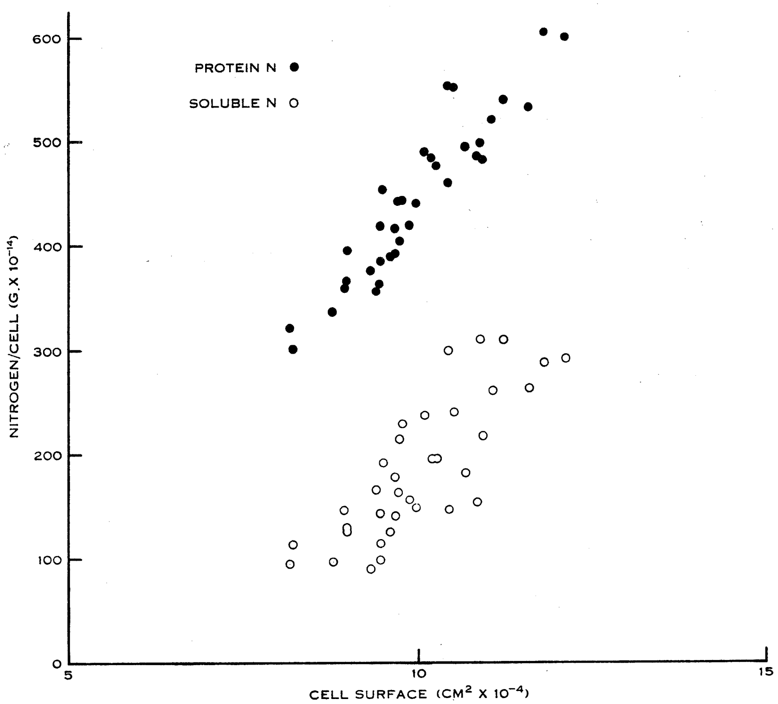

Fig. 3.—Protein and soluble nitrogen and cell surface.

(e) *Breakdown, Respiration per Unit Protein, and Cell Size*

The earlier suggestion that variation in disorder level might be related to difficulties of cell organization associated with cell growth has been supported by the intercorrelation of these facts. The decline in significance when mean fruit size is held constant by methods of partial correlation may be due to differences in level of precision in the different terms; the partial correlation of disorder and mean fruit size for constant respiration per unit protein may remain significant for the same reason.

If the differences in level of precision are not responsible for the magnitudes of the partial correlations, the explanation of why mean fruit size is such a good index of disorder susceptibility does not lie in the level of efficiency of respiration in cells of different sizes. However, the probability that light-crop trees not only have fewer fruit buds but may also have less cell division in the fruits in spite of their larger size, suggests weakness in cell organization that merits further study.

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