EFFECTS OF HUMIDITY ON METABOLISM IN TOMATO AND APPLE[1]

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(WITH ONE FIGURE)

The literature on effects of specific factors of environment includes reports which are often contradictory in greater or less degree. The reason sometimes would seem apparent. In considering the results obtained with respect to plant appearance, anatomy, or composition, the causal factor has often been assumed by the investigator to be solely that which was varied experimentally. Perhaps the most that may be said is that when one or another factor of environment is apparently dominant, as temperature, for example, metabolism and growth of the plant may under that temperature and specific total-environment show certain trends; trends, however, which can be accentuated or masked by modification of nutrition or carbon dioxide supply, light intensity, or other factors (**22**).

The quality of growth of plants, however, is always the expression of the specific character of their metabolic processes involving all assimilated products (**15**), and in part unassimilated materials (**8, 12, 14, 16**), guided and often qualitatively and quantitatively directed by the factors of the environment. A shift in one or more of these factors is reflected in the type of growth of the plant. With these points in mind, a study was made of the growth responses and accompanying chemical changes which were found to take place in tomato plants subjected to an environment in which humidity was the single variable factor for a given nutrient treatment. The nitrate reducing power of these plants, together with microscopic observations, are reported in detail by ECKERSON (**4**), although frequent reference to her work will be made in the following pages.

PART I. TOMATO

Experimental methods

Recent papers (**11, 19, 20**) record the results of experiments in which plants were grown at the same relative humidity but at different temperatures, the lowest being 45° and the highest 95° F. The rate of evaporation from leaves must be closely related to the vapor pressure deficit of the

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surrounding air. Figure 1 shows that although a constant relative humidity may be maintained, the vapor pressure deficit of air varies as the temperature changes. It was found practically impossible, in the experiments cited, to maintain the same vapor pressure deficit at the extremes of temperature employed, but relatively easy to maintain the same relative humidity. The significance of vapor pressure deficit in relation to phases of metabolism other than transpiration is problematical. Because of this fact and because of the practical impossibility of maintaining the same vapor pressure deficit under these extremes of temperature, it was empirically chosen to maintain the same relative humidity at all temperatures. This method of expression of the moisture content of the air is adhered to in this, as well as in the papers cited, and is commonly used in horticultural practice.

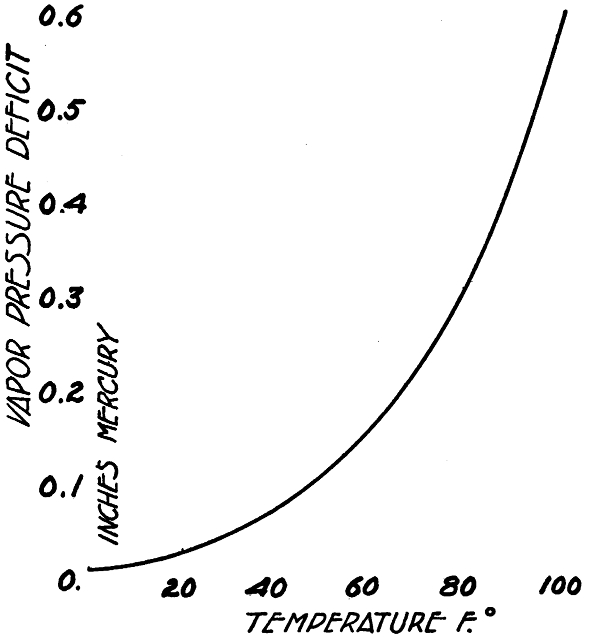

Fig. 1. Variation of vapor pressure deficit of atmosphere with rise of temperature at a maintained relative humidity of 70 per cent.

Tomato plants of the Bonny Best variety were employed for these experiments. They were grown for four weeks in sand culture with no external nitrogen supply, in new, washed, 10-inch clay pots, five plants to a pot, under prevailing greenhouse conditions. The pots were set in shallow enamelware pans which were kept constantly full of nutrient solution, which was applied daily in sufficient quantity completely to flush the sand.

On May 31, after the plants had been grown for four weeks with minus-N solution, some of them, later referred to as initial plants, were harvested for analysis and the remainder were placed in two glass inclosed chambers. The air in one chamber was maintained at 95 and in the other at 35 per cent. of saturation. The temperature was 70° F. (21° C.) in both cases. These conditions were constant day and night, with a variation in temperature of not more than ± 1° and in humidity of not more than ± 1.8 per cent. Sunlight and carbon dioxide supply were variable but the same for both humidity treatments. Condensation of moisture on the glass of the chambers did not occur as the surrounding air outside the chambers was not allowed to drop below 90° F.

Each chamber accommodated 20 pots, or 100 plants. After 12 hours under the humidity conditions indicated, half of the plants in each chamber received a complete or plus-NO_3 nutrient solution and the remainder continued to receive a solution lacking nitrogen. The nutrient solutions were applied daily, as already described, and the pans were kept constantly full of distilled water. In the low humidity chamber the surface sand at the top of the pots became dry to a depth of about 0.5 inch, but in the vicinity of the root systems the moisture was found to be in each case approximately 49 per cent. of the total water-holding capacity of the sand. With the same nutrient treatment, differences observed in growth and metabolism were brought about solely as the result of differences in humidity, although obviously all factors of environment influenced the response obtained.

Methods of chemical analysis of the tomato plants have already been described (**11**).

Results

Initial plants

The initial plants which were used for analysis and others of the same lot which were employed for experimental treatment were selected from a population of about 700, and were uniform in size, quality, and appearance. They were collected for analysis and experimental treatment begun on May 31, after the plants had been grown as already described with no external nitrogen supply. At that time they exhibited all the usual symptoms of nitrogen deficiency; and like the initial plants of the temperature experiments, may be described as follows: The stems were about 25 cm. long but stiff and woody. The base of the stem was purplish blue to light yellow. The upper two or three leaves were fairly dark green but the lower leaves were distinctly yellow with purple veins. There were practically no blossoms present. The root system was unusually large in proportion to the tops and consisted of many fine white extensive roots. The plants were high in carbohydrates but very low in all forms of organic nitrogen, and contained neither nitrate nor ammonium (tables I–IV). Cell walls of mechanical and conductive tissue were thick, and starch was observed in large quantities in all parenchymatous tissue nearly to the tip of the stem.

PLANTS DEFICIENT IN NITRATE AT 35 PER CENT. HUMIDITY

The plants deficient in nitrate were harvested for analysis on June 8, nine days after being continuously subjected to 35 per cent. humidity. During that period there was no noticeable change in appearance and but little change in chemical composition as compared with their initial condition on May 31 (tables I–IV).

PLANTS WHICH RECEIVED COMPLETE NUTRIENT SOLUTION AT 35 PER CENT. HUMIDITY

These plants absorbed nitrate instantly and in about four hours nitrate was present in abundance in all parts of the plant (**4**). Following absorption, assimilation of nitrate undoubtedly took place, for in nine days the stems increased nearly 100 per cent. in concentration of organic nitrogen (tables III, IV). The elaboration of nitrate necessarily involves utilization of carbohydrates, and as might be expected there occurred a decrease in sugars and starch (tables I, II).

Growth of these plants, however, was extremely slow for nitrate-supplied plants at 70° F. (**11**). The stems increased in length only 5–7 cm., and although there was some expansion of apical leaves they lacked vigor and succulence. They were comparatively thick, stiff to the touch, slightly mottled green in appearance, and even lower in chlorophyll (table V) than the leaf blades of the plants grown with no nitrate but with 95 per cent. humidity. It was further found that the stomatal apertures of the low humidity plants were small and that stomata were comparatively few in newly expanded leaves (**4**).

The plants exhibited no symptoms of wilting yet gave as a whole the general impression of dryness and stiffness. Microscopic observation (**4**) also showed that there was differentiation of heavy walled xylem and mechanical tissue, practically to the embryonic stem tip. In connection with this general lack of succulence, attention is called to the fact that although the plants were not low in total assimilated nitrogen, much of it was in the form of complex comparatively immobile proteins (tables III, IV).

PLANTS DEFICIENT IN NITRATE AT 95 PER CENT. HUMIDITY

During the 9-day period at 95 per cent. humidity, these plants with no external nitrogen supply decreased materially in all determined forms of

TABLE I

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER, AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE HUMIDITY				
	MAY 31	JUNE 8			
	INITIAL PLANTS	MINUS-N		PLUS-NO_3	
		35	95	35	95
	%	%	%	%	%
Dry matter	14.80	15.20	13.80	11.20	10.40
Reducing sugars	8.60	6.30	5.70	4.98	2.98
Sucrose	3.51	4.45	1.05	3.75	2.05
Total sugars	12.11	10.75	6.75	8.73	5.03
Starch and dextrin	16.61	18.34	14.57	14.90	11.57
Total carbohydrates	28.72	29.09	21.32	23.63	16.60

TABLE II

CARBOHYDRATE FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE HUMIDITY				
	MAY 31	JUNE 8			
	INITIAL PLANTS	MINUS-N		PLUS-NO_3	
		35	95	35	95
	%	%	%	%	%
Reducing sugars	1.27	0.96	0.79	0.56	0.31
Sucrose	0.52	0.67	0.14	0.42	0.21
Total sugars	1.79	1.63	0.93	0.98	0.52
Starch and dextrin	2.46	2.79	2.01	1.67	1.20
Total carbohydrates	4.25	4.42	2.94	2.65	1.72

carbohydrates, as shown in tables I and II. The percentage and quality of nitrogenous material in whole stems (tables III, IV) shows a slight increase in concentration of soluble organic nitrogen, and especially in percentage of the simpler, more mobile forms. Accompanying these changes the stems elongated from 3 to 4 cm. and lost much of the purplish blue of anthocyanin. The apical leaves also expanded, the plants became definitely darker green, and in the upper part more succulent. There was likewise found a remarkably high concentration of chlorophyll and increase in chloroplasts (**4**) in the leaf blades as compared with comparable plants lacking nitrate at 35 per cent. humidity (table V).

As might be anticipated, the stomatal apertures were found to be much more open at high than at low humidity, although they were not closed in any case (**4**).

PLANTS WHICH RECEIVED COMPLETE NUTRIENT SOLUTION AT 95 PER CENT. HUMIDITY

Absorption and translocation of nitrate, as nearly as could be determined by microscopic observation (**4**), apparently took place at about the same rate as at 35 per cent. humidity. On a percentage basis the amount of assimilated nitrogen would not appear to be greater at high than at low humidity (tables III, IV), but the plants at high humidity increased much more in volume than did those in the dry atmosphere, and, containing about the same percentage of total organic nitrogen on a green weight basis, must therefore have synthesized a considerably greater absolute amount. Necessarily accompanying comparatively rapid nitrate assimilation there occurred a marked decrease in carbohydrates (tables I, II).

TABLE III

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF DRY MATTER

	PERCENTAGE HUMIDITY				
	MAY 31	JUNE 8			
	INITIAL PLANTS	MINUS-N		PLUS-NO_3	
		35	95	35	95
	%	%	%	%	%
Total nitrate-free N	0.530	0.570	0.600	1.388	1.340
Protein N	0.418	0.478	0.480	0.788	0.420
Nitrate-free soluble N	0.112	0.092	0.120	0.600	0.920
Basic N	0.050	0.053	0.038	0.214	0.200
Amino N	0.043	0.029	0.060	0.258	0.494
Amide N	0.014	0.009	0.022	0.072	0.134
Other N	0.005	0.001	0.000	0.056	0.092
Nitrate N	None	None	None	0.466	0.500
Total N	0.530	0.570	0.600	1.854	1.840

Whereas the nitrate supplied plants at 35 per cent. humidity were woody and stiff, these were of extreme succulence. The newly expanded leaves had many stomata per unit area and the apertures were relatively large. The blades were thin, of an even dark green, and high in chlorophyll (table V). Not only were these organs thin and succulent, but there was only limited differentiation of xylem and mechanical elements in the stem tissue which developed during the 9-day period at high humidity. Further

TABLE IV

NITROGENOUS FRACTIONS OF WHOLE STEMS EXPRESSED AS PERCENTAGE OF GREEN MATTER

	PERCENTAGE HUMIDITY				
	MAY 31	JUNE 8			
	INITIAL PLANTS	MINUS-N		PLUS-NO_3	
		35	95	35	95
	%	%	%	%	%
Total nitrate-free N	0.078	0.087	0.083	0.155	0.139
Protein N	0.062	0.073	0.066	0.088	0.044
Nitrate-free soluble N	0.016	0.014	0.017	0.067	0.095
Basic N	0.007	0.008	0.005	0.024	0.021
Amino N	0.006	0.004	0.009	0.029	0.051
Amide N	0.002	0.001	0.003	0.008	0.014
Other N	0.001	0.001	0.000	0.006	0.009
Nitrate N	None	None	None	0.052	0.052
Total N	0.078	0.087	0.083	0.207	0.191

cell division at the stem tip was rapid, as is apparent from the fact that the stems elongated from 12 to 15 cm.

Correlated with these growth responses it was found (tables III, IV) that an exceptionally high percentage of the total organic nitrogen was made up of amide and comparatively simple amino acids. This is in striking contrast to the quality of organic nitrogen found in the slow growing non-succulent plants at 35 per cent. humidity. These were notably high in complex proteins.

During the brief period of these experiments the roots were not noticeably affected by any of the experimental treatments.

TABLE V

CHLOROPHYLL IN THE LEAF BLADES OF TOMATO; JUNE 8

	PERCENTAGE HUMIDITY			
	MINUS-N		PLUS-NO_3	
	35	95	35	95
	%	%	%	%
Percentage of green matter	0.087	0.102	0.093	0.125
Relative values*	88	103	94	126

* Calculated using as 100 tomato plants which were subjected to plus-nitrate treatment at 70° F. and 85 per cent. humidity (11).

Discussion

Low humidity during a period of only eight days exerted a pronounced effect that was apparent in the external appearance of the tomato plants of these experiments, in that they lacked succulence, were low in chlorophyll (table V), and grew slowly as compared with comparable plants grown at high humidity. This response seems logical, however, as transpiration is greatly accelerated under conditions of low atmospheric moisture (**6, 10**). But such a marked external effect could scarcely take place were it not accompanied by internal changes. Chemical analyses (tables I–IV) show that internal changes did occur, and that they were of a type that usually (**8, 11, 12, 14, 16**) takes place in plants under conditions which promote carbohydrate accumulation.

The plants at 35 per cent. humidity with no external nitrogen supply, and therefore unmodified in carbohydrate metabolism by nitrate assimilation (**11**), were relatively high in dry matter and correspondingly low in moisture. They contained a high percentage of carbohydrates in the stems (tables I, II) and in all other plant parts. The nitrate supplied plants at low humidity, although lower in carbohydrates, owing to assimilation of nitrate (tables III, IV), were nevertheless much higher in concentration of sugars and starch than the plants with comparable nutrient treatment but at 95 per cent. humidity.

Further contributing to the high percentage of dry matter were the relatively thick cell walls of xylem and mechanical tissue found in the plants grown with low atmospheric moisture. This seems (**2, 9, 10**) to be a very common effect of low humidity. Yet it should also be emphasized that thick cell walls were developed under conditions of high humidity when nitrate was withheld, and there was thereby (**11**) maintained in the plant a fairly high concentration of carbohydrates (tables I, II). Cell wall thickness, with the possible exception of the calcium deficient plant (**17**), seems to be intimately associated with the carbohydrate content (**8, 13, 14, 15, 16, 23**). When carbohydrates are abundant cell walls are thick and the plant is stiff; when carbohydrates are low cell walls are thin and the plant is usually succulent. A wide variety of combinations of environmental conditions may modify the carbohydrate content of plants and therefore cell wall thickness.

Although the plants of these experiments were not grown to the fruiting stage, it may be mentioned that low humidity has been said (**2, 10**) to hasten flowering and fruiting. This also seems logical, as it has been demonstrated repeatedly (**8, 13, 14, 15**) that reproduction in plants takes place under conditions where carbohydrates are available in abundance rather than where carbohydrates are deficient. It may again be pointed out, however, that by withholding nitrate, carbohydrates were maintained in high concentration in the plants even at 95 per cent. humidity (tables I, II).

When grown with a complete nutrient solution, however, the plants subjected to high atmospheric moisture were low in carbohydrates (tables I, II), especially in the newly developed tissue. They therefore produced thin cell walls. Further, new growth was rapid and the plants were extremely succulent. Coupled with a comparatively low carbohydrate and dry matter content, high percentage of moisture, rapid growth, and succulence, there was found (tables III, IV) a very high concentration of the simpler soluble forms of organic nitrogen as amino, amide, and other nitrogen but the percentage of complex, relatively immobile proteins was low. This, however, is not a situation in any respect peculiar to high humidity. The less complex water soluble forms of organic nitrogen in the plant seem generally (**1, 12, 13, 14, 21**) to be present in abundance when carbohydrates are comparatively low and the moisture content is high.

At low humidity, however, under conditions of carbohydrate accumulation, slow growth, and lack of succulence, there was found (tables III, IV) in the nitrate supplied plants a fairly high concentration of total elaborated nitrogen. Yet much of it was in the form of complex, relatively immobile, dehydrated protein. An explanation is not attempted, but the general effect of dehydration apparently might be anticipated. It may be mentioned that EMIL FISCHER and others have employed dehydration in the synthesis of polypeptides from amino acids, and that storage proteins do not appear in seeds until desiccation begins (**3**). Condensation of simple amino acids to protein seems generally (**3, 12, 13, 14**) to occur with loss in moisture and increase in carbohydrates in the plant, and is not in any sense peculiarly a result of low humidity.

PART II. APPLE

The experimental technique employed and the results obtained were much alike for both tomato and apple. A comparatively brief presentation will suffice, therefore, to show that effects of relative humidity on apple are in harmony with the responses exhibited by tomato when subjected to similar conditions of humidity.

Experimental methods

The trees employed for this work were of the Rome variety. They had been propagated in the nursery as whole root grafts and were carefully selected 24-inch whips. On April 4, 1933, after several months in a cold storage cellar, 250 uniform trees were selected, washed free of adhering soil, the fine roots removed, and the dormant trees set in nitrogen-free quartz sand, in clean 3-gallon self-draining, glazed crocks, four trees per crock. The tops of the trees were then pruned back to a uniform height of 25 cm. above the surface of the sand and all but the most distal bud was removed. This was done to eliminate variation in the number of shoots per tree, which would have made difficult the accurate comparisons of amount and quality of growth.

The crocks were placed in shallow enamelware pans and received daily applications of nutrient solution (**20**) in quantity sufficient to flush the sand. The pans were kept full at all times by the addition of distilled water. The water level in the pans did not extend more than 1 cm. above the bottom of the inside of the crocks. The sand adjacent to the roots was thus maintained at 52 per cent. of saturation.

On June 4, after the trees had been grown in sand for two months under usual greenhouse conditions, but with minus-N solution (**11, 20**), some of them, later referred to as *initial trees,* were harvested for analysis. At the time of this analysis and on all subsequent occasions, harvests were made shortly before sunrise. The remainder of the trees were placed in the humidity chambers. The air in one chamber was maintained at 40 and in the other at 95 per cent. relative humidity, with a temperature of 75° F. in both cases.

The plant population and other conditions were essentially the same as for tomato. After 12 hours in the chambers, nitrate was applied to half the plants of each group. The methods of chemical analysis of the apple trees have already been described (**18, 20**). The *current stem* and *leaves* were the only plant parts which were analyzed macrochemically. In other portions of the trees there was little noticeable difference between comparable nutrient series during the 18-day period of these experiments.

Results

INITIAL TREES

Although the initial trees had no external nitrogen supply when they were shifted to the humidity chambers on June 4, they were nevertheless all actively growing with no sign of terminal bud development. The current stems were about 60 cm. in length and rather dark red, except the upper third of the stem which was comparatively soft and green in color. A few of the lower leaves were slightly yellowish green although the foliage of the remainder of the stem was dark green. White fibrous roots were very abundant and extensive, but of comparatively small diameter. This is a condition frequently exhibited by apple trees and other plants if grown with a limited nitrogen supply and opportunity for carbohydrate accumulation. Starch was observed in abundance nearly to the tips of the finest roots. Nitrate was not present as there was none in the nutrient medium.

TABLE VI

CARBOHYDRATE FRACTIONS EXPRESSED AS PERCENTAGE OF DRY MATTER, AND DRY MATTER EXPRESSED AS PERCENTAGE OF GREEN MATTER IN TWO-YEAR-OLD ROME APPLE TREES

	JUNE 4	PERCENTAGE HUMIDITY			
		JUNE 22			
	INITIAL TREES	MINUS-N		PLUS-NO_3	
		40	95	40	95
LEAVES	%	%	%	%	%
Dry matter	27.00	39.50	34.50	33.00	29.00
Reducing sugars	5.83	4.27	3.15	3.22	1.70
Sucrose	1.29	2.41	1.20	1.50	0.82
Total sugars	7.12	6.68	4.35	4.72	2.52
Starch and dextrin	4.13	7.72	6.55	4.26	3.00
Total carbohydrates	11.25	14.40	10.90	8.98	5.52
CURRENT STEM					
Dry matter	26.50	39.00	34.50	33.50	29.50
Reducing sugars	1.90	2.10	1.90	1.00	0.78
Sucrose	0.85	1.00	0.55	0.27	0.11
Total sugars	2.75	3.10	2.45	1.27	0.89
Starch and dextrin	6.20	5.90	3.97	3.52	1.59
Total carbohydrates	8.95	9.00	6.42	4.79	2.48

TABLE VII

CARBOHYDRATE FRACTIONS EXPRESSED AS PERCENTAGE OF GREEN MATTER IN TWO-YEAR-OLD ROME APPLE TREES

	JUNE 4	PERCENTAGE HUMIDITY			
		JUNE 22			
	INITIAL TREES	MINUS-N		PLUS-NO_3	
		40	95	40	95
LEAVES	%	%	%	%	%
Reducing sugars	1.57	1.69	1.09	1.06	0.49
Sucrose	0.35	0.95	0.41	0.49	0.24
Total sugars	1.92	2.64	1.50	1.55	0.73
Starch and dextrin	1.11	3.05	2.26	1.41	0.87
Total carbohydrates	3.03	5.69	3.76	2.96	1.60
CURRENT STEM					
Reducing sugars	0.50	0.82	0.66	0.34	0.23
Sucrose	0.23	0.39	0.19	0.09	0.03
Total sugars	0.73	1.21	0.85	0.43	0.26
Starch and dextrin	1.64	2.30	1.37	1.18	0.47
Total carbohydrates	2.37	3.51	2.22	1.61	0.73

Associated with these growth responses and those exhibited by the aerial organs, there was found in the leaves and current stem an abundance of sugars and starch (tables VI and VII) and a moderate concentration of organic nitrogen but no nitrate (tables VIII and IX).

TABLE VIII

Nitrogenous fractions expressed as percentage of dry matter in two-year-old Rome apple trees

	June 4	Percentage humidity			
		June 22			
	Initial trees	Minus-N		Plus-NO$_3$	
		40	95	40	95
Leaves	%	%	%	%	%
Total nitrate-free N	2.73	2.42	2.56	3.10	3.40
Protein N	2.29	2.17	2.26	2.68	2.82
Nitrate-free soluble N	0.44	0.25	0.30	0.42	0.58
Nitrate N	None	None	None	None	None
Current stem					
Total nitrate-free N	1.39	0.78	0.94	1.05	1.42
Protein N	0.57	0.48	0.50	0.58	0.65
Nitrate-free soluble N	0.82	0.30	0.44	0.47	0.77
Nitrate N	None	None	None	None	None

TABLE IX

Nitrogenous fractions expressed as percentage of green matter in two-year-old Rome apple trees

	June 4	Percentage humidity			
		June 22			
	Initial trees	Minus-N		Plus-NO$_3$	
		40	95	40	95
Leaves	%	%	%	%	%
Total nitrate-free N	0.73	0.96	0.88	1.02	0.99
Protein N	0.62	0.86	0.78	0.88	0.82
Nitrate-free soluble N	0.11	0.10	0.10	0.14	0.17
Nitrate N	None	None	None	None	None
Current stem					
Total nitrate-free N	0.37	0.30	0.32	0.35	0.42
Protein N	0.15	0.19	0.17	0.19	0.19
Nitrate-free soluble N	0.22	0.11	0.15	0.16	0.23
Nitrate N	None	None	None	None	None

TREES DEFICIENT IN NITRATE AT 40 PER CENT. HUMIDITY

The trees, under these conditions of low humidity with no external nitrogen supply, apparently ceased terminal growth almost at once. At least the current stems made no measurable increase in length, and in 5 or 6 days it was clearly evident that all the trees of this group were forming a terminal bud. Simultaneously the foliage became yellowish green and the tip of the stem much stiffer and darker red. Internally this was associated with heavy deposition of starch and thickening of cell walls, especially near the tip of the stem. At the end of 18 days the leaves and current stem had increased greatly in percentage of dry matter and carbohydrates, as shown in tables VI and VII. On a dry weight basis there occurred a decrease in concentration of nitrogen (table VIII), although there was apparently translocation of soluble organic nitrogen from the current stem to the leaves where there was seemingly condensation to proteins, as indicated in table IX. As already pointed out in case of tomato, this type of response seems frequently to occur with loss of moisture and increase in dry matter in the plant (**3, 11, 12, 13, 14, 21**).

TREES WHICH RECEIVED COMPLETE NUTRIENT SOLUTION AT 40 PER CENT. HUMIDITY

The trees at low humidity which were supplied with nitrate absorbed it instantaneously, and nitrate as usual was observed only in the fibrous roots. That the trees assimilated nitrate was evident by the appearance of nitrite and a noticeable decrease in starch in the fibrous roots only 20 hours after nitrate was absorbed. Two days after the complete nutrient solution was applied the foliage became darker green, although dull green, and the current stem and petioles gradually decreased in intensity of red color. During the 18-day period the trees increased 5 to 8 cm. in length of current stem, and materially in area of the leaves (near the stem tip) which were only partially expanded when the experiments were commenced. At the end of 18 days, however, all of the trees had terminal buds and shortly before that time the stem tips were rapidly becoming woody and darker red. It may be pointed out that development of terminal buds was therefore much slower than in case of the trees lacking nitrate at low humidity, also that the nitrate supplied trees were much lower in dry matter and in carbohydrates (tables VI and VII). Maturity of vegetative or reproductive organs is frequently associated with accumulation of carbohydrates.

At the time of harvesting, dry matter in the current stem was considerably higher in the trees which received nitrate under the conditions of low humidity, than in the initial trees (table VI). This would seem to be due, at least in part, to the marked increase in thickness of cell walls which occurred especially in the upper third of the current stem. Accompanying increase in thickness of cell walls, there was a decrease in percentage of the determined carbohydrate fractions (tables VI and VII). Polysaccharides other than starch may very well have increased, however, as is indicated by the work of MURNEEK (**10a**).

In the same trees the increase in percentage dry matter in the leaves (table VI) may have been due partly to relatively rapid loss of water through transpiration under the conditions of low atmospheric moisture. There was, however, no noticeable sign of wilting of these trees nor of any of the others of these experiments. On the other hand, the cuticle of the leaves of both series at 40 per cent. humidity was comparatively thick as were also the cell walls of veins and mesophyll.

Cell walls are made up partly of cellulose and other carbohydrate materials which are derived presumably through condensation of the simpler carbohydrates, resulting in a decrease of those in storage unless supplied through new synthesis. It should also be pointed out that although nitrate assimilation is limited mainly to the fibrous roots in apple (**18, 19**), the aerial organs must eventually supply the carbohydrates utilized in the synthesis of proteins from nitrate. Therefore both of these factors, assimilation of nitrate and thickening of cell walls, would presumably involve utilization of carbohydrate fractions such as those determined (tables VI and VII). This increase of dry matter with decrease in percentage of determined carbohydrates is in contrast to the occurrence in an herbaceous plant such as tomato, where fluctuations in percentage dry matter commonly correspond with changes in concentration of the simpler metabolic carbohydrates such as sugars and starch.

The concentration of organic nitrogen on a dry weight basis (table VIII) is higher in the current stem and leaves of the trees supplied with nitrate at 40 per cent. humidity than in the trees lacking an external nitrogen supply under the same conditions of atmospheric moisture. Expressed as percentage of green weight matter, there is very little difference between the two groups of trees (table IX). It should be recalled, however, that the trees which received nitrate increased considerably more in volume than those lacking it. In both cases, accompanying the conditions of low humidity and associated with the development of terminal buds there was a marked decrease in soluble organic nitrogen and increase in the relatively complex protein fraction (table IX). A similar response of the tomato plants is described earlier in this paper.

Both groups of trees at 40 per cent. humidity exhibited stomata which were approximately closed day and night. The leaves near the tip of the stem which made all or most of their growth during the 18-day experimental period were relatively small in area when fully developed. Yet when completely expanded these leaves had a smaller number of stomata per unit area than comparable leaves at high humidity. The palisade and spongy mesophyll of the former were also very compact, with comparatively small cells and small intercellular spaces, and had, as already mentioned, thick cell walls and cuticle.

TREES DEFICIENT IN NITRATE AT 95 PER CENT. HUMIDITY

The trees under high humidity conditions with no external nitrogen supply gradually increased in rate of growth of the current stem. The foliage became darker green and there was considerable increase in number and area of leaves. When the trees were harvested for analysis on June 22, the current stems had increased in length from 8 to 12 cm. In no case was there any sign of a terminal bud. The new stem growth was of comparatively small diameter, and in the upper third of the stem it was considerably less woody than in the case of either group of trees at 40 per cent. humidity. It will also be recalled that during the same experimental period, terminal buds appeared on all the trees of both nutrient series at low humidity.

Accompanying the conditions of high atmospheric moisture and associated with continuous linear stem growth and development of new leaves, there was found in the current stem and leaves of the trees without nitrate a very much lower percentage of dry matter and carbohydrates than in the trees lacking nitrate at low humidity (tables VI and VII). The contrast in the upper third of the current stem, however, was much more striking than is indicated by the macroanalysis of whole stems. In the stem tip there was comparatively little starch and the cell walls were relatively thin. In the lower portion of the stem there was much starch, and considerable increase in thickness of cell walls had occurred since the initial observations were made on June 4. This would apparently account, at least in part, for the increase in percentage dry matter as compared with that found in the current stem of the initial trees (table VI).

The leaves of the trees which were subjected to high humidity with no external nitrogen supply present a situation that requires explanation. The analytical sample *leaves* consisted of young and old leaves. The latter contained considerable starch. In fact, separate analysis of a group of lower leaves which turned yellow and abscissed before the end of the experimental period showed them to be high in starch but extremely low in nitrogen. It may be remarked that this was the only series which exhibited abscission or severe yellowing of leaves. It would seem that the low concentration of nitrogen in these lower leaves may have been associated with early senescence. The low concentration of nitrogen may also have limited, directly or indirectly, diastatic digestion in those older leaves which did not absciss. There would thus be maintained in the leaf sample as a whole a comparatively high percentage of dry matter and carbohydrates (tables VI and VII).

The leaves of the upper third of the stem were dark green and extremely low in starch. The young leaves (near the tip of the stem) which expanded during the period at high humidity were of relatively large area but not so thick as comparable leaves under conditions of low humidity. The cuticle and cell walls were also thinner, and the palisade and spongy mesophyll very much less compact than in case of the leaves which developed under the conditions of low atmospheric moisture. As might be anticipated, the stomata of the trees of both nutrient series were wide open during the day at 95 per cent. humidity, although closed at night.

It may again be emphasized that the trees with no external nitrogen supply stopped linear stem growth almost immediately when shifted to low humidity, and rapidly developed a terminal bud. In striking contrast, accompanying high humidity there occurred the considerable increase in volume of tops and decrease in carbohydrates in the upper leaves and current stem that has already been described, even though there was no nitrogen in the nutrient medium. Between the roots of these two groups of trees there was little, if any, difference in appearance and chemical composition.

It has already been mentioned that there was loss of nitrogen from the older leaves of the trees grown without nitrate at 95 per cent. humidity. It is also apparent that there was a decrease in soluble organic nitrogen in the current stem as shown in tables VIII and IX. Obviously the considerable increase in volume of the trees could not have occurred without a drop in concentration of total nitrogen. This is clearly indicated in table IX. It would seem that in these apple trees, as in tomato, the condition of high humidity directly or indirectly resulted in a decrease in percentage of carbohydrates, accompanying which there occurred proteolysis and increased growth as already recorded. As discussed in greater detail in Part I, hydrolysis of proteins seems frequently to follow or accompany decrease in dry matter in the plant (**5**, **11**, **12**, **13**, **14**, **21**).

TREES WHICH RECEIVED COMPLETE NUTRIENT SOLUTION AT 95 PER CENT. HUMIDITY

The trees which received nitrate at 95 per cent. humidity absorbed it instantly and it was observed only in the fine roots. Reduction to nitrite occurred in these organs, and other observations concerning the fibrous roots were essentially the same as recorded for the trees which were supplied with the complete nutrient solution at low humidity.

The leaves became noticeably darker green about 20 hours after application of nitrate, and at the end of the 18-day period of these experiments the foliage of even the lower leaves was almost black-green. During this interval the current stems increased 12 to 16 cm. in length. There was no sign of a terminal bud and almost all red pigmentation disappeared from the current stem, which was very much softer near the tip than in case of any of the other series.

Associated with these responses there was found to be very little starch in the upper third of the stem and there the cell walls were comparatively thin. The remainder of the current stem exhibited considerable cell wall thickening and some starch, but was very much lower in starch and had thinner cell walls than comparable portions of the trees of the other groups. The relatively low percentage of carbohydrates in the current stem and leaves is clearly indicated in tables VI and VII. In general, the lower leaves contained more starch than the tip leaves but neither was high in starch.

In structure the newly expanded but fully developed leaves were similar to comparable leaves of the trees grown without nitrate under the same conditions of high humidity. The former, however, were considerably larger in area and relatively thinner. Cell walls and cuticle were very thin and the palisade and spongy mesophyll were loosely constructed of large cells and large intercellular spaces. Stomata were wide open during the day but apparently closed at night.

Associated with relatively rapid growth, nitrate assimilation, and decrease in carbohydrates, there was a much higher percentage of soluble organic nitrogen in the current stem and leaves than in case of any of the other groups of trees harvested on June 22 (tables VIII and IX). A similar situation has frequently been observed and is discussed more fully in connection with the tomato. They apparently responded in an analogous manner. Although the trees of this group increased rapidly in volume there was maintained in the current stem and leaves a high percentage of total organic nitrogen (tables VIII and IX). Presumably there was therefore a considerable increase in absolute amount of organic nitrogen newly synthesized from nitrate.

Summary

TOMATO

1. Tomato plants were grown in sand maintained at 49 per cent. of saturation in all cases. The experiments were carried on in glass inclosed chambers at a constant temperature of 70° F. (21° C.). Sunlight and carbon dioxide supply were variable but at any given time were the same for each humidity treatment. One group of plants was grown at 35 and the other at 95 per cent. humidity.

2. The plants grown with a complete nutrient solution at 35 per cent. humidity were lighter green and contained less chlorophyll than those subjected to high atmospheric moisture. They grew slowly, had relatively thick leaves, stiff stems, and in general lacked succulence. Carbohydrates were relatively high and cell walls were thick. There was a fairly high concentration of total organic nitrogen but much of it was as complex insoluble protein.

3. Comparable plants at 95 per cent. humidity were dark green and high in chlorophyll. They grew rapidly, had relatively thin leaves, and the leaf and stem tissue which developed during the period of high atmospheric moisture was comparatively thin walled and succulent. Carbohydrates were relatively low and much of the organic nitrogen was water soluble.

4. It is pointed out and shown experimentally that these responses are typical of, but not peculiar to, the respective humidity treatments.

Apple

1. Young Rome apple trees were grown in sand culture under conditions similar to those described for tomato.

2. All the trees at 40 per cent. humidity had terminal buds at the end of 18 days, although those receiving the complete nutrient solution formed them much more slowly than the trees lacking nitrate under the same conditions. Accompanying low humidity the foliage became lighter green and the current stems darker red. Associated with these responses there tended to be carbohydrate accumulation and apparently condensation of the simpler forms of organic nitrogen to complex proteins.

3. Comparable trees at 95 per cent. humidity had darker green leaves which were relatively thin. The current stems exhibited little red color and elongated rapidly even in case of the trees lacking nitrate. During the 18-day period of these experiments the current stem tips remained comparatively soft and none of the trees formed terminal buds. Correlated with these responses carbohydrates were found in relatively low concentration, there was less indication of protein condensation, and cell walls were comparatively thin.

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