Accumulation of Nitrate in Tomato Fruit and its Effect on Detinning[1,2]

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Abstract. Tomato fruit accumulated nitrate when the controlled environmental conditions combined high temperature, high N fertilization level, and low light intensity. Nitrate accumulation in the fruit was preceded by a condition of low nitrate reductase activity and nitrate accumulation in the leaves. The processed product with high concentration of nitrate caused extensive detinning of internal can surfaces after 6 months' storage at room temperature.

TOMATO products processed in plain tin cans are occasionally subject to chemical, non-microbial spoilage due to an accelerated detinning of the internal can surfaces. When detinning has progressed to the extent that the steel base is exposed to the product, evolution of hydrogen follows rapidly resulting in "hydrogen swells."

The cause of the sporadic occurrence of accelerated detinning in processed tomato products is not known. However, it is known that detinning usually results from the presence of oxidizing agents, and one oxidizing agent, the nitrate ion, has been strongly implicated in other products (6). The suspicion that nitrate is the causal agent in tomato products is strengthened by the fact that nitrate added to tomatoes before processing causes accelerated detinning of the can containing the processed product.[5]

The factors that govern the accumulation of nitrate in crops have been extensively studied, and the topic has been reviewed (8). Major effects have occasionally been observed with respect to temperature, light intensity, water supply, fertilization and herbicide (2,4-D). These effects have been found to be far from consistent and dependent on such things as species, age and soil type. Accumulation is, in most instances, favored by high N fertilization, high temperature, low light intensity and poor water supply (drought). The enzyme nitrate reductase which reduces nitrate is an adaptive enzyme whose induction is favored by high nitrate levels in the tissues, high light intensity and moderately high temperatures (1, 2, 5). At the present time, little is known about the accumulation of nitrate in fruits, although several studies have demonstrated markedly lower levels of free nitrate and of nitrate reductase in fruit than in leaves and roots (1).

In the present investigation we have attempted to study the effect of the major factors suggested by previous investigations on the accumulation of nitrate in tomato fruit. The factors selected for study were specifically temperature, fertilization (N level) and light intensity. Fruit harvested from plants grown under various conditions were canned for later studies of detinning.

Materials and Methods

Each trial consisting of 40 plants of the cultivar 'Epoch', having a dwarf vine, was brought to near maturation of the first fruiting cluster in the greenhouse. No more than 3 clusters per plant were allowed to develop. The plants were grown in a mixture of soil, compost, vermiculite and peat moss in 10-liter plastic pots and were fertilized for optimum growth. The greenhouse temperatures were maintained at 75°F day and 65° night. Light was supplemented by both fluorescent and incandescent light to give a minimum of 1000 ft-c on cloudy days. When adequate development was reached, the plants were held in 2 growth chambers (16 per chamber) until a sufficient number of ripe fruit had developed to yield at least one 303 can of processed tomatoes per treatment. One growth chamber was maintained at 80° day–70° night, the other at 70°–60°. The light intensity at the top of the plants approximated 3000 ft-c and decreased to about 1500 ft-c at the soil level. Half of each chamber was shaded and the light intensity here was reduced to 600 ft-c or less. The high N treated plants were fertilized in the chamber, except where other-wise noted, with a solution of 800 ppm N as NH_4NO_3. Nitrate analysis and nitrate reductase activity in fruit and leaves were obtained during various stages of growth and at harvest time.

Analysis of nitrate. Nitrate in fruit and leaves was determined by the method of Kamm et al. (4) and reported as micrograms nitrate nitrogen per gram of tissue (or ppm NO_3-N). In this method nitrate is reduced to nitrite by contacting the plant extracts with metallic cadmium followed by a colorimetric determination of nitrite by means of naphtylamine.

The diphenylamine spot test (7) was also used to obtain rough estimates of the nitrate levels.

Assay of nitrate reductase. The procedure of Hageman et al. (3) was used.

Canning. The harvested tomatoes were processed as whole pack in #303 plain tin cans with enamelled lids and bottoms. Suitable sized tomatoes were blanched in boiling water for 30 sec, cooled and peeled. The remainder, at least 60% of the harvested crop, was juiced by passage through a Langsenkamp finisher. The juice was heated to 190° and poured into the cans containing the peeled tomatoes. The cans were closed with a steam closing machine and processed in boiling water for 45 minutes. After cooling in water, the cans were stored at room temperature for 6 months followed by inspection of can contents and conditions of the tin lining of the container.

Tin analysis. The can contents were homogenized in a Waring blendor and 2 g, in duplicate, digested by a H_2SO_4–H_2O_2 wet digestion procedure. Tin in the final, oxidant-free solution was determined polarographically.[6]

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[5]Personal communication R. Jackson, Heekin Can Co., Cincinnati, Ohio.

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Results

When the diphenylamine spot test was applied to sections of the tomato leaf, the highest nitrate concentrations were found in the vicinity of the vascular system, particularly in the midvein and in increasing concentrations towards the base of the leaf at the petiole. Sections of pedicels taken from the plant fertilized with high levels of N indicated high concentrations (> 100 ppm NO_3–N) in the pith and in the peripheral cambium layer while the collenchymatous fiber layer always gave a negative reaction.

When the fruit tissue was tested with the diphenylamine reagent, most often no reaction or only a faint positive reaction was obtained. But occasionally, when fruits of high nitrate were tested, strong positive reactions were obtained and it was possible to form a picture of the nitrate distribution. Highest concentrations were found in the pulp close to the base of the fruit decreasing along the periphery towards the blossom end. When radial sections were tested it was sometimes possible to develop a network of blue color exhibiting the characteristics of the vascular system extending from the base through the pulp to the blossom end. This network could also occasionally be observed to penetrate into the placenta circumventing the core region.

The effect of the various treatments on the accumulation of nitrate in tomato leaves is presented in Table 1. The most striking effect is noted in Treatment 7 (high temperature, high N and low light intensity). This treatment resulted in unusually high levels of nitrate in the leaves. Temperature and light affected the nitrate level only at the high nitrogen level. Nitrate was increased by high temperatures at low levels of light intensity.

Treatment 7 resulted also in extensive deterioration of the plant tissue. The symptoms were similar to those caused by early blight with concentric ring lesions both on stems and leaves. An investigation of the affected tissue failed to establish the presence of the suspected organism *(Alternaria solani)* and the condition was judged to be, most likely, an effect of excess N associated with environmental stress.

Nitrate reductase in the leaves was affected by the light intensity as presented in Table 2. These data were collected from one of the later experiments which included only Treatments 7 and 8. The tissue yielded a consistently lower enzyme activity at low light intensity than at high light intensity. Remarkable is the absence of the enzyme in mature leaves. In no case was it possible to obtain a positive reaction from fully expanded leaves.

Nitrate accumulation in fruit tissue occurred to a much smaller extent than in leaves, amounting to only 1 to 5% of the leaf concentration (Table 1). Again, Treatment 7 resulted in a relatively high concentration of nitrate. The nitrate levels in the unripe (green) fruit tended to be slightly higher than in the ripe fruit, but the difference was not statistically significant.

Table 1. Effect of temperature, nitrogen, and light levels on accumulation of nitrate-N in tomato leaves and fruit before harvest.

Environmental factors	Treatment levels							
Temperature:	Low				High			
Nitrogen:	Low		High		Low		High	
Light:	Low	High	Low	High	Low	High	Low	High
Treatment no.:	1	2	3	4	5	6	7	8
Plant analysis (ppm)								
Leaf-NO_3-N, 1st. exp.	330	250	230	475	475	420	1100	420
, 2nd exp.	106	26	228	568	228	448	1510	500
Fruit-NO_3-N, unripe	12	20	24	6	13	22	50	10
, ripe	14	11	11	12	15	9	46	11

Table 2. Nitrate-N and nitrate reductase in leaves before and during harvest.

Days in growth chamber	Treatment 7[a]			Treatment 8[b]		
	Nitrate N (ppm)	Nitrate reductase		Nitrate N (ppm)	Nitrate reductase	
		Immature	Mature		Immature	Mature
4	880	ND[c]	ND[c]	720	.54	ND[c]
15	440	.18	ND	1000	.14	ND
22	680	.25	ND	1140	.61	ND
29	740	.26	ND	1020	.87	ND

[a]Low light, high nitrogen, high temperature.
[b]High light, high nitrogen, high temperature.
[c]Not detectable.

The assay of nitrate reductase in the fruit was hampered to some degree by a slight pink coloration of the fruit extracts, reducing the limit of detection to approximately .07 absorbance units. Significant enzyme activity could in most cases not be detected in the fruit, but was observed (.13 absorbance units) in the stem end in high nitrate fruit when the fruit was dissected and the individual part analyzed separately.

The correlation between nitrate in the raw fruit and dissolved tin in the can after 6 months storage (Table 3) was high (r = .94), strongly suggesting that the corrosion was mainly affected by nitrate. Visual inspection of the can lining (Fig. 1) supported the analytical results and further underscored the difference between treatments of high and low light intensity; every treatment with low light intensity showed more corrosion than the corresponding treatment with high light intensity.

Discussion

Since it is generally accepted that nitrate is not synthesized in the plants it follows that nitrate accumulation must be the result of a difference between nitrate absorption and nitrate utilization. Absorption of nitrate is influenced by factors such as soil nitrate level, transpiration rate (which again is influenced by ambient temperature and wind) and growth rate. Utilization is affected by growth rate, that is, demand for reduced N, and depends more directly on the activity of the nitrate reductase—nitrite reductase complex and its ability to provide the growing cell with reduced N.

These enzymes have been studied in detail in recent years (2, 4, 5). Nitrate reductase is believed to be the rate limiting enzyme. It requires the presence of the nitrate ion for its induction, and it rapidly decays in the absence of nitrate. Light is required for the nitrate ion to penetrate the cell wall and enter the cytoplasm. Of particular significance appears to be our observation of complete absence of the enzyme in non-growing leaves, since this would seem to define a general condition for nitrate ac-

Table 3. Concentrations of initial nitrate-N, and tin in canned fruit after 6 months storage.

Environmental factors	Treatment levels							
Temperature:	Low				High			
Nitrogen:	Low		High		Low		High	
Light Intensity:	Low	High	Low	High	Low	High	Low	High
Treatment no.:	1	2	3	4	5	6	7	8
Fruit analysis[a]:								
NO_3-N (ppm)	19	17	11	4	18	4	68	9
Tin (ppm)	189	148	224	67	215	80	445	115
Detinning (%)	29	23	34	10	33	12	68	18

[a]NO_3 vs. tin, r = .94.**

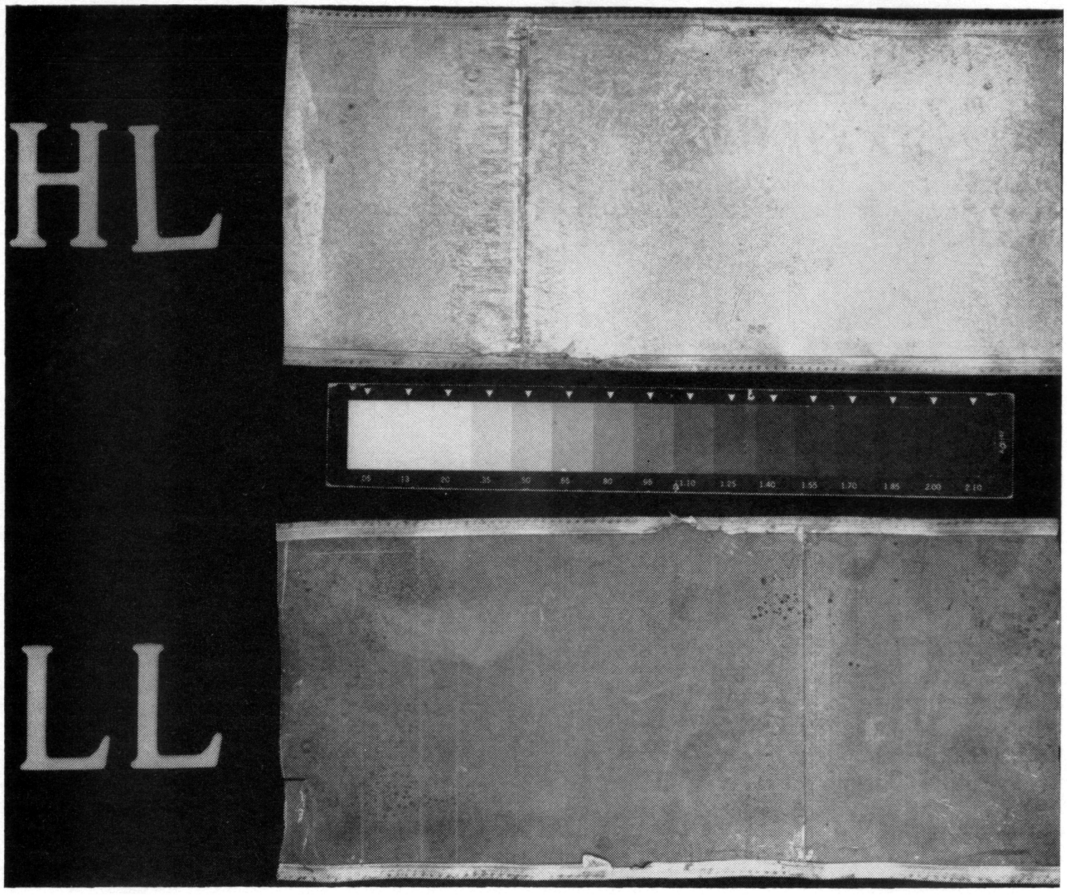

Fig. 1. Detinning of the interior can surface by processing tomato fruit, after 6 months storage. HL: high light, high nitrogen, high temperature. LL: low light, high nitrogen, high temperature. The low corrosivity of the HL treatment is indicated by the highly reflective tin surfaces, while the LL treatment resulted in virtually complete stripping of tin down to the darker tin-steel alloy layer. (Compare with the normal gray scale between the 2 cans.)

cumulation in the plant as any environmental condition which inhibits or decreases growth rate while favoring nitrate uptake from the soil. High temperatures, excessively high salt and nitrate levels in the soil, fungal disease, etc., could provide such conditions. Treatment 7 constituted an environment of this type. The growth chamber was held at a relatively high temperature; high transpiration was maintained through air circulation; high salt and nitrate levels were reached in the soil by rapid depletion of the moisture; poor vine growth was experienced by the insufficiency of light.

A barrier against nitrate translocation seems to exist between the receptacle and the developing ovary. We have observed a sharp gradient in nitrate concentration between the pedicel and the adjoining parts of the fruit. The question whether high concentrations of nitrate in the fruit tissue are caused by unusually large concentration differences across the barrier or by a breakdown of the barrier itself is not answered by the present investigation. However, the symptoms of stress noted in plants kept at high temperature at high levels of N tend to support the latter possibility.

The close correlation of the initial nitrate content of the canned product with the tin concentration after storage provides good evidence for nitrate as a causative factor for detinning in whole pack tomato products. Assuming that eight electrons are transferred in the reaction between nitrate and tin (to yield divalent stannous ions), a minimum concentration of 35 ppm NO_3-N is required for complete detinning of a #303 can with a tin coating of 1.0 lb. tin/BB[7]. Concentrations far in excess of this value were observed in the course of this investigation in samples from Treatment 7.

[7]BB: Base box, 112 sheets, 14" x 20".

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