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Action of the Non-ripening (*nor*) Mutant on Fruit Ripening of Tomato[1]

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Abstract. **Changes in respiration, ethylene production, firmness, pectolytic enzyme activity and carotenoid accumulation during ripening were monitored in fruit of a segregating population of the non-ripening (*nor*) mutant of tomato (*Lycopersicon esculentum* Mill.). In fruit from mutant (*nor/nor*) plants, no respiratory or ethylene climacteric was observed, firmness declined very slowly with only trace levels of polygalacturonase present in mature fruit and phytoene, β-carotene and neurosporene were the major carotenes. In very old fruit (120 days' post-anthesis), lycopene and β-carotene were the predominant carotenoids, however lycopene content was less than 10% of normal.**

Ripening of fruit from heterozygous (*nor*$^+$/*nor*) plants was delayed and occurred more slowly than for normal (*nor*$^+$/*nor*$^+$) fruit. The respiratory climacteric was partially suppressed, peak ethylene production was one-sixth of normal, fruit softening was retarded, and polygalacturonase activity was lower at comparable maturity stages. Fruit from heterozygous (*nor*$^+$*nor*) plants accumulated lycopene, gamma-carotene, phytofluene, and phytoene at a slower rate, but β-carotene accumulation was similar to normal. A mechanism for action of *nor* in control of ripening is proposed.

The ripening process in the tomato encompasses a number of physiological and biochemical alterations in the fruit leading to a state of optimum edibility in terms of color, texture, flavor, and aroma (13). These alterations include the rapid increase in production of CO_2 and ethylene typical of climacteric fruits (3), as well as degradation of chlorophyll, the synthesis and accumulation of carotenoids, solubilization of pectic substances, and the development of flavor components. These changes culminate in the senescence and death of the fruits.

Studies of the genetic regulation of tomato fruit ripening have been prompted by recent reports of 2 mutants which inhibit many ripening changes (10, 28). Fruit of the ripening inhibitor (*rin*) mutant fail to undergo a respiratory climacteric and concomitant ethylene evolution at a time when normal fruit ripen (10) and exhibit an abnormal pattern of chlorophyll degradation and carotenoid synthesis (24). Mutant fruit also fail to soften or exhibit any polygalacturonase activity at a time when normal fruit have ripened and started to senesce (4).

Recently, a new tomato ripening mutant *nor* (non-ripening) whose fruit also fail to undergo the characteristic softening and color development has been reported (28). In many respects, the *nor* mutant resembles *rin;* both fail to undergo fruit softening or normal chlorophyll degradation, and both exhibit a remarkably prolonged storage life. In physiological investigations involving both *rin* and *nor,* it has been noted that although excised fruit pericarp disks from both mutants are capable of converting methionine to ethylene, neither ethylene treatments (10, 17), grafting mutant scions on normal understocks (19), nor transplanting disks of mutant fruit into normal fruit (20) could induce either the *rin* or the *nor* fruit to ripen normally.

The *rin* and the *nor* mutants are similar in the behavior but are genetically distinct and compliment in tests of allelism. The gene *rin* is linked to macrocalyx (*mc* on chromosome 5 whereas *nor* is linked to the uniform ripening gene (*u*) on chromosome 10 (28). Old fruit of the *nor* mutant eventually evolve a low level of ethylene, while *rin* fruit do not (18). Very old *nor* fruit accumulate lycopene and β-carotene (28) while *rin* fruit never contain more than trace levels of lycopene even up to 180 days after anthesis (24).

Despite the amount of information that has accumulated about these mutants, the physiological effects of *nor* on fruit ripening have not been fully characterized. The current investigation was initiated to study the effects of the *nor* allele in both the heterozygous and homozygous condition upon the rate and magnitude of fruit ripening changes.

Materials and Methods

Plant material. A population segregating for *nor* was derived from a cross between the cultivars Italian Winter (*nor u*$^+$/*nor u*$^+$) and Heinz 1350 (*nor*$^+$ *u*/*nor*$^+$ *u*) with 4 subsequent backcrosses to 'Heinz 1350 to provide a relatively homogeneous genetic background. The close repulsion phase linkage between the *nor* and *u* loci (28) was used to distinguish between individuals homozygous for the normal ripening allele (*nor*$^+$/*nor*$^+$) and those heterozygous (*nor*$^+$/*nor*); non-uniform ripening fruit (*u*$^+$/–) were assumed to be heterozygous for *nor.*

Plants were grown in the Purdue Univ. greenhouses during the winter of 1976. Supplemental fluorescent lighting was supplied on a 14 hr photoperiod. The plants were trained to a single stem, and flowers were vibrated and tagged at anthesis. Fruits were thinned to 2 per cluster to reduce competition among fruits, and the time from anthesis to breaker for fruit of the first cluster in the ripening genotypes was recorded.

Respiration and ethylene studies. Twelve fruit from different plants of each of the ripening genotypes in the segregating population, were harvested at the mature green stage. Fruit of the *nor/nor* genotype were harvested at a similar chronological age after fruit set. Fruit were surface sterilized by immersion in a solution of 0.1% Benlate and 0.05% 2,6-dichloro-4-nitroaniline for 1 min, and air dried. Individual fruits were weighed and placed in glass canning jars maintained at 21 ± 1°C and ventilated with a continuous supply of CO_2-free humidified air at rates ranging from 1.35 to 1.60 liters/hr. Fruit respiration was determined daily by analyzing the CO_2 concn of the effluent air stream with an infra-red gas analyzer (Beckman model 315A). Ethylene production was assayed using a flame ionization gas chromatograph (Varian Aerograph series 1200). All rates were calculated on the basis of original fresh weight.

Firmness studies. Twelve fruit of each genotype were harvested as in the respiration studies and maintained in the dark at 21 ± 1°C and 80-85% relative humidity over a period of 2 weeks. Nondestructive measurements of firmness were taken at 3 day intervals using an Instron Universal Testing Instrument (model TTC). A prestress load of 100 g was applied with a flat

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plate on one point on the equatorial surface of the fruit, and the amount of deformation of the pericarp with a weight increment of 250 g was used as a measure of the firmness of the fruit.

Pectolytic enzymes. In these studies, the breaker stage was assumed to indicate the onset of ripening. Equatorial tissue sections of fruit, 4 and 10 days after the date of incipient color in the ripening genotypes and at a similar chronological age after fruit set in the nonripening genotypes, were homogenized (Sorvall Omni-mixer) for 60 sec in an aqueous solution containing 5% NaCl and 1% polyvinylpyrrolidone (1 g/3 ml). A minimum of 6 fruits per genotype per age category were analyzed. For pectinesterase (PE) analysis, the homogenate was filtered through cheesecloth and used as a source of enzyme. PE activity was determined from the amount of 0.05N NaOH required to maintain a 1% pectin (polygalacturonic acid methyl ester, Grade I, Sigma Chemical Co.) solution containing the enzyme at pH 7.5 for a period of 5 min, and expressed as meq of acid produced per min per g of tissue (23).

For analysis of polygalacturonase (PG) activity, the homogenate was adjusted to pH 9.0, filtered through cheesecloth after 30 min, and centrifuged at 10,000 rpm for 10 min. PG activity was determined using the method of Babbitt et al. (2). A 1 ml aliquot of the supernatant was added to 9 ml of a 1% solution of PGA (polygalacturonic acid, Grade III, Sigma Chemical Co.) buffered at pH 5.0 with 0.1 M sodium acetate in a Cannon #200 viscometer at 40°C, and the change in viscosity of the solution after 10 min was measured. One unit of PG activity was defined as the amount of enzyme required to increase the reciprocal of the specific viscosity ($1/N_{sp}$) of the polygalacturonic acid solution by 0.01 during the assay.

Carotene analysis. Fruit of each genotype were harvested 1, 5, and 9 days after the stage of incipient color; fruit from plants homozygous for the *nor* allele were harvested at comparable ages after fruit set. All fruit were frozen and maintained at −20°C until time of analysis. A minimum of 6 fruit per maturity stage studied were individually analyzed for carotenoid content.

Carotenoids were extracted, separated, and quantified as described by Tomes (29). Fruit were thawed, macerated, and a 25 g sample blended in 75 ml of acetone and 60 ml of hexane. The solution was washed free of acetone, saponified with methanolic KOH (20%), and washed again to remove the alkali. The extract was then dried with anhydrous sodium sulfate, made up to volume, and stored at 4°C until time of chromatography.

Individual carotenoids were separated on a column of 1 magnesium oxide:1 infusorial earth (by wt) and developed with increasing amounts of acetone in hexane up to 20%. Phytoene, phytofluene, and β-carotene were collected as they were eluted from the column; gamma-carotene, neurosporene, and lycopene were cut from the column and eluted with 10% ethanol in hexane. No other carotenoids were detected except in trace amounts. All fractions were washed with distilled water to remove traces of acetone or ethanol, dried with anhydrous sodium sulfate, and made up to volume.

The pigments were identified by their position on the column and by their UV and visible absorption spectra. Quantitative estimations of the various carotenoids were determined spectrophotometrically (Beckman DB spectrophotometer) utilizing the wave lengths and absorptivities reported by Tomes (29).

Results

Time from anthesis to initiation of ripening. The rate of fruit maturation as measured by time from anthesis to date of incipient color or respiratory peak was delayed by about 1 week for fruit from plants heterozygous for the *nor* allele when compared to fruit from normal (*nor*$^+$/*nor*$^+$) plants (Fig. 1). Fruit from mutant (*nor/nor*) plants remained green during the period when the normal and heterozygous *nor* fruit were undergoing ripening, but eventually showed a blush of red color about the 90th day after anthesis; this color increased slowly with time but never approached color of normal fruit.

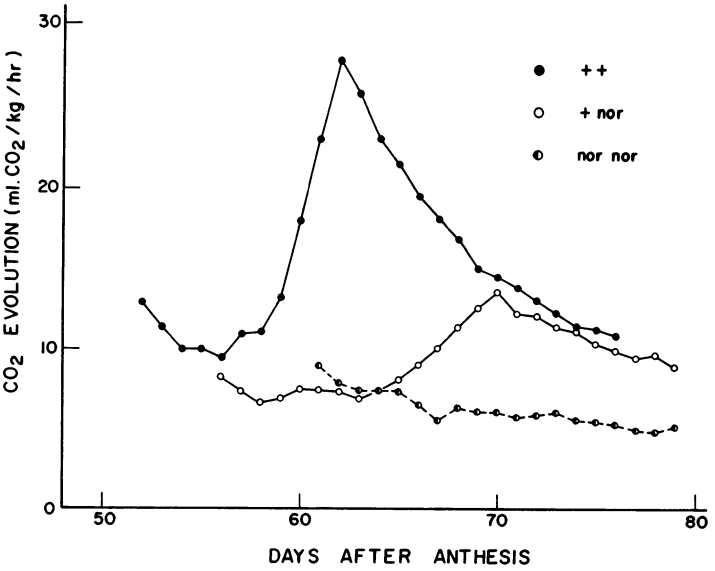

Fig. 1. Respiratory rates of *nor*$^+$/*nor*$^+$, *nor*$^+$/*nor*, and *nor/nor* fruit at 21°C.

Respiratory behavior. Virtually all aspects of the respiratory climacteric of the heterozygotes differed from normal fruit; the onset was delayed about 7 days, the time from the preclimacteric minimum rate of respiration to the climacteric peak was longer, and the preclimacteric minimum and peak respiratory rates were respectively only about 65 and 45% that of normal ripening fruit (Fig. 1). The *nor/nor* fruit exhibited no respiratory climacteric.

The pattern of ethylene evolution during the ripening of normal and heterozygous fruits resembled that of the respiration rate (Fig. 2). Increased ethylene production during ripening was delayed and partially suppressed in the heterozygotes when compared to that of normal fruit, and peak ethylene rates were only about one-sixth that of normal. In the *nor/nor* fruit, ethylene was present only in trace levels during the period when fruit of the other genotypes were undergoing ripening.

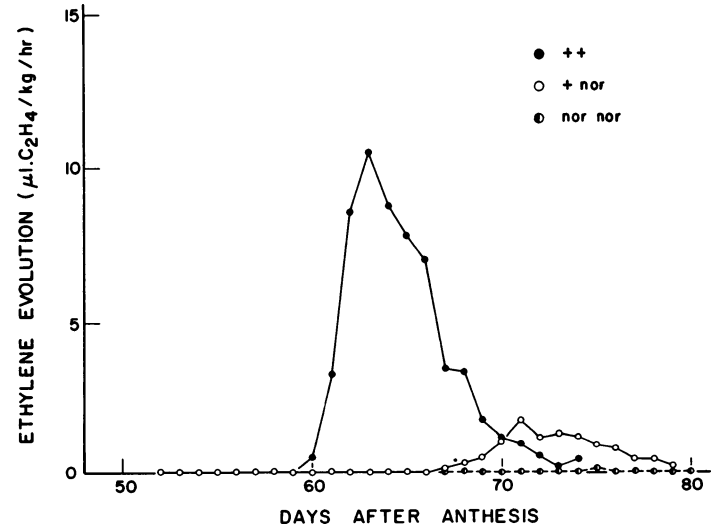

Fig. 2. Ethylene production of *nor*$^+$/*nor*$^+$, *nor*$^+$/*nor*, and *nor/nor* fruit at 21°C.

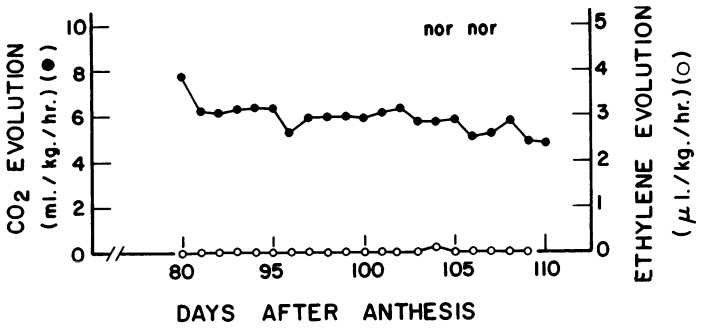

Fig. 3. CO_2 and ethylene production of fruit of *nor/nor* plants from 80 to 110 days after anthesis.

Table 1. Pectolytic enzyme activity of fruit from different genotypes of a segregating population of *nor* at 2 stages of ripening.

Genotype	Days after breaker	Days after anthesis	Pectinesterase activity (units/g tissue)	Polygalacturonase activity (units/ml extract)
nor^+/nor^+	4	60	67.6a[z]	21.0c
	10	66	85.6b	38.0d
nor^+/nor	4	64	78.4ab	y
	10	70	83.1b	6.6b
nor/nor		64	88.6b	y
		70	83.8b	y

[z]Mean separation in columns by Duncan's multiple range test, 5% level.
[y]Present in trace amounts.

Even when fruit of the mutant were harvested 80 days after anthesis and their respiration and ethylene production followed until 110 days after anthesis, respiration continued to decline slowly and only trace amounts of ethylene were detectable (Fig. 3).

Fruit softening after harvest for each genotype (Fig. 4) is expressed as deformation of the pericarp in response to an applied load, and the units used are inversely related to firmness; the higher the value of the load-deformation response, the softer the fruit. Fruit of the nor^+/nor^+ genotype softened rapidly during the ripening period with the greatest decrease in firmness occurring during the 61st to 67th day after anthesis, corresponding to the period when the fruit progressed from the breaker to the red stage of color development. In fruit from heterozygous plants, the onset of softening and of incipient color occurred several days after that of normal fruit, and fruit softened more slowly during this period when compared to normal fruit. Fruit of the *nor/nor* genotype exhibited only a slight softening during this same period of time, and retained the appearance of mature green fruit throughout the 62nd to 74th day after anthesis during which firmness was monitored.

Softening patterns of the fruit of the various genotypes may be attributed in part to the pectolytic enzyme activity within the fruit, particularly the activity of polygalacturonase (9, 12). Recent studies have shown lowered pectinesterase (PE) and an absence of polygalacturonase (PG) activity in mutant *nor* fruit (4). In these studies, PE activity appeared to be similar in fruit of each genotype during the ripening period whereas PG activity was virtually absent in mutant fruit and developed very slowly in heterozygous fruits after the breaker stage (Table 1). On the 4th day after the date of incipient color, PG activity was high in normal fruit but virtually undetectable in fruit of the heterozygote. By the 10th day after the breaker stage, when normal fruit were entering the overripe stage, heterozygous fruit remained firmer and exhibited only a small amount of PG activity; levels were one-sixth that of normal fruit at the same stage and less than one-third that of normal fruit at 4 days after the date of incipient color. Fruit from heterozygous plants harvested 20 and 30 days after breaker exhibited PG levels of 10.2 and 6.4 units respectively which represents 27 and 17% respectively of activity of normal fruit 10 days after breaker.

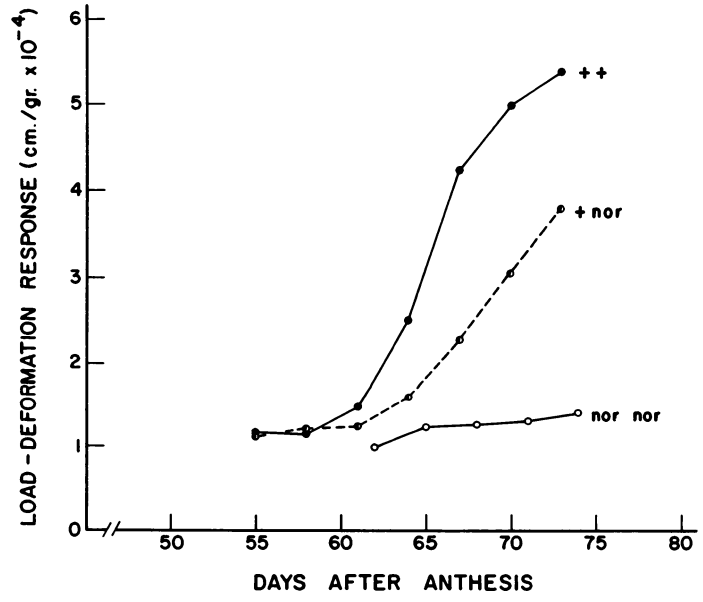

Fig. 4. Softening of fruit of the nor^+/nor^+, nor^+/nor, and *nor/nor* genotypes at 21°C and 85% relative humidity.

Heterozygous fruit, which do not appear overripe even at 30 days after the breaker stage and show improved shelf life (5) may fail to undergo senescence at the normal rate because of the low polygalacturonase activity in the ripening fruit. PG was present in only trace amounts in *nor/nor,* resulting in the virtual absence of softening and senescence of the mutant fruit.

Carotenoid synthesis. In normal fruit, total carotenoids rose rapidly from 14.9 μg/g to 64.0 μg/g on a fresh wt basis during the period from 1 to 9 days after the first sign of visible fruit color (Table 2). Most of the increase is attributable to a large increment in the lycopene content, which rose from 8.5 μg/g to 48.8 μg/g during this interval.

In fruit from plants heterozygous for the *nor* allele, the carotenoids formed during ripening were qualitatively similar to those of normal fruit, but total carotenoid concentrations at equivalent maturity stages were a fraction of normal. Total carotenoids increased from 5.5 μg/g to 34.9 μg/g from the 1st to the 9th day after breaker, and as in the case of normal fruit, the majority of this increase was due to an accelerated accumulation of lycopene (Table 3). In absolute carotenoid content on a fresh wt basis, the heterozygous fruits lagged behind the normal fruit in all categories except beta-carotene. Total carotenoids of nor^+/nor fruit were only a third of normal on the 1st and 5th day after incipient color, and only half of normal on the 9th day; lycopene content on the 9th day was less than half of normal. The relative proportions of the various carotenes were similar in normal and heterozygous fruit at 9 days post-breaker except for beta-carotene which was proportionately twice as high in heterozygous (nor^+nor) fruit.

Total carotenoids in mutant (*nor/nor*) fruit remained essentially unchanged over the period during which carotenes were monitored (Table 4). The fruit retained the appearance of a mature green tomato, and phytoene and β-carotene were the major carotenoids present, though at levels far below those of the ripening genotypes. Phytofluene and neurosporene were present in lower amounts, and only traces of lycopene were evident.

Table 2. Carotenoid composition of fruit of the *nor*$^+$/*nor*$^+$ genotype at 3 stages of ripening.

Carotenoid	1 day post-breaker (μg/g fresh wt)	(%)	5 days post-breaker (μg/g fresh wt)	(%)	9 days post-breaker (μg/g fresh wt)	(%)
Phytoene	0.9 ± 0.3[z]	6.0	10.0 ± 2.5	21.1	5.3 ± 1.7	8.3
Phytofluene	1.1 ± 0.1	7.4	4.6 ± 0.7	9.7	4.7 ± 1.1	7.3
Beta-carotene	3.3 ± 0.1	22.1	2.6 ± 0.8	5.5	3.7 ± 1.1	5.8
Gamma-carotene	1.1 ± 0.1	7.4	1.4 ± 0.2	3.0	1.5 ± 0.8	2.3
Lycopene	8.5 ± 1.3	57.0	28.7 ± 6.6	60.7	48.8 ± 6.2	76.3

[z]± SE.

Table 3. Carotenoid composition of fruit of the *nor*$^+$/*nor* genotype at 3 stages of ripening.

Carotenoid	Carotenoid level: 1 day post-breaker (μg/g fresh wt)	(%)	5 days post-breaker (μg/g fresh wt)	(%)	9 days post-breaker (μg/g fresh wt)	(%)
Phytoene	1.5 ± 0.2[z]	27.3	1.4 ± 0.4	8.2	3.1 ± 0.5	8.9
Phytofluene	0.3 ± 0.0	5.5	1.4 ± 0.3	8.2	2.8 ± 0.6	8.0
Beta-carotene	1.9 ± 0.3	34.5	3.7 ± 0.8	21.8	4.4 ± 1.0	12.6
Gamma-carotene	0.2 ± 0.1	3.6	0.5 ± 0.1	2.9	0.7 ± 0.1	2.0
Lycopene	1.6 ± 0.4	29.0	10.0 ± 1.8	58.8	23.9 ± 4.0	68.5

[z]± SE.

Table 4. Carotenoid composition of fruit of the *nor/nor* genotype at 3 stages of maturity.

Carotenoid	Carotenoid level: 69 days from anthesis (μg/g fresh wt)	(%)	74 days from anthesis (μg/g fresh wt)	(%)	79 days from anthesis (μg/g fresh wt)	(%)
Phytoene	0.4 ± 0.1[z]	33.3	0.4 ± 0.2	36.4	0.7 ± 0.4	63.6
Phytofluene	0.1 ± 0.0	8.3	0.1 ± 0.0	9.1	[y]	
Beta-carotene	0.5 ± 0.1	41.7	0.4 ± 0.1	36.4	0.3 ± 0.2	27.3
Neurosporene	0.2 ± 0.1	16.7	0.2 ± 0.1	18.2	0.1 ± 0.0	9.1
Lycopene	[y]		[y]		[y]	

[z]± SE.

[y]Present in trace amounts.

When *nor/nor* fruit attained an age of about 90 days after anthesis, they exhibited a blush of color at the blossom end. The fruit slowly yellowed after this time and the interlocular tissue of the fruit started to accumulate a red pigment. In fruit detached at 90 days after anthesis and stored at room temp and humidity for 1 month, lycopene was the major pigment, but was present in only small amounts when compared to normal fruit even one day past the breaker stage (Table 5). Beta-carotene is the other prevalent carotenoid at this stage, with phytoene and phytofluene present in smaller concentrations. Neurosporene and gamma-carotene were present in only trace amounts.

Discussion

The *nor* mutant is similar in many respects to the ripening inhibitor (*rin*) mutant. Mutant fruit are typically non-climacteric and many ripening changes are dramatically delayed in time and altered in magnitude. This effect of *nor* presents difficulties (particularly in heterozygous fruit) in sampling fruit of contrasting genotypes for physiological comparisons because both timing and the magnitude of ripening changes are altered. Where possible, fruit were compared in relation to an equivalent developmental stage (generally breaker) rather than at equivalent ages. Mutant fruit lacked an obvious respiratory climacteric and concomitant rise in ethylene at a time when normal fruit undergo ripening, and this condition persisted up to 110 days after anthesis, twice the time required for normal fruit to mature and ripen. Color development occurred slowly over this period of time, and the fruit eventually attained a yellow appearance with a blush of red color at the blossom end. Polygalacturonase activity was present in trace amounts and little softening occurred during what whould constitute the ripening period of a normal tomato.

Althouth the *nor* allele has been described as a recessive mutant (28), it exerts a profound effect on the ripening of fruit from plants heterozygous at this locus. Every characteristic of ripening measured was modulated in the heterozygous fruit. The onset of the climacteric was delayed and carotene develop-

Table 5. Carotenoid composition of 120 day old fruit of the *nor/nor* genotype.

Carotenoid	Carotenoid level (μg/g fresh wt)	(%)
Phytoene	0.9 ± 0.1[z]	12.5
Phytofluene	0.3 ± 0.1	4.2
Beta-carotene	2.4 ± 0.2	33.3
Gamma-carotene	[y]	
Neurosporene	[y]	
Lycopene	3.6 ± 0.1	50.0

[z]± SE.

[y]Present in trace amounts.

ment retarded, respiratory levels were partially suppressed at all phases of the climacteric, and peak levels of ethylene evolution were only a fraction of normal. Softening and increases in polygalacturonase activity proceeded at a slower rate in heterozygous fruit, however they eventually attain acceptable flavor, texture, and color (21).

While current information does not permit precise determination of the primary mode of action of the gene, comparisons may be drawn between the effects of *nor* and those of certain growth regulator and controlled atmosphere treatments administered to normal strains of tomato.

Gibberellin treatments have been reported to delay ripening and color development in tomato (1, 2, 8), and to partially suppress the rate of fruit softening and formation of polygalacturonase in the fruit (2). However, the delay in ripening evident in the *nor*$^+$/*nor* fruit cannot be explained simply on the basis of higher endogenous gibberellin content since the duration and peak rate of the respiratory climacteric is unaffected by gibberellin (8) and PG activity was suppressed to a greater extent in the gibberellin-treated fruit (2) than in the *nor* heterozygotes.

In many ways, the ripening of *nor*/*nor* and *nor*$^+$/*nor* tomatoes resembles the ripening of normal tomatoes stored under low oxygen atmospheres. Tomatoes stored under hypobaric conditions ripen at a slower rate than those under normal atmospheres (6, 30). Burg and Burg (6) attributed this delay in ripening to a reduction of the internal concn of ethylene in the fruit tissues due to the hypobaric conditions, but Stenvers and Bruinsma (26) theorized that the low oxygen tensions were the causal factor delaying the ripening process. At very low levels of oxygen (2.5–5%), ripening in green tomatoes is inhibited (14, 15) and fruit stored at these oxygen tensions resemble nonclimacteric fruit in that they exhibit no rise in carbon dioxide production and evolve no ethylene (15).

Ripening may be inhibited in the *nor* mutant by the limited availability of oxygen at sites of active oxidation within the tissue. The delay and partial suppression of a climacteric in the *nor* heterozygote may be attributable to limited oxygen, inducing a lack of sufficient respiratory activity during ripening, reduced levels of ethylene production, or a decline in the rate of oxidation of a growth regulator. The possibility also exists that binding of ethylene to a receptor site within the fruit is impaired by low oxygen availability (7). Further studies at the cellular level would be necessary to test these hypotheses.

A more plausible explanation for the observed effects of *nor* assumes that the primary genetic effect is on PG synthesis or activation. PG activity is absent in immature normal fruit and increases dramatically after the onset of ripening (2). Absence of PG activity in both the *rin* and *nor* mutants (4, 5); its attenuation in fruit of the *Never Ripe* (*Nr*) mutant (12) and lowered activity in normal fruit with the blotchy ripening disorder (11) suggest that this enzyme plays a vital role in the initiation of normal ripening.

Recent studies (25) suggest that physical integrity of the cell may be an important event in regulating ethylene production in preclimacteric fruits. Ethylene can stimulate its own production by its postulated ability to influence cell integrity. This effect may occur in part through PG biosynthesis. Production of defective PG by the mutants would account for the inability of mutant fruit to produce more than trace levels of ethylene. Absence of other ripening changes in mutant fruit may be secondary effects arising from failure to undergo changes in compartmentation which may occur as a result of release of cell wall bound enzymes by PG (27). Studies of PG in mutant and normal fruit are in progress to attempt to verify this model for genetic regulation of ripening by the ripening mutants.

From an applied standpoint, utilizing the *nor* allele in F_1 hybrids offers the potential of partially utilizing the desirable storage qualities of his mutant (5). Increases in storage life would have several advantages in a fresh market tomato program. Intervals of 2 weeks between harvest and marketing of tomatoes are common, and fruit harvested mature green often represent a heterogeneous collection of fruit at various maturity stages. As ripening occurs in transit, shipments often arrive with fruit at various stages of ripening within a given box, necessitating a repackaging operation at the terminal market (16). A genetically controlled delay in ripening would allow fruit to be harvested at some determined color stage, decreasing the variation in maturity within a given shipment, and increasing the uniformity of coloration of the shipment upon reaching market without undue amounts of spoilage and without special storage conditions. An added advantage might be an increase in fruit size, as tomatoes undergo a 12% increase in size during the 4 day period prior to the turning stage (16).

Experiments are currently in progress to explore the effects of the *nor* allele in hybrid combination on quality parameters during ripening. Preliminary results indicate that *nor*/*nor* fruit maintain a high level of acidity during the normal ripening period, and that *nor* hybrids tend to retain acidity to a greater extent than normal fruit. As the sugar-acid ratio plays a major role in tomato flavor (14), the retention of acidity in *nor* hybrid fruit may be an additional advantage in utilizing the *nor* mutant in a tomato breeding program.

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Influence of Exogenous Daminozide and Gibberellic Acid on Cluster Development and Yield of the 'Concord' Grape[1]

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Abstract. **In 1968 and 1970 applications of gibberellic acid (GA) at berry shatter to mature vines of *Vitis labrusca* L. cv. Concord treated with succinic acid-2,2-dimethylhydrazide (daminozide, SADH) at first-bloom appeared to reduce cluster compactness by lengthening the rachis, to increase berry size, to reduce the number of shot berries, and to increase further the number of mature berries, cluster weight and yield, as compared to daminozide alone. A single application of GA at 50 ppm at berry shatter was as effective in increasing yield as daminozide at 1000 ppm at first bloom, while GA at 100 ppm was significantly more effective. When GA was combined with daminozide were counteracted. The effect of GA on vegetative development was less pronounced than on yield. Highest yield was from the sequential treatment of daminozide 1000 ppm at first bloom and GA at 100 ppm at berry shatter. In the posttreatment year, yields were significantly higher on vines previously treated with either daminozide at 1000 ppm at first bloom, GA at 100 ppm at berry shatter, or daminozide at 1000 ppm with GA at 50 ppm at first bloom.**

Berry set on 'Concord' grapes can be increased by a prebloom application of daminozide especially when natural set is low (3, 11). However, the resulting increase in yield is often less than anticipated based on the amount of berry set (11).

Since GA has been shown to increase berry size in seedless grapes (13), it was hypothesized that a postbloom spray of GA at the berry-shatter stage would be effective in counteracting the berry size reduction effects of daminozide. Such a possibility had been indicated by work in Australia, where GA was used as a postbloom spray to counteract the berry size reduction effect obtained from a prebloom application of (2-chlorethyl)ammonium chloride (chloremequat) (6).

Methods and Materials

In both 1968 and 1970, experiments were conducted to study the effect of GA on 'Concord' grape vines which received a first-bloom application of daminozide at the recommended rate of 1000 ppm. First-bloom is when there is initial evidence of calyptra dehiscence, and full-bloom, when 50% of the calyptras has dehisced. Berry shatter is the stage when noticeable abscission or drop of the young berries is observed following bloom. The experiments in 1968 were designed to test the hypothesis. Experiments in 1970 were designed to verify the results of the 1968 experiments, and to study further the action of daminozide and GA alone, and in combination.

Ten-year-old 'Concord' grape vines used were of medium vigor, trained to the unbrella system, and had been pruned generally following a balance pruning schedule of 30 + 10 buds. The grapes were growing in the teaching vineyard at University Park. In general, vines used in 1970 were not the same as those used 2 years earlier. In all cases, vines were of similar vigor and condition within a replication. Commercial formulations of daminozide (Alar-85) and GA (Pro-Gibb) were applied as dilute aqueous sprays using a hand gun. Tween-20 at 1 cc/3.8 liters was added to each spray. Nontreated vines were not sprayed.

A randomized complete block design with 5 treatments and 4 replications was used in 1968. In 1970 a randomized complete block design with 8 treatments and 7 replications was employed. Single vine plots were used. Data were obtained on randomly selected basal (primary) and second clusters and on the entire vine, and were analyzed as a single classification analysis of variance using replications as samples. Student's 't' test was used to compare treatment means. Where variances were unequal for a particular comparison, the degrees of freedom for values in the 't' table were reduced by one-half.

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