Literature Cited

1. Downs, R. J. and V. P. Bonaminio. 1976. Phytotron procedural manual for controlled-environment research at the SouthEastern Plant Environment Laboratories. *N. C. Agr. Expt. Sta. Tech. Bul.* 244.
2. Einert, A. E. 1971. Response of pot chrysanthemums to growth retardant. EL-531. *Amer. Farm Res.* 20:7-8.
3. Gill, D. L. 1974. Ancymidol shortens Georgia Easter lillies. *Flor. Rev.* 154(3980):19, 61-64.
4. Heins, R. D., R. E. Widmer, and H. F. Wilkins. 1976. Growth regulator recommendations for floricultural crops. *Flor. Rev.* 158(4107): 36-37.
5. Hasek, R. F. and R. H. Sciaroni. 1973. Height control of pot chrysanthemums with A-rest. *Flor. Rev.* 151(3924):53-54.
6. Johnson, C. R. 1974. Response of chrysanthemums grown in clay and plastic pots to soil applications of ancymidol. *HortScience* 9:58.
7. Krauskopf, D. M. and P. V. Nelson. 1976. Chemical height control of Reiger Elatior Begonia. *J. Amer. Soc. Hort. Sci.* 101:618-619.
8. Larson, R. A. and V. P. Bonaminio. 1973. Application of A-Rest to chrysanthemums growing in a bark medium. *N. C. Flower Growers Bul.* 17:5-7.
9. Larson, R. A. and R. K. Kimmins. 1972. Response of *Chyrsanthemum morifolium* Ramat. to foliar and soil application of ancymidol. *HortScience* 7:192-193.
10. Love, J. W., R. A. Larson, and B. G. Hilliard. 1970. The effects of growth retardants and temperature on the growth and flowering of poinsettia cvs. 'Annette Hegg' and 'Eckespoint C-1'. *HortScience* 5:482-483.
11. Sanderson, K. C., W. C. Martin, Jr., K. A. Marcus and W. E. Goslin. 1975. Effects of plant growth regulators on *Lilium longiflorum* Thumb. cv. Georgia. *HortScience* 10:611-613.
12. Shanks, J. B. 1972. Chemical control of growth and flowering in hibiscus. *HortScience* 7:574.
13. Tschabold, E. E., W. C. Meredith, L. R. Guse, and E. V. Krumkalns. 1975. Ancymidol performance as altered by potting media composition. *J. Amer. Soc. Hort. Sci.* 100:142-144.
14. Tjia, B., S. Ohpanayikool, and J. Buxton. 1976. Comparison of soil applied growth regulators on height control of poinsettia. *HortScience* 11:373-374.
15. White, J. W. 1976. Lilium sp. 'Mid Century Hybrids' adopted to pot use with ancymidol. *J. Amer. Soc. Hort. Sci.* 101:126-129.
16. _____and E. J. Holcomb. 1974. Height control methods for poinsettia. *HortScience* 9:146-147.

Total and Reduced Ascorbic Acid Levels in *Rin* and Normal Tomatoes.[1]

Alfredo R. Gonzalez[2] and P. E. Brecht[3]

Department of Vegetable Crops, Cornell University, Ithaca, NY 14853

Additional index words. *Lycopersicon esculentum*, fruit ripening, postharvest physiology, ethylene

Abstract. Changes in reduced ascorbic acid in normal tomato fruits (*Lycopersicon esculentum* Mill.) during holding at 20°C seem to be related to ripening. No change in reduced ascorbic acid levels from mature green to yellow *rin* fruits was observed when detached from the plant. Apparently, a reduction mechanism from dehydro to reduced ascorbic acid is active in normal tomatoes but inactive in detached *rin* fruits. This mechanism was operative in normal and *rin* fruits left on the plant. Exogenous ethylene (10 ppm) neither affected reduced ascorbic acid levels in normal and *rin* fruits, nor the total ascorbic acid in normal fruits. Maintenance of initial levels of total ascorbic acid was noted in ethylene treated *rin* fruits. Similar total ascorbic acid levels were found in normal and *rin* fruits held on the vine to the red and yellow stages, respectively; and were higher than in comparable fruits held in storage.

Tomatoes are considered a good source of vitamin C (23). Ascorbic acid (vitamin C) is found in plant tissue in 3 forms: reduced ascorbic acid (RAA); monodehydro-ascorbic acid (an unstable intermediate); and dehydro-ascorbic acid (DHA) (11). Dehydro-ascorbic acid is more stable at low pH, but can be lost by irreversible conversion to 2,3-diketogulonic acid (11). Reduced and dehydro-ascorbic acids are physiologically active in the animal body and have antiescurbic properties (17, 20) while 2,3-diketogulonic acid does not (17).

Clow and Marlatt (5) found that total ascorbic acid (TAA)

in mature green and red tomatoes was the same. Brecht et al. (4) found similar results in 6 of 8 cultivars studied. Mapson (11) reported that the level of RAA in plant tissue is about 95% of the total ascorbic acid. However, Brecht et al. (4) found that in mature green tomatoes RAA values ranged from 21 to 72% of TAA depending on the cv. They also reported that RAA levels increased from mature green to the red stage to reach values that fluctuated from 83 to 100% of the TAA. The increase in RAA from mature green to red stages is supported by numerous reports (2, 3, 10, 21). However, others report no change (12, 22) or a decrease (16). Vine ripe tomatoes seem to have higher levels of RAA than fruits ripened in storage (2, 9, 21).

Exogenous ethylene has been reported to cause either no change (5) or a decrease (8) of TAA in treated fruits relative to non-treated. A slightly higher level of RAA was noted in ethylene treated fruits at the red stage (14, 22).

The *rin* mutant tomato lacks many of the changes associated with ripening (18). Due to this characteristic, the *rin* mutant was used in the present work to compare changes in TAA and RAA during ripening. Normal and *rin* tomatoes grown in the field or held in storage with or without ethylene treatment were studied in an attempt to relate changes of ascorbic acid to ripening.

Materials and Methods

Two isogenic lines, the *rin* and the normal line ('Fireball' x Cornell 54-149) from which the *rin* mutant originated (18), were field grown. Mature green fruits of both lines were obtained 38 days after anthesis and only fruits of about 5 cm in diameter were held at 20°C in humidified air with or without ethylene (10 ppm) for different times. Ten fruits of both lines were sampled when the normal fruits were at the mature green, turning, pink, and red stages (1). Yellow *rin* fruits were sampled after 18 days of ethylene treatment or 60 days of storage in the absence of exogenous ethylene. Representative samples of normal and *rin* fruits were left on the plant for 58 and 84 days after anthesis to reach the red and yellow color, respectively. After sampling, the fruits were frozen and ground into a fine powder, the bulk sample was stored at −20°C for up to 3 and 6 months prior to analysis of TAA and RAA, respectively. Additional information on pre and postharvest handling methods were previously described by Gonzalez et al. (6).

Duplicate frozen subsamples of 25 g were used for analysis of total and reduced ascorbic acid. In the analysis of TAA, the samples were homogenized in a Waring Blender in 100 ml of 1% oxalic acid for 3 min at high speed, and the 2,4-dinitrophenylhydrazine method of Roe and Oesterling (19) was used. Triplicate readings for each subsample were made on a Bausch and Lomb Spectronic 20 spectrophotometer. In the analysis of RAA, the samples were homogenized as previously indicated in 100 ml of 3% metaphosphoric acid and the indophenolxylene method of Nelson and Somers (15) was used. Triplicate readings for each subsample were made on a J. K. Turner Spectrophotometer Model #330. LSD 5% levels were calculated to compare mean values for TAA and RAA from 2 identical replicated experiments. Since the TAA analysis included RAA, DHA, and 2,3-diketogulonic acid, it was assumed that the differences between TAA and RAA was composed mostly of DHA with a very small amount of 2,3-diketogulonic acid as indicated by Mills et al. (13).

Results and Discussion

Normal fruits harvested at the mature green and red stages had levels of TAA similar to the *rin* fruits at mature green and yellow stages (Fig. 1, Fig. 2). In normal fruits left on the plant under field conditions, the RAA level increased 3 fold from the mature green to red stage (Fig. 1). A significant decrease in TAA was noted from mature green to red stage in fruits ripened in storage with or without exogenous ethylene when compared to fruits ripened on the plant. Levels of RAA in red fruits from the 2 storage treatments were about the same but lower than those found in the field ripened tomatoes. Levels of RAA of normal fruits held in storage increased 1.5 fold from mature green to the red stage.

Reduced ascorbic acid levels in *rin* fruits at the mature green stage was one-half of those harvested at the yellow stage (Fig. 2). Levels of TAA in *rin* fruits held in storage under humidified air decreased gradually, and the lowest level was measured after 60 days. Reduced ascorbic acid in *rin* fruits remained about the same throughout the storage period from mature green to yellow stage. Ethylene treated *rin* fruits had a significant decrease in TAA the 4th and 6th day after initiation of treatment compared to fruits left on the plant. However, due to a gradual increase in TAA, no significant differences were noted after 7 days and at the yellow stage reached at the 18th day as compared to levels in freshly harvested fruits. No significant difference in RAA was noted in ethylene treated *rin* fruits during the storage period (Fig. 2).

The respiratory behavior of the *rin* and normal fruits held in the presence or absence of exogenous ethylene was similar to the finding of Herner and Sink (7) and is presented in Fig. 1 and Fig. 2 for comparative purposes.
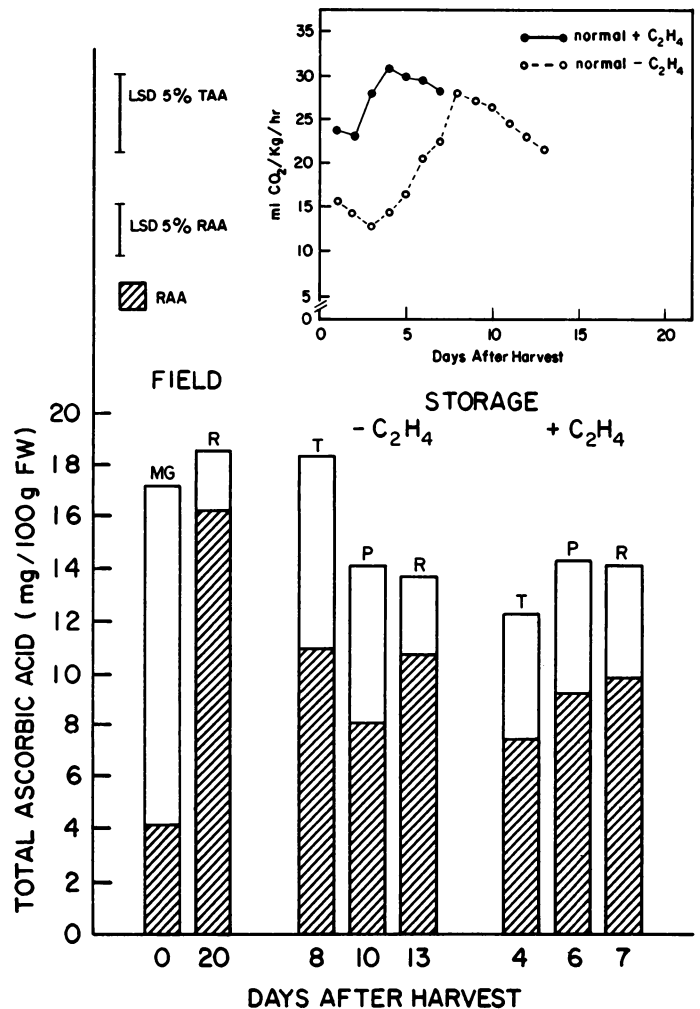

Fig. 1. Levels of ascorbic acid, total (TAA) and reduced (RAA), in normal fruits from the field and in storage. Fruits were stored with or without 10 ppm ethylene. Mature green (MG) tomatoes were harvested 38 days after anthesis. Further ripening stages were identified as turning (T), pink (P) and red (R). Inset shows respiration rates [after (6)].

Reports indicating an increase in RAA during ripening of tomatoes (2, 3, 4, 10, 21) suggested that this change is related to ripening. To test this hypothesis, levels of RAA were evaluated in normal *rin* fruits. Similar levels of RAA were noted in mature green normal and *rin* fruits. However, an increase in RAA followed by a temporary decrease at the pink stage was noted in normal fruits during ripening. In contrast, no significant change from mature green to yellow was observed in *rin* fruits (Fig. 1, Fig. 2). These observations support the initial hypothesis. We further hypothesized that the lack of increase of RAA in *rin* fruits was due to the effect of low levels of ethylene produced by *rin* fruits (7). Exogenous ethylene (10 ppm) was applied to mature green *rin* fruits to test this hypothesis. Although exogenous ethylene did increase the respiration rate of *rin* fruits, it had no effect on the RAA levels. Similarly, ethylene treatment did not change RAA levels in normal fruits (Fig. 1).

Lower TAA and RAA levels were observed in normal fruits ripened in storage than in fruits vine ripened in the field. This indicated that the parent plant helps to maintain TAA levels and also to increase the levels of RAA during ripening. Levels of RAA in *rin* fruits procured from the plant increased significantly from mature green to the yellow stage (2×), while
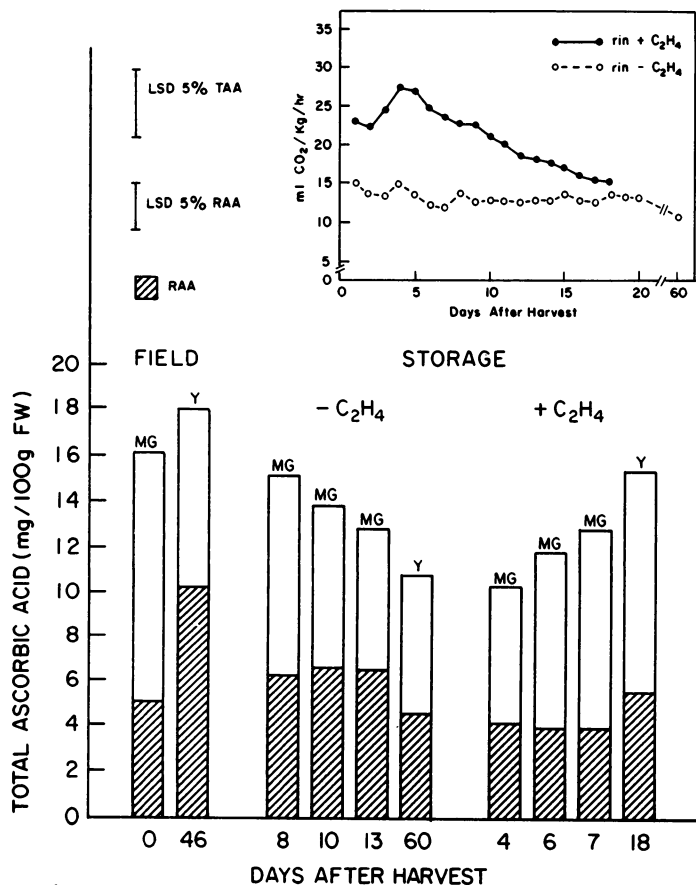
Fig. 2. Levels of ascorbic acid, total (TAA) and reduced (RAA), in *rin* fruits from the field and in storage. Fruits were stored with or without 10 ppm ethylene. Mature green (MG) tomatoes were harvested 38 days after anthesis. Yellow (Y) fruits from the field and storage were also sampled. Inset shows respiration rates [after (6)].

Apparently, this mechanism is inactive in *rin* mutant fruits after harvest, but is partially active when these fruits are attached to the parent plant. Exogenous ethylene had no effect on this mechanism. Reduced ascorbic acid data obtained by the indophenol-xylene method (15) provided a reasonable estimate of the TAA in tomatoes ripened on the vine, but it can be misleading in tomato fruits, ripened in storage.

in normal fruits the increase was even greater (3x) (Fig. 1, Fig. 2).

A significant decrease in TAA in ethylene treated normal and *rin* fruits was probably due to the induced increase in respiration. While the levels of TAA in normal fruits decreased during the ethylene treatment, an increase was noted in *rin* fruits (Fig. 1, Fig. 2). This increase was probably due to a synthesis of DHA since the RAA levels remained the same during the treatment. The increase of DHA in *rin* fruits was related to a decrease in the rate of respiration (Fig. 2).

Evaluations of TAA and RAA in normal fruits ripened on the vine indicated that RAA data can give a reasonable idea of the total level of ascorbic acid in tomatoes. However, Brecht et al. (4) reported that in some cultivars, RAA can be misleading since those values could be 17% lower than TAA. More serious consideration should be given to possible anomalies when RAA data is used as a measure of TAA in storage of ripening tomato fruits, since the conversion of DHA to RAA during ripening seems to be less efficient than in vine ripened tomatoes.

In summary, the data suggest that in normal fruits there is a reduction mechanism from DHA to RAA and the increase of RAA is associated with ripening. This conversion was greater in fruits ripened on the vine than those ripened in storage.

Literature Cited

1. Anonymous. 1975. Color classification requirements in tomatoes. United Fresh Fruit, Vegetable Association and USDA. Visual Aid TM – L-1.
2. Betancourt, L. A., M. A. Stevens, and A. A. Kader. 1977. Accumulation and loss of sugars and reduced ascorbic acid in attached and detached tomato fruits. *J. Amer. Soc. Hort. Sci.* 102:721-723.
3. Bisogni, C. A., G. Armbruster, and P. E. Brecht. 1976. Quality comparisons of room ripened and field ripened tomato fruits. *J. Food Sci.* 41:333-338.
4. Brecht, P. E., L. Keng, C. A. Bisogni, and H. M. Munger. 1976. Effect of fruit portion, stage of ripeness and growth habit on chemical composition of fresh tomatoes. *J. Food. Sci.* 41:945-948.
5. Clow, B. and A. L. Marlatt. 1930. Studies of vitamin C in fresh and canned tomatoes. *J. Agr. Res.* 40:767-775.
6. Gonzalez, A., P. E. Brecht, and C. C. Rehkugler. 1976. Free methionine levels in *rin* and normal isogenic tomato fruits ripened in the field or in storage. *Plant Physiol.* 58:648-650.
7. Herner, R. C. and K. C. Sink, Jr. 1973. Ethylene production and respiratory behavior of the *rin* tomato mutant. *Plant Physiol.* 52:38-42.
8. Jones, D. B. and E. M. Nelson. 1930. Vitamin content of ethylene treated and untreated tomatoes. *Amer. J. Pub. Health* 20:387-394.
9. Kader, A. A., M. A. Stevens, M. Albright-Holton, L L. Morris, and M. Algazi. 1977. Effect of fruit ripeness when picked on flavor and composition in fresh market tomatoes. *J. Amer. Soc. Hort. Sci.* 102:724-731.
10. Malewski, W. and P. Markakis. 1971. Ascorbic acid content of developing tomato fruit. *J. Food Sci.* 36:537.
11. Mapson, L. W. 1958. Metabolism of ascorbic acid in plants. Part 1. Function. *Annu. Rev. Plant Physiol.* 9:119-150.
12. Matthews, R. F., P. Crill, and S. J. Locascio. 1974. B-Carotene and ascorbic acid contents of tomatoes as affected by maturity. *Proc. Fla. St. Hort. Soc.* 87:214-216.
13. Mills, M. B., C. M. Damron, and J. H. Roe. 1949. Ascorbic acid, dehydroascorbic acid and diketogulonic acid. *Anal. Chem.* 21:707-7-9.
14. Morris, L. L. and A. A. Kader. 1975. Postharvest physiology of tomato fruits. Fresh Market Tomato Research. 1975. Res. Rpt., – Univ. of California, Davis. p. 69-84.
15. Nelson, N. L. and G. F. Somers. 1945. Determination of ascorbic acid. Application of the indophenol-xylene extraction method to determination in large numbers of tomato and tomato juice samples. *Ind. Eng. Chem., Anal. Ed.* 17:754.
16. Pantos, C. E. and P. Markakis. 1973. Ascorbic acid of artificially ripened tomatoes. *J. Food. Sci.* 38:550.
17. Penney, J. R. and S. S. Zilva. 1943. The chemical behavior of dehydro-L-ascorbic acid *in vitro* and *in vivo*. *Biochem. J.* 37:403-417.
18. Robinson, R. W. and M. L. Tomes. 1968. Ripening inhibitor: a gene with multiple effect on ripening. *Tomato Genet. Coop.* 18:36-37.
19. Roe, J. H. and M. J. Oesterling. 1944. The determination of dehydroascorbic acid and ascorbic acid in plant tissue by the 2,4-dinitrophenylhydrazine method. *J. Biol. Chem.* 152:511-517.
20. Schultze, M. O., E. Stotz, and C. G. King. 1938. Studies on the reduction of dehydroascorbic acid by Guinea pig tissues. *J. Biol. Chem.* 122:395-406.
21. Scott, L. E. and A. Kramer. 1949. The effect of storage upon the ascorbic acid content of tomatoes harvested at different stages of maturity. *Proc. Amer. Soc. Hort. Sci.* 54:277-280.
22. Watada, A. E., B. B. Aulenbach, and J. T. Worthington. 1976. Vitamin A and C in ripe tomatoes as affected by stage of ripeness at harvest and by supplementary ethylene. *J. Food Sci.* 41:856-858.
23. Watt, B. K. and A. L. Merril. 1963. Composition of foods. U.S. Dept. Agr., Agr. Handb. 8 USDA.

758

J. Amer. Soc. Hort. Sci. 103(6):756–758. 1978.