J. Amer. Soc. Hort. Sci. 102(5):523–525. 1977.

Carbon Dioxide, Ethylene, and Color Development in Ripening Mature Green Bell Peppers[1]

Mikal E. Saltveit, Jr.[2]
Department of Horticulture, Michigan State University, East Lansing, MI 48824

Additional index words. Capsicum annuum, climacteric, wounding

Abstract. **Mature green bell pepper fruit (*Capsicum annuum* L. cv. Yolo Wonder) exhibited a non-climacteric pattern of ethylene and carbon dioxide production during normal ripening and red color development at 24°C. Exposing detached mature green fruit to 500 ppm propylene in air for 48 hours, did not induce an increase in ethylene or carbon dioxide production. Wounding excised plugs of ovary wall tissue caused an increase in carbon dioxide production within one day, and an increase in ethylene production by the second day.**

Red-ripe pepper fruit are valued for the color and flavor they impart to foods. Completely red fruit are necessary for some uses and more desirable than partially green fruit for others. Since fruits mature throughout the growing season, once-over mechanical harvesting yields fruit at different stages of maturity. Certain pre- and postharvest treatments can increase the yield of red fruit from some pepper cultivars by either increasing the number of mature fruit before harvest or by inducing red color development after harvest; however, results are often inconsistent.

Ethylene and the ethylene-releasing chemical (2-chloroethyl) phosphonic acid (ethephon) enhance maturation, ripening (3, 5, 9), and color development in many fruit (1). Foliar application of ethephon to plants of bell pepper (10, 18), paprika pepper (23), pimiento pepper (11), or chili or pimiento pepper (21) significantly increased red color development and/or percentage of red fruit harvested. Harvested pimiento peppers, at or beyond the breaker stage, developed normal red color when treated with ethephon (11). However, mature green pimiento or bell peppers failed to develop acceptable red color when treated with ethephon or ethylene (10, 11).

Preliminary observations showed that injury or disease, which have been reported to promote ethylene and carbon dioxide production in plants (12, 13), appeared to hasten ripening and color development in pepper fruit. Propylene, an active ethylene analog, has been used to induce ethylene-like responses in other fruits while allowing endogenous ethylene production to be measured (15). A 500 ppm propylene mixture in air is about analogous in effect to a 5 ppm ethylene mixture (4) and has been reported to promote a typical respiratory increase in climacteric fruit (15). In non-climacteric fruit, eg. citrus, it slightly increased respiration but had little effect on ethylene production. Propylene increased respiration in immature tomato fruit from normal strains and in fruit from the abnormal ripening mutant *rin*, but had no effect on endogenous ethylene production by either (7, 14).

Ripening factors in tomato have been investigated using tissue transfers between different cultivars or between fruits at different stages of maturity. Reciprocal transfers of tissue between *rin* fruit and fruit from a normal ripening tomato strain showed that *rin* fruit do not contain translocatable ripening inhibitors or lack translocatable ripening factors (17).

The ripening of green bell peppers was investigated to provide information on how color development could be induced in detached fruit.

Materials and Methods

Freshly harvested, green or red-ripe, unblemished 'Yolo Wonder' pepper fruits of uniform size were washed in a 0.5% solution of sodium hypochlorite, and the stems were trimmed and covered with a thin layer of silicon stopcock grease to reduce desiccation, prevent entry of disease, and mimic gas diffusion of fruit left on the plant. The peppers were then assigned to blocked replicates on the basis of weight. All laboratory experiments were run at 24°C and at 80 to 90% relative humidity. For experiments with attached peppers, mature plants with developing green peppers were taken from the field, pruned of excess foliage, and maintained in the greenhouse.

The carbon dioxide and ethylene concn in ripening peppers was studied by gas chromatographic analysis of samples from the locular cavity. Samples were initially taken by inserting the needle of a 1 ml gas tight syringe through the ovary wall. To circumvent possible effects of periodic wounding from this sampling technique, later experiments used fruit fitted with a rubber serum stopper. A 1.4 cm (diam) sterile serum stopper was inserted into a 1.2 cm (diam) hole cut in the ovary wall with a cork borer. Very little microbial contamination resulted from this procedure. Each fruit was tested for gas-tightness by injecting 20 ml of air into the pepper while holding it under water; those that leaked were discarded. The ripening of each fruit was followed by rating its maturity on a 1 to 7 scale of color development: 1 = mature green, 4 = half red, and 7 = full red-ripe.

Tissue transfer. Three plugs of ovary wall tissue from mature green or red-ripe peppers were transplanted into mature green peppers to determine if ripening would be affected. The transfers were done under aseptic conditions. A 1.8 cm (diam) ovary wall plug from either a mature green or red ripe pepper was inserted into a 1.6 cm (diam) hole in the wall of a mature green fruit. The exposed incision in the epidermis was covered with a thin layer of silicon stopcock grease to insure a gas-tight fit. A similar amount of grease was applied to the uninjured control. The stage of ripening was periodically noted. The internal ethylene and carbon dioxide concn were determined 3 days after transfer of the plugs for 12 replicates of each treatment.

Endogenous ethylene and carbon dioxide. Internal ethylene and carbon dioxide concn, and the degree of ripening were periodically determined for each of 50 mature green peppers. The experiment was repeated 3 times using a) detached peppers, b) detached peppers fitted with serum stoppers, and c) peppers attached to the plant and fitted with serum stoppers.

Propylene treatment. Desiccators (ca. 10 liters) containing 6 mature green peppers fitted with serum stoppers were continuously flushed with ethylene-free air (ca. 100 ml/min). After 2 days one-half of the desiccators were flushed with 500 ppm propylene in air for 48 hr. The internal ethylene and carbon dioxide concn and the degree of ripening were periodically determined. The experiment was done twice with 2 replicates each.

Wound stress. Wound induced ethylene and carbon dioxide production was studied using 1.5 cm (diam) plugs of mature green pepper ovary wall tissue. Whole plugs, plugs with 4 radial

[1]Received for publication February 28, 1977. Michigan Agricultural Experiment Station Journal Article No. 8000.

[2]I wish to thank Drs. D. R. Dilley and H. C. Price for their assistance and Dennis Werner for bringing the difference between cultivated and wild peppers to my attention.

incisions, and plugs cut into 4 radial segments were enclosed with moist filter paper in separate 50 ml Erlenmeyer flasks. Plugs for each set of treatments were taken from the same locule. After gas samples were taken for analysis, the flasks were flushed with ethylene-free air and recapped with serum stoppers for the next sampling interval. The experiment was done twice with 4 replicates each.

Results and Discussion

Tissue transfer. Transfer of tissue from mature green or red ripe peppers to mature green fruit did not significantly affect ethylene production or color development (Table 1). Examination of the peppers at the conclusion of the experiment revealed that the plugs and wall tissue were in intimate contact. It appeared that neither a translocatable factor, nor an emanation from ripening tissue promoted ripening. Similar results were obtained with the *rin* tomato mutant (17).

After 17 days peppers with green or red plugs had respectively a 20% or 35% higher average color score than the control (Table 1). These large differences were not statistically different at the 5% level because of a coefficient of variability of 34% in the experiment. Researchers have encountered similar developmental heterogenity in peppers (8, 11, 20, 23). Howard and Yamaguchi (8), finding no adequate method to select pepper fruits of the same physiological age, used fruit weight and surface appearance in their studies. Since fruits in this experiment had already been selected on the basis of weight and surface uniformity, patterns of respiration and ethylene production were studied to see if they could be used to select a more physiologically homogeneous population.

Endogenous ethylene and carbon dioxide. Ripening mature green bell peppers did not show a climacteric rise in either ethylene or carbon dioxide concn prior to, or after color development commenced (Fig. 1). Before the data were analyzed, the internal ethylene and carbon dioxide concn and the degree of color development for each pepper was shifted on the time axis, so that day zero became the common starting date of ripening for each of the 50 peppers. The beginning of color development was taken as day one. For example, data for a mature green pepper with an internal ethylene concn of 21 ppb on day 1 after harvest, 25 ppb on day 5, 30 ppb on day 10, and 35 ppb on day 15, and with the beginning of color development 9 days after harvest, would be arranged on the time axis so that the 21 ppb concn of ethylene would occur on day −7, 25 ppb on day −3, 30 ppb on day +2, and the 35 ppb on day +7, 25 ppb on day −3, 30 ppb on day +2, and the 35 ppb on day +7. After all the data were recorded, an average was calculated for each day.

Both the ethylene and carbon dioxide concn increased during color development. The carbon dioxide concn increased about 10%, while the ethylene concn nearly doubled. Both changes were significant, though small compared to the dramatic increases (10 to 100-fold) in ethylene and carbon dioxide production found for most climacteric fruits as they ripen (19). The increase in carbon dioxide and ethylene was partially the result

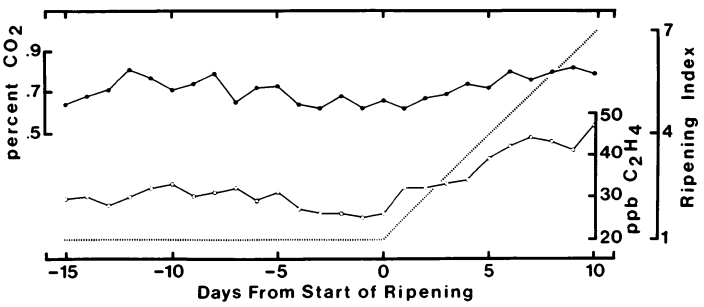

Fig. 1. Red color development and ethylene and carbon dioxide concentration in ripening bell pepper fruits. The data is the average of 3 experiments. The time course of ripening of each pepper was adjusted so that color development started on day 1 (see text for further details).

of general deterioration and microorganism infection in the 10% to 20% of the mature green peppers which ripened to more than 70% red.

Bell peppers would be classified as a non-climacteric fruit on the basis of these very small changes in carbon dioxide and ethylene concn (2). Howard and Yamaguchi (8) have reported a similar nonclimacteric respiratory pattern for *C. annuum* cv. California Wonder. Using excised tissue, they showed that the rate of oxygen consumption decreased until the mature green stage, then after a slight rise it declined as the fruit senesced. Pratt and Lyons (20) have also reported that ripening bell peppers did not exhibit a climacteric rise in respiration. Lockwood and Vines (11) reported that a 1 min dip in 3000 ppm ethephon induced a climacteric rise in the respiration of predominantly green detached pimiento peppers. However, since ethylene treatment increases respiration of non-climacteric fruit, ethylene from decomposition of the ethephon may have induced the transient "climacteric."

Bell peppers, which show a distinct color change as they ripen, appear to be non-climacteric, while the tomato, which is also a member of the Solanaceae, is climacteric. A possible explanation could lie in the history of the pepper's domestication. Domesticated peppers have persistent, pendulant fruit, while wild peppers have decidious, erect fruit (6). Smith (22) has proposed that persistent, large fruited cultivars were selected by the American natives in their domestication of the pepper. If a climacteric rise in ethylene induces abscision in wild peppers, as it does in other species (1), then the selection for persistent fruit may also have inadvertently selected for a non-climacteric ethylene and carbon dioxide pattern.

Detached fruit not fitted with serum stoppers had a significantly higher concn of ethylene and carbon dioxide than detached or attached peppers fitted with serum stoppers (Table 2). Peppers on the plant had a significantly lower ethylene concn than harvested fruits, with or without serum stoppers. Clearly, wounding the tissue to the extent required to implant

Table 1. Ethylene concn and color development in mature green peppers at various days after transfer of plugs. Plugs were transfered to mature green pepper from mature green or red ripe fruit.

Tissue	Ethylene (nl/liter)	Avg color score[z]	
	3 days	10 days	17 days
Intact green	61	2.1	3.4
Green on green	63	2.2	4.1
Red on green	61	2.3	4.6
F test (5% level)	N.S.	N.S.	N.S.

[z]Color score: 1 = green. 7 = full red.

Table 2. Ethylene and carbon dioxide concn in mature green peppers which were: a) detached, b) detached and fitted with a serum stopper in the ovary wall, or c) attached to the parent plant and fitted with a serum stopper.

Treatment	Ethylene (nl/liter)	Carbon dioxide (ml/liter)
Detached fruit	34.3a[z]	8.3a
Detached fruit with serum stoppers	27.4b	6.3b
Attached fruit with serum stoppers	18.8c	6.8b

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

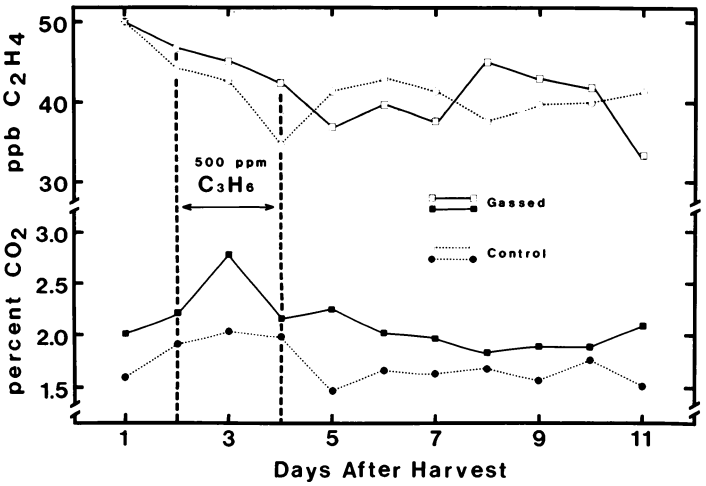

Fig. 2. Ethylene and carbon dioxide concn in mature green bell peppers exposed to air with, or without 500 ppm propylene for 48 hr. Each point is the average of 12 observations.

the serum stoppers does not stimulate ethylene and CO_2 production.

Propylene treatment. A 48 hr exposure to 500 ppm propylene in air did not induce a climacteric rise in ethylene or carbon dioxide (Fig. 2). Ethylene concn in the treated peppers did not differ significantly from those in the air controls. Carbon dioxide concn increased 1 day after exposure to propylene, returned to their initial levels while still exposed to propylene, and subsequently remained close to the levels found in the air controls. The transitory increase in respiration is not unexpected since, with the exception of cranberries, ethylene increased respiration in all fruit tested (1).

Mature green bell peppers respond to ethylene and ethylene analogues in a manner similar to non-climacteric fruit, immature climacteric fruit, or vegetative tissue. Abeles (1) concluded that ethylene has no effect on the respiration of most vegetative tissue. However, it was observed that mature green bell peppers did partially ripen as indicated by the increase in red pigmentation, but the pattern was abnormal.

Wound stress. Additional wounding, beyond excision of the tissue, caused an immediate increase in carbon dioxide production which persisted for the duration of the experiment (Table 3). Ethylene production increased by the second day. There was no statistical difference in ethylene or carbon dioxide production between wounding with 4 radial incisions and by cutting the plug into four segments.

Conclusions

Ripening green bell pepper fruit are non-climacteric in their pattern of carbon dioxide or ethylene production. Exposing fruit to 500 ppm propylene elicited a respiratory and ethylene production response characteristic of vegetative tissue. Wounding increased the rate of carbon dioxide and ethylene production from excised plugs of ovary wall tissue. Horticulturally mature green peppers appear to be physiologically immature and remain so upon removal from the plant; they are incapable of ripening normally. In this respect they are similar to *rin* tomato fruit, which partially ripen if treated with ethylene while attached to their parent plant (16) but react to ethylene like non-climacteric fruit once harvested (7). Also like *rin* fruit, transfer of tissue from mature green or red ripe peppers to mature green fruit did not promote ripening.

Table 3. Ethylene and carbon dioxide production by mature green pepper plugs, plugs additionally wounded with 4 radial incisions or plugs cut into 4 radial segments.

	Ethylene production (nl/g hr)			Carbon dioxide production (μl/g hr)		
	Days after wounding			Days after wounding		
Wound stress	1	2	3	1	2	3
Control	1.95a	1.94a	1.93a	36.2a	26.2a	27.0a
Wounded	2.72a	7.09b	7.14b	37.4a	42.8b	47.6b
Segmented	2.89a	7.23b	5.72b	42.7b	46.0b	41.7b

[z]Mean separation in columns by Duncan' multiple range test, 5% level.

Literature Cited

1. Abeles, F. B. 1973. Ethylene in plant biology. Academic press, New York. p. 161-178.
2. Biale, J. B. 1964. Growth, maturation, and senescence in fruit. *Science* 146:880-888.
3. Burg, S. P. 1962. The physiology of ethylene formation. *Annu. Rev. Plant Physiol.* 13:265-302.
4. _____ and E. A. Burg. 1967. Molecular requirements for the biological activity of ethylene. *Plant Physiol.* 42:144-152.
5. Edgerton, L. J. and G. D. Blanpied. 1968. Regulation of growth and fruit maturation with 2-chloroethanephosphonic acid. *Nature* 219: 1064-1065.
6. Eshbaugh, W. H. 1975. XII. Genetic and biochemical systematic studies of chili-peppers (*Capsicum* – Solanaceae). *Bul. Torrey Bot. Club* 102:396-403.
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. Howard, F. D. and M. Yamaguchi. 1957. Respiration and the oxidative activity of particulate fraction from developing pepper fruits (*Capsicum annuum* L.). *Plant Physiol.* 32:418-423.
9. Iwahori, S. and J. M. Lyons. 1970. Maturation and quality of tomatoes with preharvest treatment of 2-chloroethylphosphonic acid. *J. Amer. Soc. Hort. Sci.* 95:88-91.
10. Knavel, D. E. and T. R. Kemp. 1973. Ethephon and CPTA on color development in bell pepper fruits. *HortScience* 8:403-404.
11. Lockwood, D. and H. M. Vines. 1972. Red color enhancement of pimiento peppers with (2-chloroethyl)phosphonic acid. *J. Amer. Soc. Hort. Sci.* 97:192-197.
12. Lund, B. M. and L. W. Mapson. 1970. Stimulation by *Erwinia carotovora* of the synthesis of ethylene in cauliflower tissue. *Biochem. J.* 119:251-263.
13. Maxie, E. C. R. Amezquita, B. M. Hassan, and C. F. Johnson. 1967. Effect of gamma irradiation on the ripening of banana fruits. *J. Amer. Soc. Hort. Sci.* 92:235-254.
14. McGlasson, W. B., H. C. Dostal, and E. C. Tigchelaar. 1975. Comparison of propylene-induced responses of immature fruit of normal and *rin* mutant tomatoes. *Plant Physiol.* 55:218-222.
15. McMurchie, E. J., W. B. McGlasson, and I. L. Eaks. 1972. Treatment of fruit with propylene gives information about the biogenesis of ethylene. *Nature* 237:235-236.
16. Mizrahi, Y., H. C. Dostal, and J. H. Cherry. 1975. Ethylene induced ripening in attached *rin* fruits, a non-ripening mutant of tomato. *HortScience* 10:414-415.
17. _____, _____, W. B. McGlasson, and J. H. Cherry. 1975. Transplantation studies with immature fruit of normal, and *rin* and *nor* mutant tomatoes. *Plant Physiol.* 55:1120-1122.
18. Osterli, P. P., R. M. Rice, and K. W. Dunster. 1975. Effect of ethephon on bell pepper fruit ripening. *Calif. Agr.* 29(7):3.
19. Pratt, H. K. and J. D. Goeschl. 1969. Physiological roles of ethylene in plants. *Annu. Rev. Plant Physiol.* 20:541-584.
20. _____and J. M. Lyons. 1959. Respiration and ripening of fruits of bell pepper (*Capsicum annuum* L.). IX Inter. Bot. Congr. Proc. Vol. 2. p. 308-309.
21. Sims, W. L., H. B. Collins, and B. L. Gledhill. 1970. Ethrel effects on fruit ripening of peppers. *Calif. Agr.* 24(2):4-5.
22. Smith, P. G. 1951. Deciduous ripe fruit character in peppers. *Proc. Amer. Soc. Hort. Sci.* 57:343-344.
23. Worku, Z., R. C. Herner, and R. L. Carolus. 1975. Effect of stage ripening and ethephon treatment on color content of paprika pepper. *Scientia Hort.* 3:239-245.