J Plant Growth Regul (1989) 8:37–44

Journal of
Plant
Growth
Regulation

Ethylene Binding Changes in Apple and Morning Glory During Ripening and Senescence

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Received January 20, 1988; accepted June 24, 1988

Abstract. Ethylene binding sites were measured during fruit ripening and morning glory flower senescence. Little change in ethylene binding was noted during these developmental stages, except a slight decline during the later stages of fruit ripening or flower senescence. The concentration of ethylene required to achieve 50% saturation of the binding sites was 0.14 μl/liter for both apple pulp and morning glory flowers. Ethylene binding sites were calculated to be 3.2×10^{-11} moles/kg and 3.8×10^{-9} moles/kg in apple and morning glory, respectively. It does not appear that changes seen in ethylene sensitivity during fruit ripening can be readily ascribed to changes in the number of ethylene binding sites in the tissue.

Ethylene involvement in fruit ripening and senescence of plant parts, such as flowers, has been known for many years. Ethylene biosynthesis has been studied extensively; however, less is known about the changes in ethylene binding that occur during ripening and senescence. Ethylene binding has been studied using a radioisotope technique in tobacco (Sisler 1979, Goren and Sisler 1984), citrus leaves (Goren and Sisler 1986), tomatoes (Sisler 1982), cell-free preparations of bean (Bengochea et al. 1980a, b), and carnation flowers (Sisler et al. 1986). While there is no conclusive evidence that this method measures the physiological binding site per se, binding measured in this way does seem to meet the criteria for a receptor site (Sisler and Wood 1987). The concentration of ethylene needed to ½ saturate the binding sites can be calculated from the Kd (Venis 1985) and has been found to be 0.28 μl/liter in tobacco leaves (Sisler 1979), 0.31 μl/liter in tomato fruit (Sisler 1982), 0.14 μl/liter in carnation petals, and 0.13 μl/liter in carnation leaves (Sisler et al. 1986).

Paper No. 11398 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601, USA.

The significance of the large amounts of ethylene produced during the climacteric as the inducer of fruit ripening is somewhat questionable (Romani 1984, Blankenship and Unrath 1988). The idea of increased ethylene sensitivity with fruit ripening and senescence has been expressed for a number of years (Burg and Burg 1965). Increased ability to bind ethylene or an increase in the number of binding sites with ripening or senescence could make the plant more sensitive to lower levels of ethylene present. This study was designed to determine if the number of ethylene binding sites changed during the course of fruit ripening or flower senescence.

Materials and Methods

Plant Materials

"Starkrimson" Red Delicious apples were harvested weekly from 2 commercial orchards and held at 5°C overnight prior to analysis. The orchard in location 1 was in a relatively warm growing environment (elevation of 183 m), while the orchard in location 2 was in a cool growing environment (elevation 945 m). Morning glory cv. Heavenly Blue plants were grown outdoors. Flowers were harvested at the stages described by Beyer and Sundin (1978).

Internal Ethylene Measurement

Fruit internal ethylene was extracted under vacuum (Saltveit 1982). Ethylene in the resulting gas was measured by gas chromatography on an activated alumina column with an oven temperature of 130°C. Means of 20 fruit per harvest date are presented.

Ethylene Binding Measurement

Procedures described below are modifications of methods previously described (Sisler 1979, Sisler et al. 1986).

Apple Sample. A composite sample of 500 g of apple flesh was cut from 10 apples. Tissue was twice put through a juicer that separated juice from the pulp. Since intact apples bind so little ethylene, it has not proven practical to measure binding in intact tissue; therefore, binding was measured in the pulp. The pulp was further squeezed in a press until the weight of the pulp from 500 g of apples was approximately 80 g. The pulp was brought to pH 6.0 with calcium carbonate. The pulp was then blended with approximately 70 g cellulose powder as a spacer to facilitate diffusion of ethylene to the site. The resulting fluffy mixture was divided into 4 equal samples. Samples were tied in cheesecloth bags for exposure to ethylene. The juice was brought to pH 6.0 with calcium carbonate. The juice was placed on trays at 0°C and evaporated with a stream of air. After about 16 hr the juice sample became viscous. Approximately 70 g cellulose powder was added and the preparation blended to give a

fluffy mixture. This was also placed in cheesecloth bags for exposure to ethylene.

Assay. Duplicate samples were placed in each of two 5 liter desiccators containing 1.0 μCi [^{14}C]ethylene–mercuric perchlorate complex (110 mci/mmol) in a 25 ml Erlenmeyer flask. 2 N NaOH (1 ml) on cheesecloth was placed in the desiccator to absorb impurities that may be present in the released ethylene. After being sealed, an excess of saturated LiCl (1 ml) was injected through a silicone rubber stopper into the ethylene–mercury perchlorate complex to release the ethylene. A magnet was used to stir the mixture for at least 10 min to release the ethylene. Unlabeled ethylene 1000 μl/liter was included in 1 of the desiccators to determine nonspecific bound ethylene. After 4 h the desiccators were opened and aired for 30 min to allow dissipation of unbound ethylene. Initial experiments taken from 1 min to 24 h indicated that bound ethylene diffused from the site very slowly at room temperature (T ½ > 24 h) while the unbound ethylene diffused away after about 30 min. Samples were aired 30 min to reduce the background to a very low value. The 30 min airing would result in the loss of only a very small amount of bound ethylene. Samples were placed in 500 ml jars with 0.2 ml of mercuric perchlorate on a piece of fiberglass filter in a scintillation vial. After being sealed, jars were placed in an oven at 60°C for 6 h, and at least 6 additional h were allowed for collection of the ethylene. Vials were then removed, scintillation fluid added, and the samples counted in a scintillation counter.

Morning Glory. Flowers were picked in the early morning and allowed to stand for 5 h to dissipate wound ethylene. Samples (20 flowers) were exposed to ethylene (0.5 μCi/2.5 liters) for 2–3 h in the presence and absence of 1000 μl/liter of unlabeled ethylene. The samples were aired 2.5 min before being placed in jars with mercuric perchlorate (½ of the bound ethylene diffuses out in 10 min). After the samples stood overnight, the scintillation fluid was included and the samples counted.

Scatchard plots were obtained by exposing samples to various amounts of unlabeled ethylene in addition to labeled ethylene. For apple samples 0, 0.02, 0.05, 0.1, 0.2, 2.0, or 1000 μl/liter unlabeled ethylene was added to the dessicator atmosphere in addition to labeled ethylene. In the morning glory concentrations of 0, 0.05, 0.1, 0.2, 0.4, or 1.0 μl/liter unlabeled ethylene were used.

Apple experiments were performed on fruit from 2 different orchards with 4 replications/orchard/date. Morning glory experiments were conducted 3 times with 3 replicates.

Results

Ethylene binding did not substantially change in apple fruit pulp during maturation (Fig. 1). No ethylene binding ability was found in the fruit juice. In fruit from location 1, the ethylene binding decreased slightly with maturity. In fruit from location 2, ethylene binding was more variable and overall showed little consistent change during maturation. We believe that the fluctuations in binding in location 2 are not significant since these fruit also showed more variation than location 1 in internal ethylene concentration (Fig. 2). Both loca-

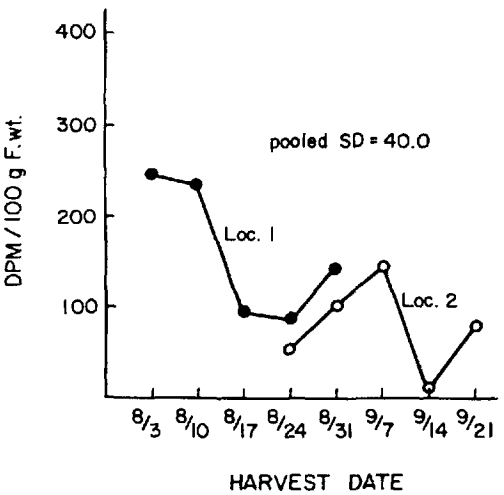

Fig. 1. Ethylene binding of pulp from apples harvested at weekly intervals from two orchard locations. Means and pooled SD are given.

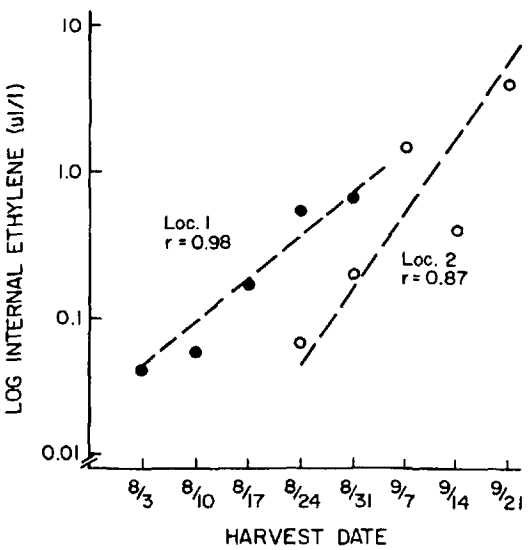

Fig. 2. Apple internal ethylene concentrations at weekly intervals from 2 orchard locations.

tions had internal ethylene concentrations that were increasing linearly when plotted on a logarithmic scale (Fig. 2), and by the end of the sampling period the apples would have been considered as entering or in the climacteric rise.

By converting dpm into moles, it can be calculated from Fig. 3 that there are 3.2×10^{-11} moles/kg FW binding sites in apple pulp under the assay conditions. The details of this type of calculation are given by Sisler (1979). In apples, the concentration of ethylene required to saturate ½ of the binding sites was calculated from the Kd to be 0.14 µl/liter (Fig. 3) (Sisler 1979, Venis 1985). It can be calculated that 94% saturation of the binding sites would require 1.1 µl/liter ethylene. As predicted from the linear relationship in Fig. 2, apple internal ethylene levels were high enough to give 50% saturation of

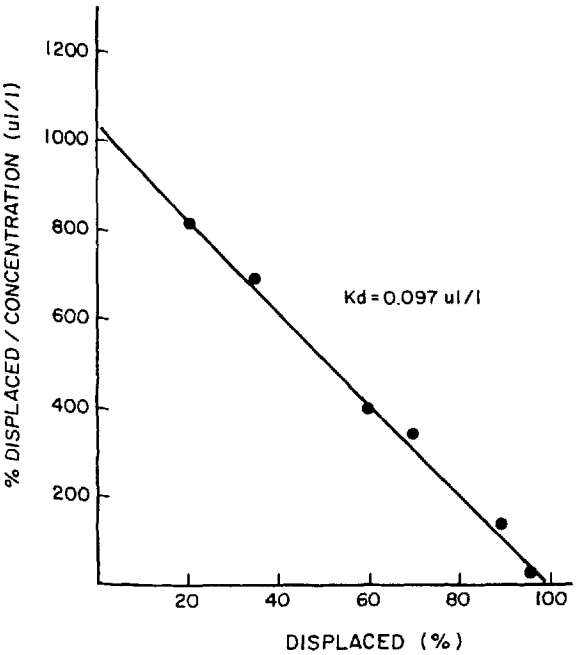

Fig. 3. Scatchard plot of ethylene binding by apple pulp.

binding sites on 14 Aug in location 1 and 30 Aug in location 2. The 94% saturation of binding sites would have occurred on 4 Sept and 11 Sept, respectively, for locations 1 and 2. Apples from location 1 were judged horticulturally mature by fruit firmness, soluble solid content, and starch–iodine rating (data not shown) by the time 94% saturation of the binding sites had taken place. However, in location 2 94% saturation would have occurred about 11 Sept and it was not until sometime between 14 Sept and 21 Sept that location 2 fruit would have been judged as mature.

During the 24 h before full bloom until 24 h after full bloom, there was little change in the ability of morning glory flowers to bind ethylene, except perhaps for a slight decline as the flower became very senescent (Fig. 4). The concentration of ethylene required for 50% saturation of the binding sites in morning glory was 0.14 μl/liter (Fig. 5). This is comparable to the value found for apple (Fig. 3) and carnation petals (Sisler et al. 1986). It was calculated that there were 3.8×10^{-9} moles/kg binding sites in morning glory under the assay conditions.

Discussion

Overall, during the developmental processes of apple fruit ripening and morning glory flower senescence there was little change in ethylene binding in the tissues. A slight decrease in binding sites during maturation was noted in location 1 apple pulp. It has been shown in leaves that as leaf size increases dpm/g decreases, but dpm/leaf increases (Goren and Sisler 1984). If this rela-

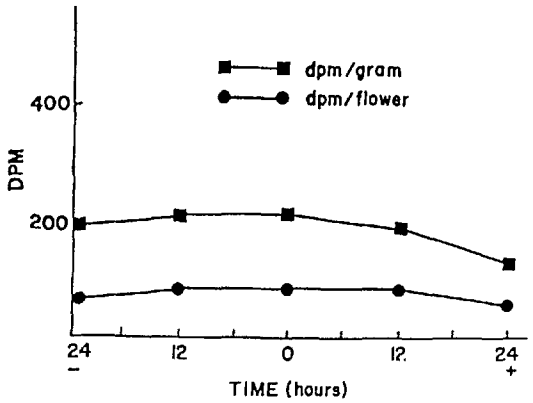

Fig. 4. Ethylene binding of morning glory flower during senescence. Pooled SD = 20 dpm/gram for intact tissue; pooled SD = 7 dpm/flower for ground tissue.

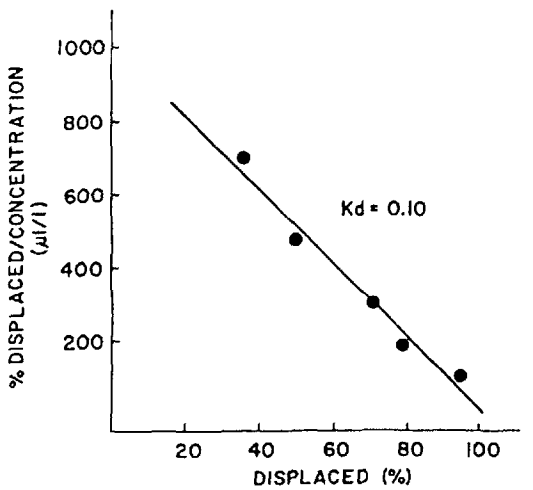

Fig. 5. Scatchard plot of ethylene binding of morning glory flower.

tionship is true in apple, it would be likely that a decrease in binding would occur as apples matured if data were expressed on a gram basis, as was seen to a limited extent in location 1. Since the decrease in binding we saw in location 1 was small and did not hold true in location 2, it would be expected that if data were expressed as dpm/apple, any increase in binding would be small. Overall, little change in ethylene binding occurred during maturation.

A small decline in the ability to bind ethylene may occur with advanced senescence, as seen in the morning glory flowers, and is probably due to the general lack of tissue integrity that accompanies senescence. The decline in ethylene binding with age has also been noted in tobacco leaves (Goren and Sisler 1984).

The threshold for ethylene action in many types of fruits has been reported to be around 0.1–1 µl/liter (Burg and Burg 1965, Harkett et al. 1971). Our data are in agreement with these amounts, since 0.14 (50% saturation)–1.1 µl/liter (94% saturation) ethylene was required in apple pulp to saturate the ethylene

binding sites. Any ethylene in excess of these concentrations would be unable to bind to the sites. Therefore, the high concentrations of ethylene that occur during the climacteric appear to serve little purpose in the initiation of ripening. This would be in agreement with the finding that fruit is often horticulturally mature before any rise in ethylene is apparent (Blankenship and Unrath 1988). Ethylene sensitivity of a tissue has often been postulated to be of more importance than the actual amount of ethylene present (Romani 1984, Burg and Burg 1965). It does not appear that the change in ethylene sensitivity often noted is a result of changes in the number of ethylene binding sites. It has been noted, however, that there are differences in attached and detached fruit in regard to ethylene sensitivity (Burg and Burg 1965) and it is possible that if ethylene binding sites could be measured in intact apples still attached to the tree, results would differ. Many other factors, besides ethylene binding ability, could account for changes in ethylene sensitivity and requirements for action. Recently, Smith et al. (1987) have reported that some ethylene binds very slowly in pea and diffuses very slowly. This slow diffusion component has been noted in a number of other tissues (Sisler 1982, Goren and Sisler 1984, 1986, Sisler et al. 1986). The Kd for this binding is reported to be 0.63, or more than 5 times the value for the lower binding component; whether or not this has any physiological significance is unknown. Most ethylene responses studied to date occur at much lower ethylene concentrations; however, some do appear to be in this range. There may be a role of the high-Kd-binding ethylene that is not yet recognized.

Acknowledgments. The authors would like to thank David Arrington, Autumn Duhe, and Carmen Wood for their technical assistance.

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