Degreening Response of 'Hamlin' Oranges in Relation to Temperature, Ethylene Concentration, and Fruit Maturity[1]

Otto L. Jahn[2], William G. Chace, Jr.[3], and Randall H. Cubbedge[2]
Agricultural Research Service
U. S. Department of Agriculture
Orlando, Florida

Abstract. 'Hamlin' oranges were degreened for 1, 2, or 4 days in ethylene concn of 0 to 50 ppm and at 21, 24, 27, and 30°C. The initial rate of chlorophyll loss was more rapid at 30°C than at 21°C. Subsequent changes at 21°C were such that the fruit reached an acceptable color almost as rapidly as at 30°C. After 4 days' degreening, fruit at both temp showed the same response pattern to the range of ethylene concn used, with the optimum response attained with ethylene concn between 5 and 10 ppm. After short degreening periods, changes continued after removal from the ethylene atmosphere. Responses at 24 and 27°C were similar to those at 21 and 30°C, respectively. No evidence of a seasonal change in the response to ethylene was shown. Stem-end decay generally was greater with increasing ethylene concn, temp, and length of the degreening period. Interactions among these variables were such that increasing the temp or length of degreening period resulted in greater increases in decay at high rather than at low ethylene levels.

Ethylene is widely and effectively used as a postharvest treatment for the removal of chlorophyll from the rind (degreening) of early season citrus fruits. In Florida, the recommended degreening conditions are an ethylene concn of 1 to 5 ppm at 30°C and relative humidity of 85% or above. In practice ethylene concn have varied widely from these recommendations. Available information is not adequate to show how these variations affect color changes and fruit quality or that the recommended conditions are optimum for degreening all citrus fruits.

In our previous work (5) the max response to ethylene was found at 5 to 10 ppm. There was an indication that the response was affected by fruit maturity and temp. The studies reported here were undertaken to relate effects of these factors to the response of 'Hamlin' oranges to ethylene.

Materials and Methods

Experimental procedures were similar to those used in previous studies (5). 'Hamlin' orange trees, *Citrus sinensis* Osb., grown on rough lemon rootstock, *C. jambhiri* Lush., were used as a fruit source. This cultivar generally reaches maturity in late October or early November but is not degreened in the field until December. Samples were harvested and treated as shown in Table 1. The experimental design was varied to emphasize the range of ethylene concn in tests 2, 4, and 6 and temp in tests 3 and 5. All fruits were washed before 20-fruit sub-samples were selected visually for treatment. Each treatment included a range of fruit color, but this range was comparable for all treatments in a test. Statistical analyses were run on data from individual tests and also on combined data from tests 2, 4, and 6 and tests 3 and 5, using tests as replicates.

As in earlier work, the fruit samples were degreened in gastight chambers, using 2 connected chambers where 2 treatment times were studied (5). Measurements made on the samples removed first, after 1 or 2 days' degreening, were used as an estimate of the comparable values for the second samples which were continuously exposed to the atmospheres for 2 or 4 days. Each unit of 1 or 2 chambers had a pump for air circulation, a CO_2 absorber, and provision for atmosphere sampling. The humidity was maintained above 95% in all tests. All fruits were transferred to 21°C after removal from the degreening atmosphere and held until 2 weeks from harvest. Fruits were held in trays so residual ethylene could readily diffuse from the fruit. The samples were evaluated for decay at 1 and 2 weeks from harvest. The fruits were neither waxed nor treated with a fungicide.

Chlorophyll losses during degreening were followed using a light-transmittance difference meter with an integrating-sphere sample-presentation system (5, 8). Data, recorded as △ OD 695-740 nm, were used as an indication of the relative chlorophyll level in the fruit. Individual fruit measurements were made on all samples before treatment and after varying intervals up to 1 week after harvest. By this measurement fruit reached an acceptable color at readings of .3 to .2 △ OD, the values decreasing as the fruit matured, apparently due to changes in the distribution of the chlorophyll in the fruit tissues.

The composition of the atmosphere was established and monitored using a gas chromatograph (5). An activated alumina column and a flame ionization detector were used to detect ethylene. A silica gel and a molecular sieve column were used with a thermal conductivity detector for O_2 and CO_2 detection. Oxygen was added as necessary to maintain the atmosphere during the longer degreening periods.

Results and Discussion

The chlorophyll losses (△ △ OD 695-740 nm) in 'Hamlin' oranges during the 2- and 4-day periods (tests 2, 4, and 6) are shown in Fig. 1A. At 30°C the max change occurred with 10 ppm during both 2 and 4 days' degreening. Higher ethylene levels contributed little, if any, to the loss of chlorophyll. In test 6 (Fig. 1B), however, fruit degreened at 30°C for 1 day showed no additional response above 5 ppm ethylene. These results agree with earlier observations (5) and indicate that at 30°C 'Hamlin' oranges require a min of 5 ppm ethylene for max color changes.

Response patterns at 21° differed from those at 30°C. After 2 days, the rate of change increased at concn up to 5 ppm ethylene (Fig. 1A, B), a response equivalent to the changes during 1 day at 30°C (Fig. 1B). However, after 4 days the response increased up to 10 ppm, resulting in total chlorophyll losses comparable to those at 30°C. In comparison, fruit degreened at 21°C for 1 day (Fig. 1B) did not show any

[1]Received for publication October 3, 1972.
[2]Research Horticulturist and Biological Laboratory Technician, respectively.
[3]Research Horticulturist, European Marketing Research Center, ARS, USDA, Rotterdam, The Netherlands.

Table 1. Degreening tests on 'Hamlin' oranges, harvest dates, temp, ethylene concn, and degreening time.[z] Each test included one 20-fruit sample for each combination of treatments listed, i.e., 12 treatments in test 1.

Test	Harvest date	Temp °C	Ethylene ppm	Days degreening
1	September 30, 1968	21, 30	0, 10, 50	2 and 4
2	October 7, 1968	21, 30	0, 1, 5, 10, 20, 50	2 and 4
3	October 14, 1968	21, 24, 27, 30	0, 5, 10	2 and 4
4	October 21, 1968	21, 30	0, 1, 5, 10, 20, 50	2 and 4
5	October 28, 1968	21, 24, 27, 30	0, 5, 10	2 and 4
6	November 4, 1968	21, 30	0, 1, 5, 10, 20, 50	1, 2 and 4
7	November 12, 1968	21, 30	0	2
8	November 25, 1968	21, 30	0, 10	1 and 2
9	December 2, 1968	21, 24, 27, 30	0, 1, 5, 10, 20, 50	1 and 2
10	November 17, 1969	21, 30	0, 1, 5, 10, 50	2 and 4

[z]Only pertinent treatments are listed for some tests.

additional response at concn above 1 ppm ethylene. These results suggest that using 5 to 10 ppm ethylene would be more effective during 3 to 4 days' degreening than for 1-day degreening periods.

When the data shown in Fig. 1A are plotted with ethylene concn on a log scale (not shown), the curves approach a straight line from 1 to 10 ppm. Assuming that the degreening effect of low levels of ethylene was a logarithmic response, these curves were used to estimate the level of ethylene which would be associated with chlorophyll changes in untreated fruit. On this basis, untreated fruit at 30°C showed changes equivalent to 0.03 to 0.05 ppm ethylene. This coincides with the level of ethylene found internally in untreated fruits (unpublished data). On the same basis, the chlorophyll losses in untreated fruit at 21°C were equivalent to about 0.01 ppm ethylene after 4 days but only a fraction of this level after 2 days. Since the ethylene levels in the fruit are higher than this, some other factor, possibly enzyme synthesis, limits the initial response at 21°C.

The changes in chlorophyll levels (△ OD 695-740) during the entire test period for ethylene concn up to 10 ppm (tests 2, 4, and 6) are presented in Fig. 2. At 30°C the rate of change

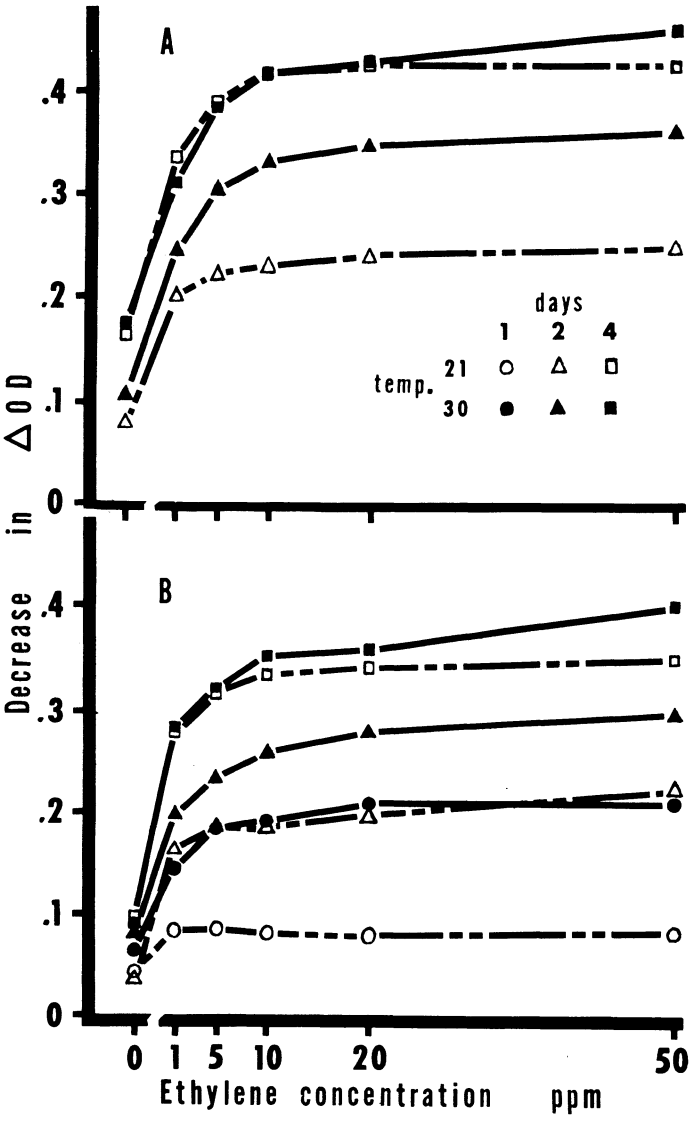

Fig. 1. Chlorophyll losses in 'Hamlin' oranges in response to concn of 0 to 50 ppm ethylene at 21 and 30°C. A - Responses during 2 and 4 days' degreening (tests 2, 4, and 6). B - Responses during 1, 2, and 4 days' degreening (test 6).

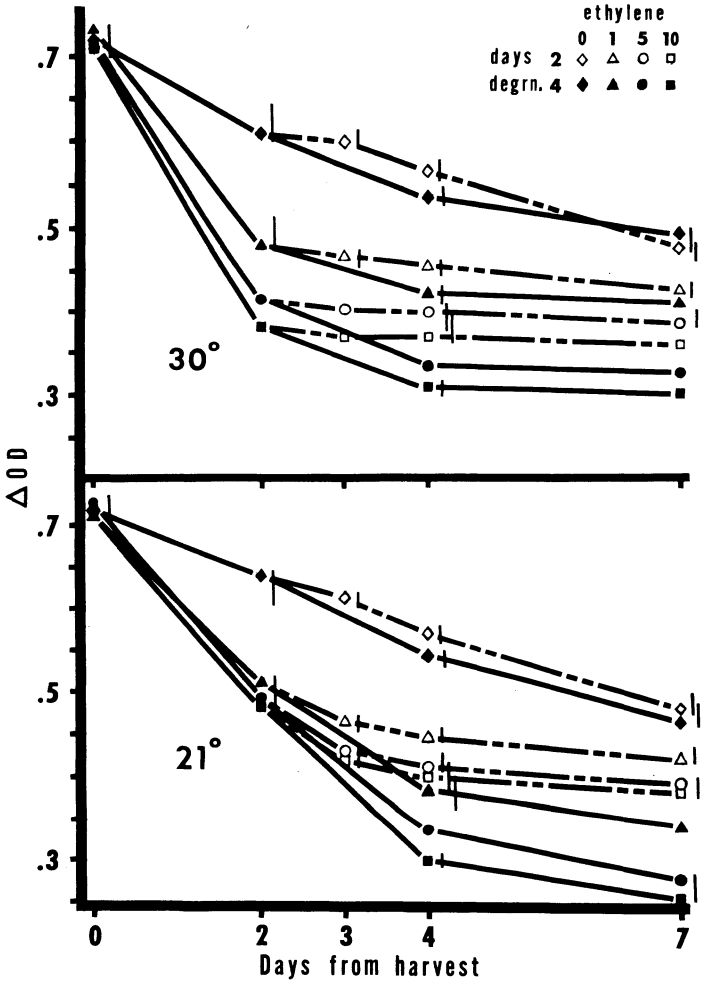

Fig. 2. Chlorophyll (△ OD) levels in 'Hamlin' oranges during 2 and 4 days' degreening at 21 and 30°C with 0 to 10 ppm ethylene (tests 2, 4, and 6).

increased as the ethylene concn was increased. Although significant, the increase at concn above 5 ppm may not be of practical value. Chlorophyll losses were most rapid during the first 2 days of treatment. Removal from the ethylene chambers at this time essentially stopped the response, although some chlorophyll remained.

At 21°C there was no significant difference in the changes in chlorophyll between ethylene concn above 1 ppm at 2 days (Fig. 2). After 4 days, however, differences were evident with the max response again appearing between 5 and 10 ppm. This development of a response between 2 and 4 days at 21°C is similar to the changes found between 1 and 2 days at 30°C (5). The response after 4 days was similar at the 2 temp. This similarity does not mean that the fruit was degreened in the same period of time since the fruit at 30°C may have been colored in 3 to 3 1/2 days. However, differences of even 24 hr in degreening time are relatively small considering the difference in temp.

The fruit removed from ethylene after 2 days at 21°C continued to degreen for 1 additional day. A similar response was observed in 'Hamlin' oranges after 1 day at 30°C and 'Dancy' tangerines after 2 days at 21°C (5). Apparently at the start of the process, the system is self-sustaining for a short time.

Data in Fig. 3 show in detail the response patterns at 21 and 30°C during 1, 2, and 4 days' degreening. At 30°C rapid changes occurred during the first day of degreening. The rate of response to 1 ppm ethylene dropped off after 1 day compared to the continued changes during the second day with 10 ppm. After 2 days' degreening, the relative rates at 1 and 10 ppm remained constant. At 21°C there was no difference in response to 1 and 10 ppm ethylene after 1 day, but these samples changed more after removal than fruit at 30°C. After 2 days' degreening at 21°C, an additional response to 10 ppm ethylene was evident. With continued changes after removal, the differences between temp disappeared. After 4 days' degreening, no differences between temp were evident. These results support the view that most of the differences between temp are due to the slow initial response at 21°C.

Fruit not treated with ethylene also lost chlorophyll slowly (Fig. 2 and 3). Because of this natural change and the attainment of a min chlorophyll level, all treatments may eventually reach a similar level as shown by the fruit degreened 1 day (Fig. 3). Ethylene, however, is necessary to attain the desired color change in a reasonable time. Degreening times for oranges should be kept under 3 to 4 days for economic and fruit quality reasons.

Data for fruit degreened at 21, 24, 27, and 30°C (tests 3 and 5) are shown in Fig. 4. These results do not show the intermediate response expected with temp of 24 and 27°C. Instead 2 groups of response patterns were found with the response at 24 resembling that at 21°C and the response at 27 resembling that at 30°C. This suggests that the major change in the pattern of response occurs between 24 and 27°C and that fruit could be degreened with temp at least as low as 27°C with little effect on the rate of degreening. The general pattern of changes shown in Fig. 4 is similar to that shown in Fig. 2, supporting the conclusions from those tests.

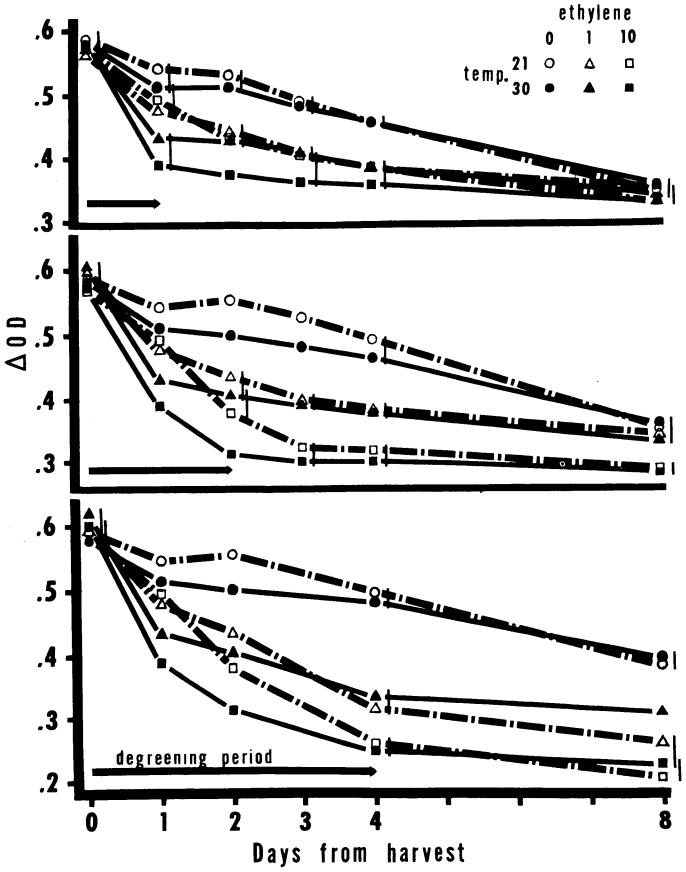

Fig. 3. Chlorophyll (△ OD) levels in 'Hamlin' oranges during 1, 2, and 4 days' degreening at 21 and 30°C with ethylene concn of 0 to 10 ppm (test 6). Fruit degreened for 1, 2, or 4 days as shown by arrows in each graph. Fruit was then transferred to 21°C for remaining period.

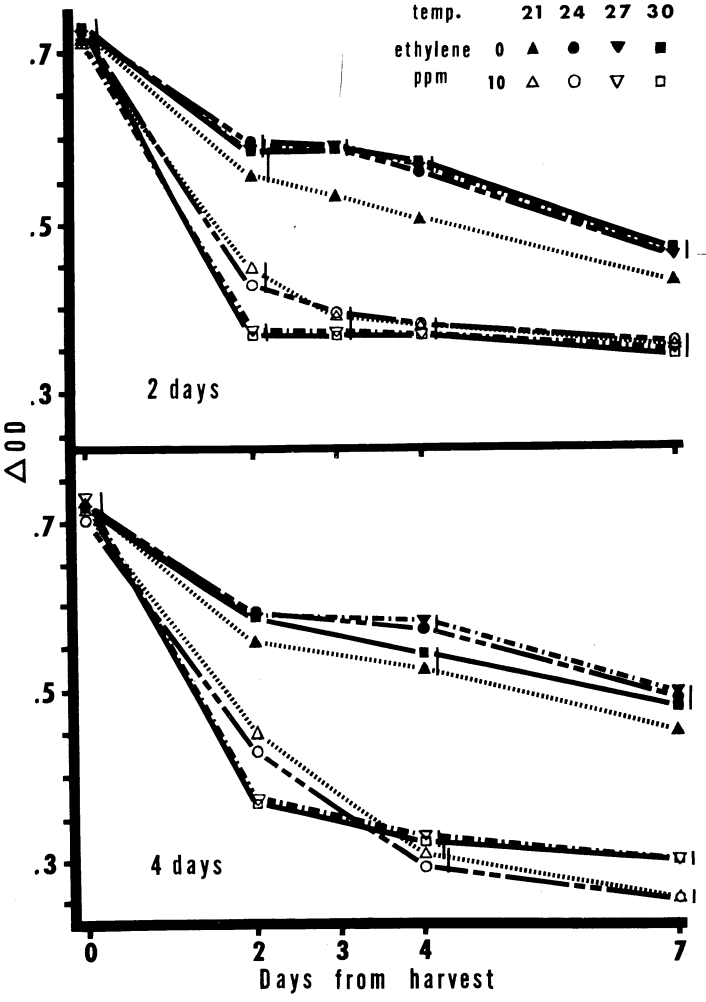

Fig. 4. Chlorophyll (△ OD) levels in 'Hamlin' oranges during 2 and 4 days' degreening at 21, 24, 27, and 30°C with 0 and 10 ppm ethylene (tests 3 and 5).

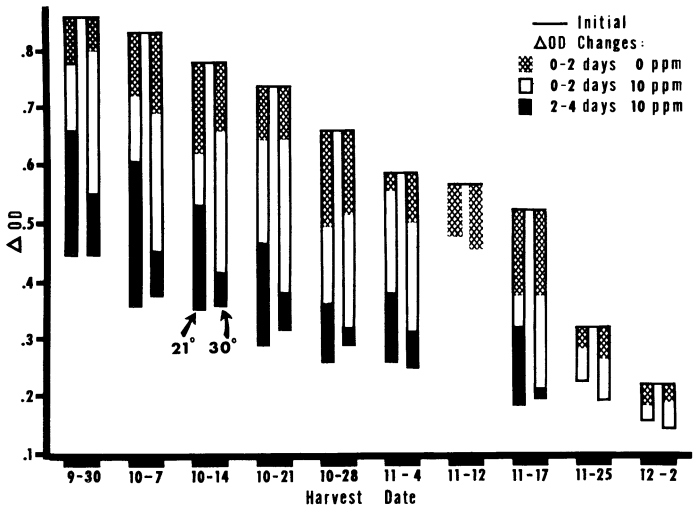

Fig. 5. Seasonal changes in chlorophyll (△ OD) levels in 'Hamlin' oranges and the associated changes during 2 and 4 days' degreening with 0 and 10 ppm ethylene at 21 and 30°C (1968, except November 17, 1969, test).

These studies do not show as great an effect of temp during degreening as has been reported (2). These results, however, are based on changes in chlorophyll rather than the combined chlorophyll-carotenoid color range that has been used. Other work (6) has suggested that these pigment systems do not necessarily respond at the same rate during degreening treatments. In the present tests, fruit fully degreened at 21°C frequently showed less orange color than those at 30°C. These small color differences apparently were due to slower ethylene-induced carotenoid synthesis (11) at 21 than at 30°C in comparison to the effects on chlorophyll degradation. Fruit stored at temp of 10 to 21°C will develop better orange color than at higher temp if given sufficient time (unpublished data), which is comparable to results shown to occur on the tree (10). In practice, low temp for early-season degreening would not be practical because high ambient temp would require refrigeration. However, these results suggest that ambient temp down to 27 or possibly 24°C could be used effectively, and this would reduce the cost and humidity-control problems resulting from heating the degreening rooms to the present 30°C. Fruit degreened under such conditions should show good color development and less decay during marketing.

The decline in initial chlorophyll level through the season (Fig. 5) resembles that found in other studies (4). This decline reflects the combined effect of increased maturity and declining field temp, both of which may influence the color development before harvest. However, no changes in the postharvest pattern of response were evident from the immature fruit on September 30 to the fully mature fruit in December. Other studies, also, have not indicated any change in the pattern of response during the degreening season (7, 9). The min chlorophyll level attained with 4 days' degreening gradually declined as the season advanced. This is not due entirely to the need for a longer degreening period in the early tests. As shown in Fig. 2 and 4 and in earlier work (7, 9), the chlorophyll levels may reach a stable level before degreening is completed. This suggests that the chlorophyll degradation system may become less effective after 3 to 4 days' degreening. It also may be due to the presence of chlorophyll in tissues other than the flavedo or in other forms which are not readily affected by ethylene, possibly in the vesicles (4). This pattern supports the view that fruit must have matured to the point where degreening is effective before it is harvested.

The incidence of decay in fruit during the 21°C holding period was recorded as *Penicillium* or stem-end (*Diplodia* and *Phomopsis*) types. Losses to *Penicillium* decay were too small to show treatment effects and are not considered here. Losses from stem-end decay were significantly increased by increased ethylene concn, temp, and degreening time independently (Table 2). There were also significant interactions between ethylene concn and days of degreening and between ethylene concn and temp. These interactions show that at higher ethylene levels decay was greater with 4 days' degreening time or at 30°C than after 2 days or 21°C treatment. Results were similar in tests 3 and 5. These data support reports of increased decay resulting from degreening (1, 3) and emphasize the importance of regulating ethylene levels and minimizing the degreening time.

The increased response noted earlier between 1 and 5 ppm ethylene is such that the reduced degreening time may offset the greater decay rates. Increased response rates between 5 and 10 ppm ethylene were small but often significant. However, the greatly increased decay suggests that the use of 10 ppm may not be practical. As noted, no fungicide was used in these tests. The use of a fungicide (normal practice) would reduce the amount of decay but probably would not influence the relative response to degreening conditions.

Table 2. Percent stem-end decay of 'Hamlin' oranges in response to ethylene concn, days' degreening, and degreening temp. Total decay, 2 weeks from harvest, tests 2, 4, and 6, 1968.

Days degreening	Temp °C	Ethylene concn (ppm) 0	1	5	10	20	50	Means: Days X temp[z]	Means: Days degreening
2	21	1.7	1.7	0	0	0.	6.7	1.7	
	30	0	0	8.3	6.7	21.7	33.3	11.7	
	Mean[y]	0.8	0.8	4.2	3.3	10.8	20.0		6.7**
4	21	1.7	5.0	10.0	13.3	26.7	20.0	12.8	
	30	0	1.7	15.0	38.3	50.0	40.0	24.2	
	Mean[y]	0.8	3.3	12.5	25.8	38.3	30.0		18.5
Temperature X ethylene concentration**								Temp	
	21	1.7	3.3	5.0	6.7	13.3	13.3	7.2*	
	30	0	0.8	11.7	22.5	35.8	36.7	17.9	
Ethylene*		0.8	2.1	8.3	14.6	24.6	25.0		

[z]Interaction of days degreening X temp ns.
[y]Interaction of days degreening X ethylene concn**.
*Significant at 5%; ** significant at 1%; ns, nonsignificant.

Literature Cited

1. Grierson, W., and W. F. Newhall. 1955. Tolerance to ethylene of

various types of citrus fruits. *Proc. Amer. Soc. Hort. Sci.* 65:244-250.
2. _____, and _____. 1960. Degreening of Florida citrus fruits. *Fla. Agr. Expt. Sta. Bul.* 620.
3. Hopkins, E. F., E. W. Loucks, and C. R. Stearns. 1944. A study of certain methods for the control of stem-end rot and blue mold of oranges. *Proc. Fla. State Hort. Soc.* 57:87-98.
4. Jahn, O. L. 1970. Effects of maturity changes on nondestructive measurements of citrus fruit quality. *U. S. Dept. Agr. Tech. Bul.* 1410.
5. _____, W. G. Chace, Jr., and R. H. Cubbedge. 1969. Degreening of citrus fruits in response to varying levels of oxygen and ethylene. *J. Amer. Soc. Hort. Sci.* 94:123-125.
6. _____, R. H. Cubbedge, and J. J. Smoot. 1970. Effects of washing sequence on the degreening response and decay of some citrus fruits. *Proc. Fla. State Hort. Soc.* 83:217-221.
7. _____, and J. J. Gaffney. 1972. Photoelectric color sorting of citrus fruits. *U. S. Dept. Agr. Tech. Bul.* 1448:
8. _____, and M. B. Sunday. 1965. Color changes in citrus fruits as measured by light transmittance techniques. *Proc. Fla. State Hort. Soc.* 78:229-232.
9. _____, G. E. Yost, and J. Soule. 1967. Degreening response of color-sorted Florida oranges. *U. S. Dept. Agr.* ARS 51-14.
10. Young, L. B., and L. C. Erickson. 1961. Influence of temperature on color changes in Valencia oranges. *Proc. Amer. Soc. Hort. Sci.* 78:197-200.
11. Young, R., and O. Jahn. 1972. Ethylene-induced carotenoid accumulation in citrus fruit rinds. *J. Amer. Soc. Hort. Sci.* 97:258-261.

Transport of Arginine and α-Aminoisobutyric Acid from Cotyledon to Axis in Germinating *Phaseolus lunatus*[1]

Aref A. Abdul-Baki[2]
Agricultural Research Service, U. S. Department of Agriculture
Beltsville, Maryland

and

Ashwani Kumar Srivastava[3]
Department of Botany and Plant Pathology
Punjab Agricultural University, Ludhiana, India

Abstract. The transport of arginine and α-aminoisobutyric acid from the cotyledon to the axis of *Phaseolus lunatus* L. was followed during a 4-day germination. Transport of both amino acids to the axis was slow during the first 2 days and increased rapidly during the third and fourth days of germination. Both amino acids accumulated in the axis of viable seeds. Alpha-aminoisobutyric acid was not metabolized by the cotyledon or axis, whereas arginine was rapidly fixed by both tissues into protein. Percent incorporation of arginine into protein was lower in the cotyledon than in the axis. Rapid synthesis of proteins in the cotyledon appears to limit transport and availability of amino acids to the axis. No transport or accumulation of α-aminoisobutyric acid was observed in seeds that lost germinability through accelerated aging.

Protein, which in most legumes comprises 20% or more of the seed dry wt (7), is localized mainly in the storage organs--cotyledons. During germination, amino acids from protein hydrolysis provide C and N for respiratory and synthetic reactions (8, 14, 15, 16, 17).

Lawrence and Grant (14) attributed the large differences in composition between free and protein amino acids of germinating pea seeds to the extensive interconversions that amino acids undergo in their metabolism. Subsequent work (16), using labeled amino acids and following their transport from the cotyledon into the axis, confirmed the interconversions of amino acids during germination.

The work of Stewart and Beevers (16) on transport of amino acids from castor bean endosperm to the embryo during germination indicates that certain amino acids undergo high interconversions whereas others are more stable. Because gluconeogenesis is very active in castor bean endosperm, those amino acids which on deamination give rise to intermediates convertible to sugar were transported as sugar and the N was transported as glutamine. Arginine, proline, valine, and phenylalanine did not undergo any significant interconversions.

Lima beans *(Phaseolus lunatus),* in which protein is the major food reserve, are well suited for studies of amino acid transport. The cotyledons seldom crack during imbibition and have little photosynthetic activity during early germination. These properties make them suitable for introducing radioactive compounds into the cotyledons and following transport of these compounds into the axes.

We report on transport of arginine and α-aminoisobutyric acid (AIBA) that were introduced into cotyledons of seeds that had been imbibed for 1 to 4 days. These amino acids undergo very little interconversion, and differ in that arginine is fixed into protein, whereas AIBA is not (6). In this respect, transport of arginine from the cotyledon to the axis would be influenced by the demand in the cotyledons for arginine for protein synthesis, whereas AIBA movement represents that of an "inert" amino acid that is not fixed into protein or any other cellular component and its transport, therefore, is not restricted by fixation.

Materials and Methods

Materials. 'Green Fordhook 861' lima bean *(Phaseolus lunatus)* seeds, supplied by Charter Seed Co., Idaho, were produced in 1971 and stored in airtight glass jars at 5°C. A subsample was accelerated-aged for 15 days, at 45°C and 100% RH, and lost germinability. Uniformly labeled ^{14}C-arginine, specific activity 220mCi/mM (New England Nuclear), and ^{14}C-1-α-aminoisobutyric acid, specific activity 14.2 mCi/mM (CalAtomic), were used.

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[2]Plant Physiologist, Post Harvest Physiology Laboratory, Agricultural Marketing Research Institute, U. S. Department of Agriculture, Beltsville, Md. 20705.

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