well as in moving-air conditions. In this modified form, shown in figure 1, opposite to the side tube used as an inlet for a current of air, the cup was provided with an inverted ground stopper with a tiny container sealed onto its tip. Test oil could be placed in this container whenever its vapor was to be applied. The procedure of the following experiments was essentially similar to that used before.

The resulting graphs of three experiments obtained when either rosemary or thyme vapor was applied in still-air conditions are shown in figure 5. In all these experiments the air stream was stopped, after a period of moving air, either at the same or before the time of introducing the oil into the porometer cup. Here, again, the graphs have the same characteristics as those shown before, rising rapidly and considerably on stoppage of circulating air. When rosemary or thyme vapor was applied, the immediate effect on the flow rate through stomata was either a slight temporary increase or no change for about 15 to 25 minutes, followed by a marked decrease. In experiment 11, following the usual closing response to rosemary vapor, stomata showed an appreciable but only partial reopening with time. Only in experiments 13 and 14, portions of the leaf areas treated with thyme vapor showed definite brown spots of injury. In all other experiments of the present work, neither rosemary nor thyme vapor seemed to have any apparent injurious effect on cherry laurel leaves.

The general conclusion to be drawn from all above experiments is that exposure of illuminated leaves of cherry laurel to still or moving air charged with rosemary or thyme vapor brought about a marked stomatal closing response. The closing movement was induced only slightly less readily in still than in moving air.

Although the closing response of stomata of cherry laurel to oil vapors in the present study agrees with the results reported earlier by the author (2) for other oils on other plants, it does not agree with the results of Audus and Cheetham (1) who reported a lack of response by the stomata of the same plant to the vapors of the same oils tested. An explanation for this cannot be obtained from the present work. However, the lack of response, as far as the present experiments indicate, cannot be interpreted, contrary to previous suggestion, on the basis that the opening movement of stomata within a confined space of porometer cup may mask the closing effect of applied vapors.

Summary

1) The effects of applying vapors of rosemary and thyme oils on the stomata of cherry laurel leaves were tested by means of porometer cups.

2) The effects of still and moving air on the stomatal behavior by the same plant were also tested. Stomata opened much wider in still than in moving air.

3) On applying rosemary and thyme vapors to illuminated leaves of cherry laurel, the stomata closed considerably. The closing effect was slightly more rapid in moving than in still air.

4) The gradual opening movement of stomata in still air could not mask their definite closing response to rosemary or thyme vapor treatments.

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PHOTOCONTROL OF ANTHOCYANIN SYNTHESIS IN APPLE SKIN[1]

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Red color of apples requires light for its formation and is localized in the areas most exposed to the sun. To enhance the color, some growers expose apples to diffuse sunlight for several days after harvest. The variation in color of apple varieties from green or yellow to various shades of red is indicative of varying capacities for anthocyanin synthesis. The red pigment of the apple skin is cyanidin-3-galactoside, idaein (17).

The literature on the influence of radiation on anthocyanin formation in apple skin was reviewed by Arthur (1). The greatest influence was considered to be between 2900 and 3120 Å, with some response to radiation in the region of 4000 to 6000 Å. Magness (11) found that "dilute applications of ultraviolet light" were effective in promoting the coloration of Jonathan apples. He found also that apples did not color as well under glass as in direct sunlight. Pearce and Streeter (16) considered the maximum action for reddening to be in the region of 3600 to 4500 Å. The action spectrum for anthocyanin synthesis, which expresses the amount of anthocyanin formed for a given energy in various wavelength regions, is reported

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here for the apple skin at wavelengths greater than 4000 Å. A maximum effectiveness is found in the region of 6500 Å.

Materials and Methods

Early-harvested Jonathan, Rome Beauty and Arkansas apples were held in bushel lots at 0° C sealed in bags made of 0.38-mm polyethylene. Skin on whole apples was used in a few cases, but generally the green areas of the apple skin were removed in strips 15 mm wide with a White Mountain apple peeler. The strips were cut into uniform pieces 7.5 × 7.5 mm or 10 × 15 mm with razor blades mounted in a holder. The pieces, which had some cortical tissue attached, were washed at least three times with about 500 ml of 0.3 M sucrose during a period of about one hour. They were used immediately after washing.

Skin pieces for each experiment were taken from 40 or more apples. They were randomized and 14 pieces were taken for each treatment. The pieces were placed skin side up in 10-cm Petri dishes or in 30-cm Pyrex glass trays containing 0.3 M sucrose and having a filter paper lining in the bottom.

Irradiation was effected with a white fluorescent source of radiant energy giving a maximum illumination of about 1000 foot-candles (ft-c) or with a spectrographic source using a carbon arc source of radiant energy (15). The spectrograph was used as a single-prism instrument and had a dispersion of 13 Å/mm at 7000 Å, 5 Å/mm at 5000 Å, and 2 Å/mm at 4000 Å. Transmission of the prisms limited the region studied to wavelengths longer than 4000 Å. Two slit widths, which subtended 2-cm and 4-cm at the focal position, were used. The 4-cm slit width sacrificed resolution for intensity. The effective widths of the 2-cm slit were 260 Å at 7000 Å, 100 Å at 5000 Å, and 40 Å at 4000 Å. Combination of neutral filters and reflections from front-surfaced mirrors were used to give approximately uniform irradiances between 4100 and 5000 Å. The somewhat greater focal length in the red combined with variations in the sharpness of focus made possible approximately the same intensity from 5800 to 7300 Å. The intensity at 5500 to 5600 Å was 85 % of that at 6100 to 6200 Å. The irradiances were 0.30 milliwatt/cm^2 for the 2-cm slit and 0.57 milliwatt/cm^2 for the 4-cm slit in the region of 6100 to 6200 Å. Exposures to the spectrographic source of radiant energy were generally for 12 hours, giving an energy of 13 joules/cm^2 when the narrow slit was used.

Formation of anthocyanin in apple skin requires a preliminary irradiation period of about 20 hours, the induction period, during which anthocyanin is not formed. After the induction period, anthocyanin synthesis is a linear function of time of irradiation at constant irradiance; for convenience, this is called the linear period.

The action spectrum of the induction period cannot be measured directly. It can be determined only by measuring the amount of anthocyanin formed early in the linear period as a function of the effectiveness of various wavelengths of light in overcoming the radiation requirements of the induction period. The action spectrum of the induction period was measured by irradiating groups of apple skin pieces across the spectrum with the spectrographic source of radiation for 12 hours. The pieces were then irradiated with the fluorescent source of radiation for 20 or 28 hours, after which they were kept 24 hours in darkness.

The action spectrum of the linear period can be measured by determining the effectiveness of various wavelengths of light in promoting anthocyanin formation during the linear period. It was determined by irradiating about 1000 pieces of apple skin with the fluorescent source of radiant energy for 32 or 40 hours. The pieces of apple skin were in the linear period of anthocyanin synthesis after this irradiation. Appreciable red color was evident at this time, and uniformly colored pieces were carefully selected. Groups of 14 selected 7.5 × 7.5 mm pieces were arranged across the spectrum and were irradiated with the spectrographic source of radiation or placed in darkness.

At the conclusion of all irradiation periods, the pieces of apple skin or the whole apples were returned to darkness for 24 hours at 15° C except as noted, to permit synthesis of anthocyanin induced by radiation. The pieces were then removed from the solution and dried by blotting.

The anthocyanin content of the skin was determined on an extract from pieces in each group in 5 ml of 1 % HCl in methanol. When whole apples were used, the skin was removed after the dark incubation, cut into slices, and extracted as just described. The optical density of the extract was generally in the range of 0.1 to 1.0 at 5300 Å. The optical density was also measured at 6500 Å to assess the degree of scattering. The optical density measurements were converted to mole idaein/cm^2 of apple skin by the use of a molecular-extinction coefficient of 3.43 × 10^4 at a wavelength of maximum absorption, 5300 Å. The molecular-extinction coefficient was determined on an authentic sample of idaein ($C_{21}H_{21}O_{11} \cdot 2.5\ H_2O$). The copper content of apple skin was determined spectrographically.

Results

The time courses of anthocyanin synthesis in pieces of apple skin and in whole apples are shown in figure 1. Groups of skin pieces or whole apples were irradiated with the fluorescent source of radiant energy for various periods. The three apple varieties examined have an induction period of about 20 hours during which practically no anthocyanin is formed. The induction period is followed by a linear period of anthocyanin formation at constant irradiance. The rate of anthocyanin synthesis in the Rome Beauty apple skin was about 12 % of the rate in the Jonathan apple skin. The linear period of anthocyanin formation in the skin of whole Arkansas apples

is closely parallel to that in pieces of skin, but the duration of the induction period is slightly greater.

The formation of anthocyanin continued in the dark following the irradiation period. Temperature and duration of the dark period following irradiation have considerable influence on the total amount of anthocyanin formed (table I).

The amount of anthocyanin formed as a result of an 84-hour irradiation with the fluorescent source of radiant energy was observed to vary linearly with intensity from an illumination of 270 to 1000 ft-c for skin pieces of Jonathan apple. Apparently a threshold of about 100 ft-c was required before anthocyanin synthesis occurred. The amounts of anthocyanin formed at each intensity expressed as 10^{-8} mole idaein/cm^2 of apple skin in excess of the non-irradiated sample were 120 ft-c, 0.24; 270 ft-c, 1.56; 500 ft-c, 2.88; and 1000 ft-c, 5.88.

Observations during the formation of anthocyanin indicated that the red coloration of the individual pieces was variable. Groups of skin pieces of Jonathan apples were irradiated with the fluorescent source of radiant energy for 40 hours and then selected for maximum and minimum red color. Portions of the two lots of selected pieces were irradiated for 8, 24, and 48 additional hours with the fluorescent source of radiant energy. Anthocyanin formation in both lots of pieces was linear with time and the rate was 2.9 times as great in the pieces showing the maximum initial red color as in those showing the minimum color.

Injury can promote the formation of anthocyanin in apple skin. Hail and insect damage are frequently recognized by an increase in red color at the site of the injury. Observations on pieces of apple skin showed that anthocyanin formation was evident first at the edge of the slice, where damage occurred in cutting.

The rate of anthocyanin formation in skin pieces held in the dark for 20 hours before irradiation was compared with that of pieces irradiated immediately after preparation. The pieces were floated on 0.3 M sucrose solution containing 1×10^{-3} M pyruvate adjusted to pH 4. The rate of anthocyanin formation was the same for both treatments (fig 2). However, the induction period of the pieces held in darkness for 20 hours before irradiation was reduced about 40 %. A strong odor of apple volatiles developed during the dark incubation. This was not evident in the sample irradiated immediately after preparation.

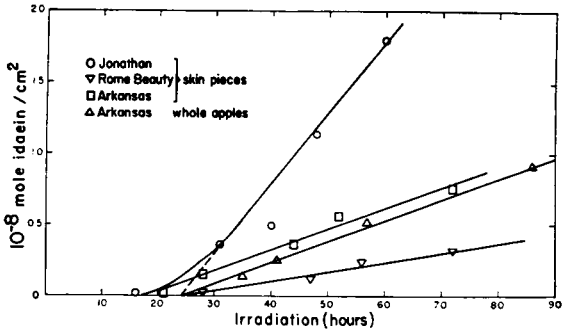
The copper content and dry weight per unit area of apple skin, scraped to remove some of the cortical tissue, were determined for two apple varieties. The dry weight of the skin was 0.0106 g/cm^2 and the copper content 7 ppm for the Arkansas, and 0.0076 g/cm^2 and 10 ppm for the Jonathan. Examination of cross sections of scraped apple skin revealed that the hypodermal cells, which contain the anthocyanin, did not exceed 15 % of the total volume of the tissue.

Anthocyanin synthesis in pieces of apple skin was found to be unresponsive to the state of the pigment

TABLE I

IDAEIN FORMATION IN PIECES OF JONATHAN APPLE SKIN FLOATING ON 0.3 M SUCROSE AS AFFECTED BY DURATION OF DARK PERIOD AND TEMPERATURE FOLLOWING 48 HOURS OF IRRADIATION WITH THE FLUORESCENT SOURCE OF RADIANT ENERGY

DARK PERIOD	IDAEIN FORMED AT INDICATED TEMPERATURE		
	5° C	15° C	25° C
hours	*10^{-8} mole/cm^2*	*10^{-8} mole/cm^2*	*10^{-8} mole/cm^2*
0	...	1.32	...
24	...	2.10	...
48	1.44	2.70	2.16
72	...	2.64	...



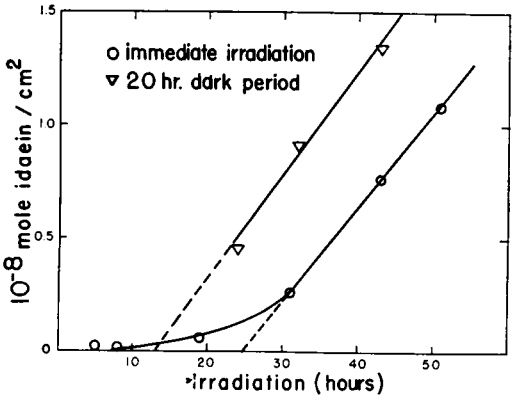


FIG. 1. The dependence of anthocyanin formation in apple skin on the time of exposure under constant irradiance with a fluorescent light source equivalent in photochemical effectiveness to 0.6 milliwatt/cm^2 at 7000 Å. Results are shown for skin pieces of Jonathan, Rome Beauty and Arkansas varieties floating on 0.3 M sucrose and for whole Arkansas apples.

FIG. 2. The dependence of anthocyanin formation in pieces of Arkansas apple skin floating on 0.3 M sucrose solution on the time of exposure at constant irradiance immediately after the preparation of the pieces or after holding them on the sucrose solution in darkness for 20 hours. The fluorescent light source used gave an irradiance equivalent in photochemical effectiveness to 0.6 milliwatt/cm^2 at 7000 Å.

for the low-energy photomorphogenic or photoperiodic response. Anthocyanin synthesis in red cabbage seedlings, but not in turnip seedlings, is responsive to the state of the low-energy response pigment (18). Fourteen groups of skin pieces from Jonathan apples were irradiated for 44 hours with the fluorescent source of radiation. One set of 7 of these groups was then exposed for 5 minutes to a far-red source of radiation and the other set to a red source of radiation. The corresponding energies between 6900 and 8000 Å for the far-red source of radiation and between 5800 and 6900 Å for the red source of radiation were about 0.1 joule/cm^2. The idaein contents of the two sets were far-red, 0.570×10^{-8} mole cm^2; and red, 0.574×10^{-8} mole/cm^2; with a standard error of 0.004×10^{-8} mole/cm^2.

The action spectra for anthocyanin synthesis were measured with skin pieces from Jonathan and Arkansas apples. Three measurements of the action spectrum were made during the induction period. Seven measurements of the action spectrum were made during the linear period of anthocyanin formation. Representative results obtained in several action-spectrum experiments are shown in figures 3, 4,

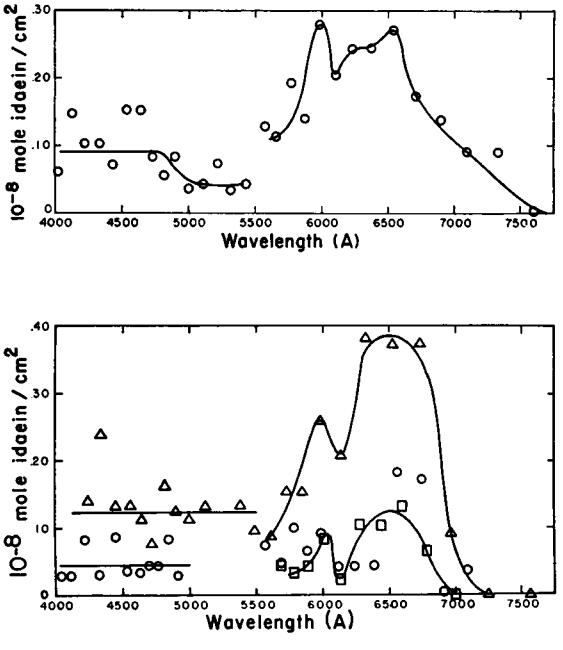


FIG. 3. The action spectrum during the linear phase of anthocyanin formation in pieces of Arkansas apple skin bathed in 0.3 M sucrose. The energy incident on the pieces was about 26 joules/cm^2 throughout the wavelength region examined.

FIG. 4. The action spectrum during the linear phase of anthocyanin formation in pieces of Jonathan apple skin, floating on 0.3 M sucrose, at an energy of about 26 joules/cm^2 (upper curve) and about 13 joules/cm^2 (lower curve) throughout the wavelength region examined. Results from two experiments with the lower energy are shown by circles and squares, respectively.

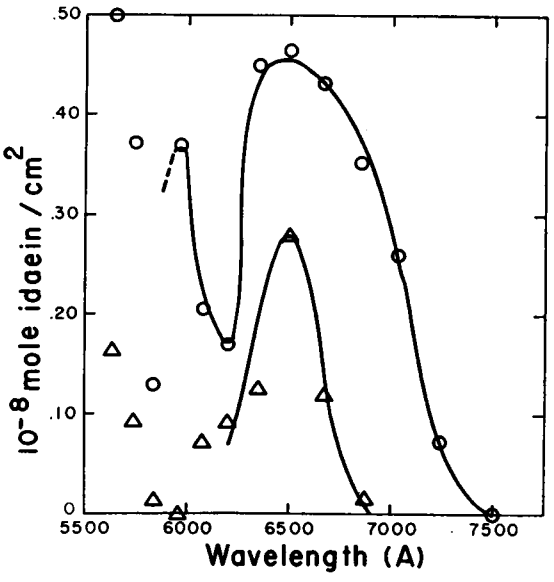


FIG. 5. Action spectrum during the induction phase of anthocyanin formation in pieces of Arkansas apple skin, floating on 0.3 M sucrose, at an energy of about 26 joules/cm^2 (upper curve) and about 13 joules/cm^2 (lower curve) throughout the wavelength region examined.

and 5. The wavelengths of light most effective in promoting anthocyanin formation were between about 6400 and 6700 Å. A subsidiary action maximum occurred near 6000 Å. In 8 of 10 action-spectrum measurements, anthocyanin formation induced in the region of 6200 Å was lower than that in the region of 6000 Å. In one measurement the values were the same and in another the formation at 6200 Å exceeded that at 6000 Å by 40 %. An estimate of precision calculated for one measurement indicated that the deviation from a smooth curve for the minimum near 6200 Å was significant at the 1 % level, while the deviation for the maximum near 6000 Å was significant at the 5 % level.

DISCUSSION

The synthesis of anthocyanin in apple skin can be separated into two radiation-dependent phases. The time course of anthocyanin formation has an induction period of about 20 hours in which practically no anthocyanin is formed. The induction period is followed by the linear period, in which anthocyanin formation is a linear function of time at constant irradiance. The induction period is interpreted as the time required to increase the concentration of a substrate to a level permitting the steady state formation of anthocyanin at a rate proportional to intensity.

The nature of the precursors of anthocyanin is suggested in these experiments by partial control of the induction period. The duration of the induction period can be reduced by subjecting apple skin pieces to a dark period before irradiation. In the presence of sucrose a strong odor of apple volatiles develops

during the dark incubation. The relation between the production of volatiles and the reduction of the required induction period is noteworthy. The odor of the apple volatiles was not detected when the apple skin pieces were exposed to the radiation source immediately after preparation. The volatile products of apples are known (9, 12, 13, 20, 21, 23) and consist predominantly of acyl compounds and alcohols. These products provided some suggestions about anthocyanin precursors or alternate products when the photoreaction is withheld. The active fatty-acid metabolism of apple skin, as evidenced by the production of oil and wax (10), also supports the view that acyl groups are precursors of anthocyanin.

Acyl compounds are probably in part the precursors of flavonoid compounds as suggested by Birch (4). Incorporation of acetate into the A ring of flavonoid compounds has been shown by Watkins, Underhill, and Neish (22), Grisebach (7) and Geissman and Swain (5). Grisebach demonstrated by degradation studies that the A ring of cyanidin was derived from a head to tail linkage of three acetate units. The B ring of the flavonoid compounds is derived from the shikimic acid pathway as shown by the work of Watkins, Underhill, and Neish (22) and Geissman and Swain (5). Flavonoid compounds in plants thus arise from a combination of two independent pathways of aromatic biosynthesis.

The action spectrum during the linear period of anthocyanin formation has marked and consistent features. Radiation throughout the visible region of the spectrum is effective in promoting anthocyanin formation, with maximum effectiveness in the red region of the spectrum between 6400 and 6700 Å and a subsidiary maximum near 6000 Å. The action spectrum of anthocyanin formation during the induction period was essentially the same as the action spectrum of the linear phase in the region of 6000 to 7500 Å.

The action spectrum of the linear period of anthocyanin synthesis in apple skin is related to the action spectrum of anthocyanin formation in red cabbage (18), turnip (18), and mustard seedlings (14). The similarities and differences are evident in figure 6. The position of the principal action maximum for the several tissues is in the region of 6500 to 7300 Å. The position of the subsidiary action maximum is near 6000 Å for apple and near 6200 Å for turnip and mustard seedlings. The differences in position of the subsidiary action maximum might not be real but might arise from the proximity of the principal action maximum.

The photoreceptive pigment for anthocyanin synthesis is probably a flavoprotein similar to the acyl coenzyme A (Co A) dehydrogenase enzyme isolated from liver mitochondria by Green, Mii, and Mahler (6). The enzyme is effective in catalyzing the dehydrogenations of acyl Co A compounds such as butyryl Co A. Mahler (12) found that the enzyme contains copper atoms which are effective in single electron transfer. Later work by Steyn-Parve and Beinert

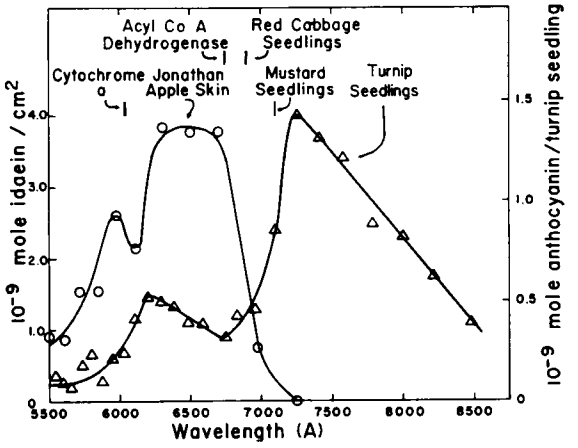


FIG. 6. The action spectrum for anthocyanin formation in pieces of Jonathan apple skin floating on 0.3 M sucrose (radiant energy = 26 joules/cm²) contrasted with that in turnip seedlings (radiant energy = 10 joules/cm²). The wavelengths for action maxima of anthocyanin formation in red cabbage and mustard seedlings and of absorption maxima of cytochrome a and acyl coenzyme A dehydrogenase are shown for comparison.

(19) does not support the presence of copper. The green color of the enzyme may be due to a semiquinoid form of the flavin moiety (3). The oxidized form of the enzyme was shown to activate hydrogen transfer through the functioning of its flavin moiety (12). The absorption spectrum of oxidized butyryl coenzyme A dehydrogenase as determined by Mahler (12) has absorption maxima near 6800 and 4600 Å.

A material balance of anthocyanin synthesis in respect to incident energy and effectively absorbed energy provides pertinent information. The hypodermal tissue, which synthesizes the anthocyanin, consists of 5 or 6 layers of cells and is about 1.0×10^{-2} cm in thickness (2). The maximum concentration of anthocyanin attained during the linear phase after 12 hours of irradiation in the region of 6500 Å is 8×10^{-4} molal in the effective cells. If, during the induction period, an acyl II Co A substrate for anthocyanin synthesis is produced from an acyl I Co A precursor by photoactivation with the same quantum efficiency for both, acyl I Co A would need to reach optimum concentration levels (10^{-3} to 10^{-4} molal) to serve as a substrate for further photoactivation in about 10 hours for the irradiances used. The photoactivation process thus is probably being used at least twice in the course of anthocyanin formation.

SUMMARY

Photocontrol of anthocyanin formation in apple skin was examined. Two distinct radiation-dependent phases were found. The first phase is an induction period of about 20 hours without anthocyanin production. In the second phase anthocyanin formation is a linear function of time of irradiation at constant irradiance. The action spectra for the two phases

were similar in the region of 6000 to 7500 Å. The action spectrum for anthocyanin formation in apple skin has a principal maximum near 6500 Å, a subsidiary maximum near 6000 Å and weak action throughout the visible region. The photoreceptor is probably a flavoprotein similar to acyl coenzyme A dehydrogenase.

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EFFECT OF 6-(SUBSTITUTED)PURINES AND GIBBERELLIN ON THE RATE OF SEED GERMINATION[1]

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Stimulation of lettuce seed germination by some 6-(substituted)aminopurines (3, 5) as well as 6-(substituted)thiopurines (4) has recently been reported. Further, gibberellin has also recently been demonstrated to increase the rate of lettuce seed germination to about the same extent as do the purine derivatives (2). In certain cases, these compounds appear to overcome the light requirements for germination which has been shown to be necessary for certain varieties of seed (1), and in addition, they also exert an effect which augments that of light (5).

In order to study the relationship of the germination effects observed upon lettuce seeds pre-treated with 6-(substituted)purines and gibberellin, the ef-

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