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SEQUENCE OF DEVELOPMENT OF AUTUMN COLORATION IN *EUONYMUS*

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Abstract—Low temperature inductive treatment of resting *Euonymus* plants sequentially resulted in the loss of chlorophyll, an increase in carbohydrate content, an increase in the activity of phenylalanine ammonialyase followed by an accumulation of cinnamic acids and flavonols and finally by the accumulation of flavolans and anthocyanins These changes were contrasted with changes resulting from low temperature treatments of actively growing plants which are physiologically not capable of the sequence leading to normal autumn coloration

INTRODUCTION

THE CHARACTERISTIC reddening of the leaves of certain temperate region plants in the fall season has attracted the attention of observers for many years. It was quickly realized that the occurrence of low temperatures was associated with the onset of coloration, and the temperature regulated starch-sugar conversion was considered to explain the phenomenon.[1] This idea was supported by the demonstration that sugars frequently stimulated red color formation in isolated tissue experiments. This stimulation, however, was not restricted to those species demonstrating autumn coloration, and the role of sugars was probably not singularly exclusive. A low temperature stimulation in the synthesis or accumulation of anthocyanins has been studied in apple skin[2,3] and of hydroxylated cinnamic acids in gherkin seedlings.[4] The mechanisms proposed to explain these phenomena[3,4] may have application in autumn coloration, although neither depend on starch-sugar changes.

Euonymus is a small bush which has been horticulturally selected specifically for its attractive autumn coloration. These studies with *Euonymus* report on the changes taking place in *Euonymus* leaves on intact plants placed in an environment resulting in development of autumn coloration. A contrast is made with *Fragaria vesca* leaves which show transient reddening under low temperature conditions but do not undergo autumn coloration.

Although many plants demonstrate reddening in response to low temperature conditions, this should not be considered as autumn coloration. Many seedlings show anthocyanin synthesis in response to many stress conditions including low temperatures. *F. vesca*

[1] OVERTON, E (1899) *Nature* **59**, 296.
[2] CREASY, L. L (1968) *Proc Am Soc Hort. Sci.* **93**, 716.
[3] CHAN, B G (1970) Regulation of L-Phenylalanine Ammonia-Lyase in Apple by Temperature and Other Factors, Thesis, Cornell University.
[4] ENGELSMA, G (1970) *Planta* **91**, 246

leaves, and leaves of many other plants show rapid anthocyanin synthesis when detached as disks.[5] This response is largely influenced by carbohydrate,[6] and massive carbohydrate accumulation is due to the isolation of a small tissue capable of rapid photosynthesis (the leaf disk) without the normal mechanisms responsible for dispersal of photosynthate to other parts of the plant (translocation). This accumulation swamps the synthetic metabolism resulting in the possibly untimely synthesis of a large number of products.

RESULTS AND DISCUSSION

The strawberry leaf while attached to a plant placed in cool temperatures is only part of a total organism which reacts differently from an isolated tissue and the rapid and massive changes characteristic of leaf disks do not take place (Table 1). The total plant reacts to changes in its environment but soon adapts, usually by some adjustment of growth rate. Like the isolated leaf disk the response seems correlated with *in situ* carbohydrate content and many synthetic (anabolic) products result. Growing plants of *Euonymus* placed in conditions favorable for autumn coloration respond similarly to the intact strawberry plants (Table 1). There is a quantitative change in the accumulation of many products, an increase of *in situ* carbohydrate and a reduction in growth. The growth reduction, a direct result of the lower temperatures is likely to be the significant reason for the accumulation of products. The growing *Euonymus* plant does not respond to low temperature resulting in autumn coloration. It is probably very much like the intact strawberry plant.

Whereas *Fragaria* is an evergreen which can continue shoot growth (and associated leaf production) indefinitely, *Euonymus* is a deciduous plant which, following a short period of shoot elongation goes into rest and produces no more leaves regardless of favorable environmental conditions until a long period of cold exposure has been completed. The mature leaves on the plant in rest are subject to induction by low temperature to undergo autumn coloration.

TABLE 1. TEMPERATURE DEPENDENCE OF CHANGES TAKING PLACE IN LEAVES OF DETACHED GROWING *Euonymus* AND *Fragaria* PLANTS. *Euonymus* treatment was for 24 16-hr days: 16 day, 8° night inductive and 22° constant temperature for non-inductive. *Fragaria* plants were treated in constant light at the constant temperatures for 10 days.

Plant treatment	PAL* (nmol hr cm^2)	Flavolans (nmol cm^2)	Flavonols (nmol cm^2)	Cinnamic acids (nmol cm^2)	Anthocyanin (nmol cm^2)	Sugar (mg cm^2)
Euonymus inductive	14·7	173	32	73	0	0·35
Euonymus non-inductive	4·7	138	17	15	0	0·27
Fragaria/10	66·0	224	115		1·3	0·46
Fragaria/25	22·0	131	40		0·9	0·32

* PAL is phenylalanine ammonia-lyase.

The environmental conditions necessary to induce autumn coloration possibly differ for different plants. The conditions used for *Euonymus* may not be optimum but are the result of trials with this plant. Analysis of leaves from plants placed in inductive and non-inductive conditions are shown in Figs. 1 and 2. The time sequence would only be valid for these particular inductive conditions. The changes which take place are an integral part of the physiological process of senescence. About 4 days after initiation of inductive conditions,

[5] CREASY L. L. (1965) *Phytochemistry* **4**, 517.
[6] CREASY L. L. (1968) *Phytochemistry* **7**, 1743.

chlorophyll loss started to occur. Other changes which took place at this time were increases in the activity of phenylalanine ammonia-lyase (PAL) and a gradual increase in soluble carbohydrate. The increase in PAL was sufficient to result in higher net activity even at the lower ambient temperatures. These changes continued, and the next observed changes to take place were an increase in flavonol content which continued until about 2 weeks following the start. At this point the accumulation of flavonols ceased and there was a rapid synthesis of anthocyanin and flavans. None of these changes took place in leaves of plants in a non-inductive environment Three weeks after the start of treatment a decline in the activity of PAL began which continued until abscission. The accumulation of cinnamic acids and flavonoids also stopped before abscission. Sampling was stopped in this experiment at 31 days because many leaves had dropped and it was no longer possible to get a representative sample

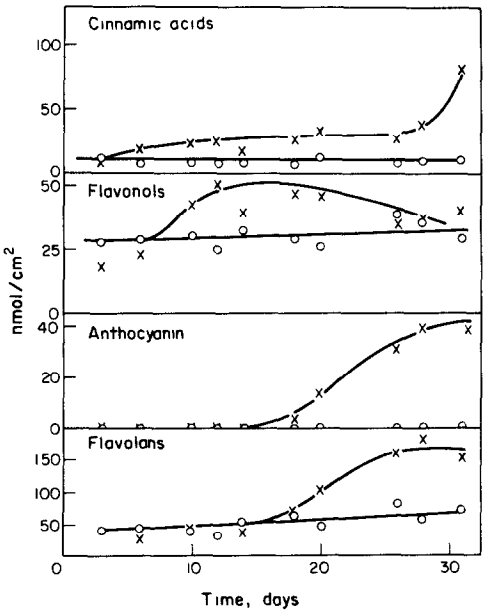

FIG 1 PHENOLIC CHANGES IN LEAVES OF RESTING *Euonymus* PLANTS TREATED WITH INDUCTIVE (×) OR NON-INDUCTIVE (O) TEMPERATURES
The day-length in each treatment was 10 hr and the inductive temperatures were 15 17° day and 0–2° night The non-inductive temperature treatment was 22–24 throughout the day and night

There were some significant differences between the content of the measured constituents of growing and resting *Euonymus* plants placed in warm or non-inductive conditions. The growing plants have higher flavolan and lower flavonol content than resting plants. Under inductive conditions growing plants increase in content in most components as do resting plants but do not synthesize anthocyanin and do not have as high a level of PAL as the resting plants. The leaves of growing plants also do not lose chlorophyll and if placed back in warm conditions will resume normal growth.

Compared with the leaves of growing *Euonymus* plants, the leaves of resting plants therefore respond differently to low temperatures. The response is characterized by a sequence of changes in their phenolic metabolism accompanied by senescence expressed as a loss

of chlorophyll and is terminated by the rapid synthesis of flavolans and anthocyanins and ultimately by the abscission of the leaves. The accumulation of these products in an organ which is abscissed by the plant is of unknown value. The biochemical mechanism of induction of these changes is unknown although the hypothesis of a specific PAL inactivation system with a temperature coefficient higher than the temperature coefficient for a PAL synthesis or activation process could find application in explaining the phenolic changes.[4]

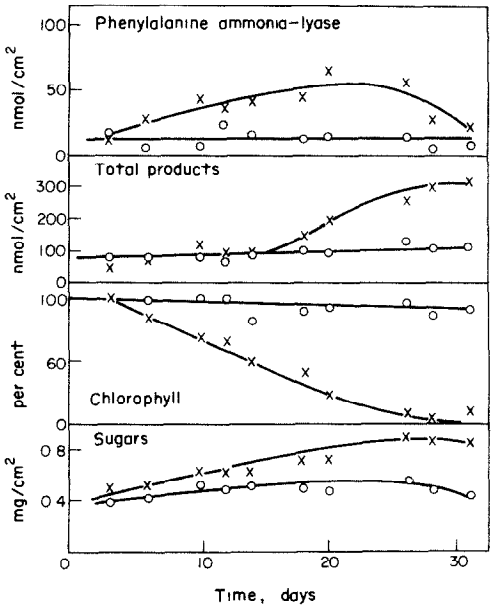

FIG. 2. CHARACTERISTIC CHANGES OCCURRING IN RESTING *Euonymus* PLANTS GIVEN DIFFERENT TEMPERATURE TREATMENTS.

Plants were treated with inductive (×) or non-inductive (○) temperatures (see Fig. 1). The total products are the sum of all the phenylpropanoid units accumulated in the tissue (see Fig. 1).

EXPERIMENTAL

Experimental material. Strawberry plants (*Fragaria vesca* var. Alpine) and *Euonymus* plants (*E. alatus-compactus*) were grown in the greenhouse at Ithaca, New York until the start of the experiment. All plants received 2 mW/cm² of fluorescent light in controlled environment chambers. Non-inductive conditions were 10 hr days, 14 hr nights with a constant temp. of 22–24°. Inductive conditions were 10 hr days, 14 hr nights, 15–17° days and 0–2° nights.

Quantitative measurements. Cinnamic acids,[7] flavonoids[5, 8] and soluble carbohydrates[6] were determined as previously described. Relative chlorophyll content was measured by determining the $A_{670}-A_{700}$ of MeOH extracts of leaf disks, the values given are the percentages of the values at the beginning of treatment.

PAL-assay. At the time of sampling 10 11 mm dia. leaf disks were frozen for each sample. For assay, the disks were placed in liquid N_2 and ground in an electric mortar at near liquid N_2 temps. with 100 mg Polyclar AT (GAF Corp.) and 25 mg borax. When this appeared homogeneous, more liquid N_2 was added and 6 ml of borate buffer (0·02 M, pH 8·8) was drop-frozen in the liquid N_2 in the mortar. The frozen buffer was then ground into a homogeneous powder with the disks. This procedure, initiated to assay samples of high phenolic content,[3] affords rapid contact between extraction solution and protein upon thawing.

Once fully ground the samples may be stored frozen for several days without loss of activity. At the time of assay the samples were placed in a centrifuge and centrifuged at 20000 *g* for 20 min. The spectrophotometric assay was then carried out as previously described.[9]

[7] CREASY, L. L. (1971) *Phytochemistry* **10**, 2705.
[8] CREASY, L. L. (1966) *Phytochemistry* **5**, 501.
[9] CREASY, L. L. (1968) *Phytochemistry* **7**, 441.