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THE RISE IN TOCOPHEROL CONTENT IN WILTING AND IN NON-ILLUMINATED LEAVES

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Abstract—The concentration of α-tocopherol in detached leaves of spinach, mustard, maize, yew, cress, clover, lucerne and grass increased after one day's storage. Tocopherol contents also rose in whole growing plants that were placed in darkness, but fell when they were illuminated.

DURING a study on the tocopherol content of vegetables it became desirable to investigate the effect of wilting. It was found that α-tocopherol, unlike carotene,[1] increased in leaves detached from the plant, a fact that is consistent with the observed increase in tocopherol in senescent leaves.[2] Further investigation, described in this report, showed that the tocopherol content rose when leaves, whether detached or not, were shaded from sunlight.

RESULTS

Detached Leaves

A sample from a batch of spinach leaves (*Spinacia oleracea*) was withdrawn and analysed immediately after harvesting. The remainder was left to wilt at room temperature, uncovered but out of direct sunlight, and further samples were analysed daily. The results in Table 1 show a steady rise in tocopherol content.

TABLE 1. α-TOCOPHEROL CONTENTS OF WILTING SPINACH LEAF

Analysed on day	Dry matter, %	α-Tocopherol in ppm based on fresh weight	α-Tocopherol in ppm based on dry matter
0	9·6	16·3	170
1	11·2	23·3	208
2	12·2	27·1	222
3	13·3	34·1	256

A similar experiment with leaf of lucerne (*Medicago sativa*) produced these values for α-tocopherol—initial 130; 1 day 215; 2 days 165 ppm; the values continuing to fall thereafter: indeed only in spinach leaf was a further rise observed after one day. Cocksfoot grass (*Dactylis glomerata*) similarly treated had these tocopherol contents: initial 65; after 1 day 140 ppm (mean of three experiments).

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[1] V. H. BOOTH, *J. Soc. Chem. Ind.* 64, 162T (1945).
[2] V. H. BOOTH and A. HOBSON-FROHOCK, *J. Sci. Fd. Agri.* 12, 253 (1961).

Errors due to evaluation of moisture, and to possible evaporation of other volatiles through respiration, were eliminated by weighing the four necessary samples for each test at the same time and directly into extraction beakers.[3] One pair was extracted and assayed at once, the other after 24 hr covered storage in the dark. Species having small leaves were used in order to obviate cutting before storage, because tocopherol is lost in severely damaged leaves.[4] Five such experiments were done, and the results are shown in Table 2. The average rise in tocopherol content during a day's storage was 16%.

Table 2. α-Tocopherol in leaves before and after storage for 1 day

Species	α-Tocopherol, ppm	
	Initial	Next day*
Mustard (*Sinapis alba*)	250	255
Cress (*Lepidium sativum*)	125	160
Clover (*Trifolium repens*)	160	170
Lucerne (*Medicago sativa*)	145	170
Yew (*Taxus baccata*)	180	230

* Based on initial dry matter.

Effect of Light

Several plants of maize (*Zea mais*) were grown in soil in a box, and when 20 cm high a sample of leaves was assayed for α-tocopherol. The box with its plants was then removed to a dark room for 24 hr and another sample of leaves was prepared in the dimmest green light practicable and immediately assayed. The plants were then replaced in daylight. Next day they were sampled, transferred again to the dark, and sampled again the following day while still in the dark room. The α-tocopherol contents were: in the light 100, in the dark 140, light 80, dark 110 ppm. Thus the concentration in leaves that were growing in light was only 72% of that in darkened leaves.

Mustard was grown in soil in two pots under conditions as identical as possible. When the plants were 5 cm high one pot was placed in darkness, the other left in daylight. The leaves of the latter, 5 hr after sunrise, had a tocopherol content of 525 ppm, while the former had 560, or 6% more. A sample of lucerne leaves was gathered in bright sunlight at 9 a.m.: dry matter was 19·3% and tocopherol 265 ppm. Another sample, gathered from the same plants after 3 hr in the shade, had dry matter 16·5% and tocopherol 445 ppm.

Plants of *Impatiens parviflora* were grown under artificial illumination. Leaves removed and analysed at once had a tocopherol content of 140 ppm. In similar leaves taken from plants after 24 hr in darkness the tocopherol content had risen to 200 ppm.

The Source and Fate of α-Tocopherol

No tocopherols other than alpha have been found in appreciable amounts in leaves except those of *Taxus baccata* and *Hedera helix*, but several other u.v.-absorbing spots are

[3] V. H. Booth, *Analyst* **84**, 464 (1959).
[4] V. H. Booth, *Biochem. J.* **84**, 85P (1962).

seen on the chromatograms.[5] Some of these spots become blue when sprayed with leucomethylene blue: they include the plastoquinones and other quinones.[6] One of these quinones,[5] q4, runs to the same position as does an oxidation product of α-tocopherol, and q3, probably a plastoquinone, runs close to it. None of these lipids has been found systematically to decrease when α-tocopherol in leaves increases (in darkness) or to increase when α-tocopherol decreases. Indeed we have the impression that the q3 and q4 spots are strongest when α-tocopherol is strong. A reducing substance appeared close to α-tocopherol in chromatograms from extracts of mustard leaves whose α-tocopherol had diminished. This substance has been found in three other species but not in those used in the present study.

Thus at present both the source and the fate of α-tocopherol in leaves are unknown.

DISCUSSION

The results provide an answer to the original question—when leafy vegetables wilt the tocopherol content first increases and later declines.

The results also indicate one source of the considerable variation found in the tocopherol contents of leafy vegetables on separate occasions.[7] And they serve as a warning that a single tocopherol determination may not be representative; leaves harvested in bright sunshine may be weaker in tocopherol than those harvested in dull illumination or kept in storage.

The α-tocopherol concentration was higher in leaves of shaded plants than in those that were growing in the light. The increase in tocopherol in resting leaves, whether detached from or attached to the plant, is in keeping with earlier observations that tocopherol is present in higher concentration in dormant than in actively-growing leaves and highest in senescent and dying leaves.[2] A notable difference between illuminated and non-illuminated leaves is in photosynthetic activity. It is not suggested that α-tocopherol takes part in photosynthesis, but its loss during periods of illumination supports the hypothesis already partially expressed[8] that tocopherol, acting as antioxidant, may be protecting chlorophyll from destruction by the oxygen produced during photosynthesis. The tocopherol while so functioning may be itself consumed. When leaves are in darkness, oxygen production necessarily ceases, and tocopherol accumulates.

The α-tocopherol in isolated chloroplasts diminished with time.[8] [α-Tocopherol always diminishes when leaves are damaged.[4]] The content of chlorophyll in these chloroplasts remained steady which it would if the protection given by tocopherol were effective. Dilley and Crane[9] found no significant changes in the levels of α-tocopherol in chloroplasts: perhaps the chloroplasts in their preparation were less damaged than mine[8] so that the enzymic loss of tocopherol only just balanced the accretion if any.

METHODS

α-Tocopherol

Lipids were extracted from small samples with cold acetone and light petroleum.[3] The extract was chromatographed on paper in two dimensions. α-Tocopherol spots were cut out

[5] V. H. Booth, *Biochem. J.* **84**, 444 (1962).
[6] M. D. Henniger, R. A. Dilley and F. L. Crane, *Biochem. Biophys. Res. Comm.* **10**, 237 (1963).
[7] V. H. Booth and M. P. Bradford, *Brit. J. Nutrition* **17**, 575 (1963).
[8] V. H. Booth, *Phytochem.* **2**, 421 (1963).
[9] R. A. Dilley and F. L. Crane, *Biochem. Biophys. Acta* **75**, 142 (1963).

and determined with dipyridyl and ferric chloride.[10] Tocopherol contents are expressed as ppm of dry matter. All analyses were done at least in duplicate. The pooled coefficient of variation of a mean, estimated from the replicated determinations, was ±6%.

Dry Matter

Samples were weighed before and after heating for 5 hr at 100°.

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[10] V. H. Booth, *Analyst*, **88**, 627 (1963).