Table 2 Specific Extinction Coefficients of the Copper Band at 346 mμ (Purified Preparations, Diluted with a Saturated Borax Buffer, *p*H 9·18, 20° C.)

Preparation	Year of collection	Protein concentration (per cent (w/v))	*E*(1 per cent, 1 cm.)
β-Hæmocyanin	1952	0·1060	2·98
	1956	0·321	2·88
	1957	0·0563	2·90
(very fresh)	1957	0·0399	3·30
	1959	0·281	2·79
α-Hæmocyanin	1952	0·240	1·78
	1956	0·397	2·20
	1956	0·334	2·58
	1957	0·100	2·60
	1957	0·202	2·07
	1958	0·236	2·61
	1959	0·194	2·21
	1959	0·156	2·10

These observations clearly establish the parallel decrease of the copper bands of *Helix pomatia* hæmocyanin under ordinary storage conditions, β-hæmocyanin changing more slowly than α-hæmocyanin. To explain the decrease a bacterial action may well be ruled out, as the effect is highest in the presence of half-saturated ammonium sulphate. An organic substance, possibly aromatic, which is salted out by ammonium sulphate, could be responsible for this spectral decrease. Evidence has indeed been given for the presence of phenolic amines, for example tyramine, in the blood of some cephalopods[3].

Related observations have been made on the oxygen binding of hæmocyanins. A preparation of *Limulus polyphemus*, stored for several months under half-saturated ammonium sulphate, showed a 30 per cent decrease of the absorption band in the visible, which was accompanied by a 26 per cent lowering of the oxygen-binding capacity[4]. The hæmocyanin of *Helix pomatia* has further been reported to show during storage at − 10° a change from the sigmoid oxygenation curve to a rectangular hyperbola[5].

The ratio, however, of α- and β-hæmocyanin was completely normal in the preparations *A* to *E* after 300 days storage, as determined by dissociation tests in a Spinco ultracentrifuge model *E* and *M* sodium chloride at *p*H 5·8.

The antigenicity moreover of some hæmocyanin solutions, kept under toluene at 4°, did not undergo any noticeable change after ten years[6].

In conclusion it may well be stressed that the usual way of storing hæmocyanins as an ammonium sulphate precipitate, without any other treatment, proves to be the least satisfactory, if properties connected with the oxygen-binding sites are to be studied.

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K. Heirwegh
R. Lontie

Laboratory of Biochemistry II,
University of Louvain,
Belgium.

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PLANT PHYSIOLOGY

Vitamin E and Flowering of *Fragaria vesca* L. var. *semperflorens* Duch.

The development of *Fragaria vesca* L. var. *semperflorens* Duch. is very sensitive to day-length[1]. In short-day conditions the plants do not reach the reproductive stage. Days more than 12 hr. long are required. Short-day treatment also inhibits flower formation of *Fragaria vesca* plants which have previously reached the flowering stage in long-day conditions. During a season in the open field, the number of flowers found on such flowering plants varies according to the day-length as shown in Fig. 1. A maximum of flowers is found in the inflorescences growing during August–October, which have been initiated during June–July, in the longest days.

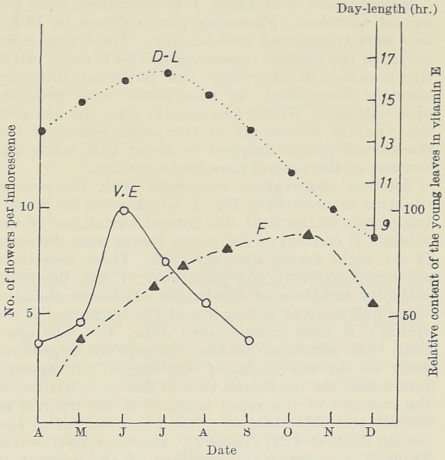

Fig. 1. Relations between day-length, number of flowers per inflorescence and vitamin E content of the young leaves in flowering plants of *Fragaria vesca* L. var. *semperflorens* Duch. (for explanations, see the text; *D-L*, day-length; *V.E.*, vitamin E content; *F*, number of flowers per inflorescence)

On the other hand, in some experimental conditions, runner plants of *Fragaria vesca* have been shown to improve their flowering capacity when vitamin E is applied externally to the leaves in low concentration[1]. It seemed, therefore, interesting to investigate the two following points: (1) Is the vitamin E content of *Fragaria vesca* plants grown in 8-hr. day (remaining vegetative) different from that of plants grown in 16-hr. day (beginning to form flowers)? (2) Is the vitamin E content of flowering plants related in some way to their flowering intensity?

Vitamin E was measured by the method of Meunier and Vinet[2] in the unsaponifiable fraction of the leaves extracted as described by Sironval[1]. The separation of the vitamin was obtained on 'Floridin *XS*' prepared following Kjölhede's method[3].

Table 1, measurements 1–3, answers the first question. The measurements were made in adult leaves of *Fragaria vesca* plants grown in 8-hr. days (remaining vegetative) or in 16-hr. days (beginning to form their first flowers). The temperature was maintained constant (20° C.) for measurement 1. Measure-

Table 1. Effect of Day-Length on the Vitamin E Content of the Adult Leaves of *Fragaria vesca* L. var. *semperflorens* Duch. (per γ in 100 mgm. of the Unsaponifiable Fraction)

Measurement No.	Temperature	8-hr. day	16-hr. day
1	20° C. constant	219	430
2	variable	255	490
3	variable	202	480
4	variable	185*	420

* This series has first been grown in long-days. It was transferred to short-days at the flowering stage. The measurement is done 2 months after the transfer (see text).

ments 2 and 3 were made on plants grown in the open field. It appears from Table 1 that the content of vitamin E per 100 mgm. of the unsaponifiable fraction is regularly about twice as high in long-days than in short-days. Calculated on a dry-weight basis, the recorded difference is still more marked.

Measurement 4 of Table 1 is concerned with the second question. Flowering plants grown in 16-hr. day were transferred to 8-hr. day. They were compared to the control remaining in 16-hr. day. After the transfer to short-day the flowering intensity strongly diminished, in accordance with previous observations[1]. Within two months after the transfer, the vitamin E content of the adult leaves of the transferred plants dropped to about half, while the content of the control remained constant.

Another observation concerned with the second question is recorded in Fig. 1, which gives for flowering plants in the field the normal variations of the vitamin E content of the young leaves near the apex (4 cm. high) during the year 1958. These variations are compared with the variations of the flowering intensity and those of day-length. It is seen that the vitamin E content is first low at the beginning of the spring; it rises rapidly until June, and falls after that. The maximum in June corresponds approximately to the maximum of day-length. It appears just before the maximum rate of flower initiation, at the moment of the rapid increase of the number of flowers per inflorescence. This has been found also during the year 1957 (Table 2). However, as shown in Table 2, the seasonal variations of the content in vitamin E is less evident in adult and old leaves than in young ones. The content drops with the ageing of the leaves, as we see when considering the underlined figures of Table 2, which are concerned approximately with the same set of ageing leaves.

It is concluded that the vitamin E content of *Fragaria vesca* leaves is very sensitive to day-length, and that this feature is related in some way to the action of day-length on flower formation. Normal flower formation seems to require a high vitamin E content in the leaves. This is seen not only in experiments with extreme day-length (8-hr. and 16-hr.), but also in ordinary culture in the field. In this last case, the occurrence of a high content in vitamin E in the young leaves surrounding the apex, at the moment of the active increase of flower initiation in June, strongly supports the idea that the vitamin plays a part in the promotion of flowering.

Table 2. Variations of the Vitamin E Content of the Leaves of Flowering *Fragaria vesca* L. cultivated in the Field during the Years 1957 and 1958 (100 for 1957, 62γ per 100 mgm. dry weight; for 1958, 71γ per 100 mgm. dry weight)

Time of observation	Young leaves		Adult, just expanded leaves		Old leaves	
	1957	1958	1957	1958	1957	1958
April	39	37	—	—	—	—
May	28	45	21	27	30	10
June	100	100	25	44	10	38
July	26	73	42	53	12	32
August	18	56	16	38	21	45
September	—	38	26	23	14	11

C. Sironval
J. El Tannir-Lomba
Laboratory of Plant Physiology,
University of Liège.
Actual address:
Laboratory of Plant Physiology (IRSIA),
Gorsem-St.-Trond, Belgium.

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Chlorogenic Acid of Lettuce Seeds

The high concentration of chlorogenic acid in lettuce seed has not previously been reported. Fig. 1 compares the ultra-violet absorption spectrum of a methanol solution of chlorogenic acid, 0·02 gm./l., with that of a methanol extract of 1 gm. of lettuce seed made up to 1 litre. The lettuce seed had been extracted continuously with methanol for 16 hr. in a Soxhlet apparatus. Paper chromatography of the extract showed the predominant fluorescent component to be chlorogenic acid. Several other fluorescent components which after elution show the same ultra-violet absorption spectra as chlorogenic acid are also present on the chromatograms. These are probably isomers of chlorogenic acid, but could be other depsides of caffeic acid. Caffeic acid itself is not a major component. Assuming that the compounds which have the same absorption spectrum as chlorogenic acid are isomers and have the same molecular extinction coefficient at 330 mμ, 2 per cent of the weight of the dry seed is chlorogenic acid and its isomers.

In order to investigate the possible role of this large amount of chlorogenic acid, lettuce seeds were germinated for as long as two weeks and the seedlings from 1 gm. of seeds were extracted with methanol.

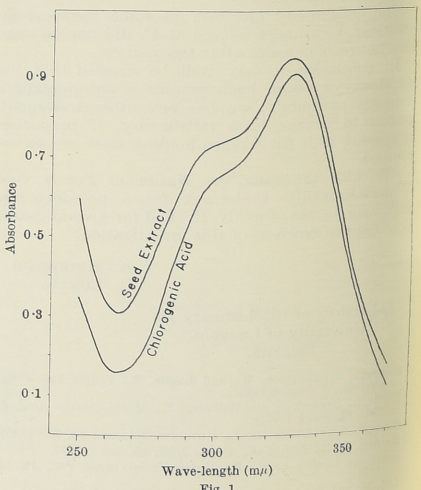

Fig. 1