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Hormonal Regulation of Growth by Vitamin E

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I. Introduction

Stowe and Obreiter (1962) published several facts showing that "lipids of both the fatty acid ester and isoprenoid vitamin type in trace quantities can mimic or enhance auxin or gibberellin action in a number of different ways". Their paper demonstrated namely that vitamin E given at a low concentration stimulated pea section growth and enhanced auxin or gibberellin induced growth.

The authors referred among others to our work (Sironval 1957) in which we showed that the crude insaponifiable extract of adult leaves of flowering *Fragaria vesca* plants modified the growth pattern and increased the flowering of a strawberry test (see below). The action of the crude insaponifiable fraction on flowering was attributed to its vitamin E content but we did not investigate whether this vitamin was able to affect growth in the strawberry test.

Recently, Bruinsma (1963) observed that vitamin E, as well as gibberellic acid, promoted flowering of unvernalsed Petkus winter rye. Bruinsma also found that the growth of the winter rye was appreciably enhanced by vitamin E.

We expose in this paper some facts relevant to the action of vitamin E on the growth of the strawberry test, and compare this action to that of gibberellic acid (GA_3) on the same object.

II. Material and Methods

1. The strawberry test

We previously showed (Sironval 1948) that the separation of very young runner plantlets from flowering strawberry mother plants (*Fragaria vesca* L. var. *semper-*

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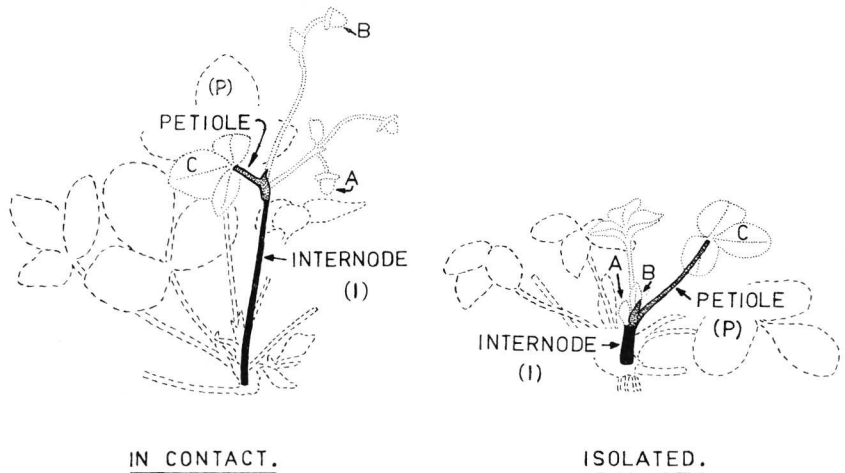


Figure 1. Effect of the isolation of young runner plantlets from the mother plant on the form of the first flower truss. A = terminal flower of the truss; B = axillary bud of the truss; C = first leaf of the truss. Separation of the plantlet at the beginning of the growth of its second leaf. Strawberry variety: "A Fruit Rouges".

florens Duch.) resulted in abnormal growth and flowering of the first flower truss of the isolated plantlets. The situation is depicted in Figure 1 for the variety "A Fruits Rouges" from Gonthier, Belgium. The flower truss of the isolated plantlets had an abnormally short internode (I) and an abnormally long petiole of the trifoliate leaf (P). The terminal flower of the truss (A) did not grow in isolated plantlets (it often died!) while the bud B, instead of forming flowers, formed trifoliate leaves. We concluded that the contact with the mother plant is necessary for a normal growth and flowering of the first flower truss and that the isolation deprives the plantlets of some substance (or substances) normally provided by the mother plant. Isolated plantlets therefore constitute a suitable test for the trying out of products suspected to be favorable to normal growth and flowering. The action of a given product is measured by the transformations induced in the characteristics of the first flower truss of the test.

2. Preparation of the test

The state of the strawberry mother plants and the conditions of isolation of the plantlets must be carefully standardised in order to obtain the most homogenous test objects possible.

This was done in the following way: mother strawberry plants (*Fragaria vesca* L. var. *semperflorens* Duch.) of the variety "La brillante" (from Vilmorin, Paris), were cultivated in the open field. The plants were planted in lines at about 40 cm from one another, the lines being 50 cm from one another. This disposition was necessary to allow an easy preparation of the plantlets for the test. The culture was conducted in such a way that the mother plants were very healthy and that they flowered abundantly. A culture of about 500 mother-plants appeared sufficient to get the required plantlets at the right time.

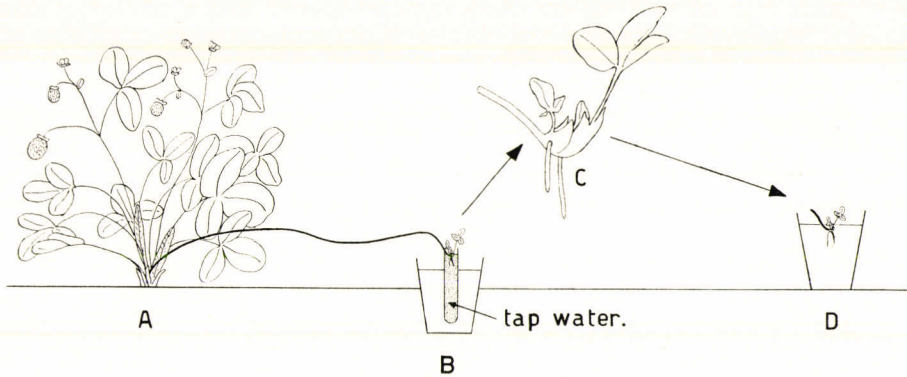


Figure 2. Method of preparation of the test. A: the mother plant; B: the plantlet dipped in water; C: the plantlet as it is isolated; D: the disposition of the plantlet in a pot.

When the mother plants were in full bloom (at the end of May), it was possible to start the choice of the experimental plantlets. Series of 30 to 50 plantlets were regularly chosen twice a week in the field from the spring to the autumn. Only the first plantlets along the runners were chosen when their first leaf was fully adult, the second beginning to open. They were immediately dipped in tap water as seen in Figure 2. Three to four days later, one or two roots were growing. At this time the plantlets were isolated from the mother plants and planted separately in small pots (5 cm diameter) containing ordinary garden earth. The pots were disposed in a greenhouse.

3. Application of gibberellic acid and vitamin E to the strawberry test

The application of GA_3 and vitamin E to the test began immediately after the isolation of the plantlets.

GA_3 was dissolved in distilled water. Each treatment consisted of spraying 1 ml of the suitable GA_3 solution on 10 test-objects.

The concentration of the solutions and the frequency of the treatments are given below in the text for each experiment.

Vitamin E (d, γ -tocopherol, Eastman Kodak) was given in ether solution. The solution was prepared immediately before its application. The ether used was analytically pure and previously de-oxidated. Each treatment consisted of spraying 1 ml of the solution of vitamin E on 20 test objects. In some experiments the ether was immediately evaporated by an air stream, but in general this practice could be omitted, since the quantity of ether sprayed per test object was very small, and did not damage the plants. The concentrations of the vitamin E solution and the frequency of the treatments are given below in the text for each experiment.

III. Results

1. First experiment

A preliminary experiment showed that in our conditions of culture, the average length of the first internode (I) of the first flower truss of control

Table 1. Action of vitamin E and GA_3 on the average lengths of the internode I and the petiole P of the first flower truss of the *Fragaria* test. 16 treatments were given; length in cm.

Plantlets	Average length of the first internode (I)	Average length of the petiole (P)	Average ratio (I/P)
Control plantlets in contact with the mother plant ...	5.3	0.6	10.8
Control isolated plantlets ...	3.3	1.9	2.8
Isolated plantlets + vitamin E (5 μ g/plantlet)	5.7	1.2	6.4
Isolated plantlets + GA_3 (25 μ g/plantlet)	4.8	2.6	2.9

plantlets connected to flowering mother plants was about 5 cm, the average length of the petiole (P) of the first leaf being 0.6 cm. The mean ratio I/P was found to be about 11.

When the plantlets were experimentally isolated, the length of I decreased, while that of P increased (Table 1). As a result of this, I/P decreased to a value of about 3. Treatment of isolated plantlets by vitamin E (5 μ g per plantlet) twice a week during 8 weeks resulted in a modification of both the length of I and P: I increased to normal "connected" values, while P decreased, the average I/P ratio being greater than 6. Treatment of isolated plantlets by GA_3 twice a week (during 8 weeks; 25 μ g per plantlet) gave a somewhat different result; the average length of P increased as well as the average length of I, the average I/P ratio being about 3.

The action of GA_3 seemed somewhat unspecific inducing indifferently an increase of the length of the two organs studied I and P, as well as an abnormal growth of the bud designated by B in Figure 1. (Under the influence of GA_3 , but not of vitamin E, the bud B produced regularly a sort of runner; we do not discuss this particular effect here). Vitamin E acted much more specifically inducing only the growth of I but not that of P, nor that of B. In this respect the action of vitamin E was similar to the action of the flowering mother plants when connected to the plantlets.

2. Second experiment

A factorial experiment 3×3 (nine series) was designed to test statistically the conclusions of the first experiment. In this experiment GA_3 and vitamin E were both given twice a week (during 8 weeks) to each series of isolated plantlets (concentrations for vitamin E: 1.25–2.50 and 5.00 μ g per plantlet; for GA_3 : 5–10 and 25 μ g per plantlet). Vitamin E was applied between 10 and 11 a.m.; GA_3 was applied about 4 hours after the application of vitamin E.

The statistical analysis first showed the occurrence of a significant correlation between the length of I and that of P. The regression line representing the relation between the lengths of I and P in the factorial experiment is given in Figure 3 (without consideration of the quantities of GA_3 and vitamin E applied to the plantlets).

Figure 3. Regression line representing the relation between I (length of the internode of the first flower truss) and P (length of the petiole of the first leaf of the first flower truss. Cf. Figure 1).

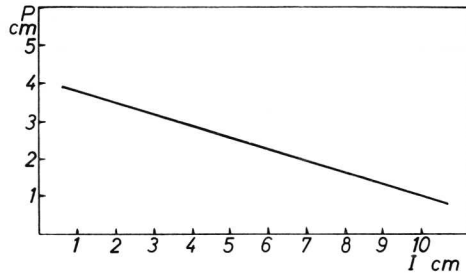


Figure 4 shows that, notwithstanding the concentration of GA_3 , the length of I increased linearly while the length of P decreased linearly with increasing concentration of vitamin E. The effects of vitamin E were found significant at the 5% level both for P and I , the analytical analysis being made on the original values of P and I . The linearity of the increase of I under the influence of vitamin E was found significant at the 1% level and the linearity of the decrease of P significant at the 5% level.

Such clear effect was not met with in the case of GA_3 . GA_3 tended to increase indifferently the lengths of P and I , but the increase was not statistically significant. It must be noted that, as in the first experiment, GA_3 acted much more on the growth of bud B than on the length of P and I .

The statistical analysis indicated further that vitamin E and GA_3 did not interact in their action on P and I ; the actions of the two factors were simply additive.

The results confirmed the preliminary observations concerning the specificity of the action of vitamin E when compared with that of GA_3 in our

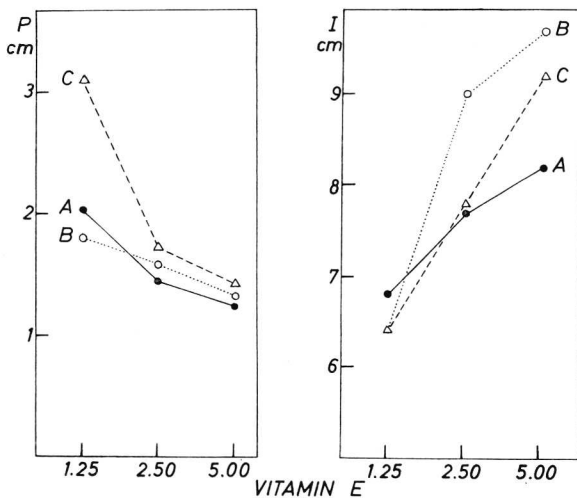


Figure 4. Effect of vitamin E on the growth of I and P (see Figure 1), for 3 different concentrations of GA_3 . A = 5; B = 10 and C = 25 γ GA_3 per plantlet. Vitamin E in μg per plantlet.

test. It is obvious that increasing the concentration of vitamin E raised the I/P ratio as follows:

Vitamin E $\mu\text{g/plantlet}$	1.25	2.50	5.00
I/P	4.1	6.1	8.1

The lowest concentration gave a ratio analogous to that found in control isolated plantlets; the highest concentration gave a ratio analogous to that of normal plantlets in contact with flowering mother plants (see Table 1).

The linearity of the variations of the I/P ratio in function of the increasing doses of vitamin E was found significant at the 5 % level.

This shows that vitamin E is really able to replace the flowering mother plant for the control of the relative length of I and P in the first flower truss of the strawberry plantlets. Such a control is not obtained with GA_3 ; this product tends to increase unspecifically the length of both I and P.

IV. Discussion and Conclusions

The hormonal nature of vitamin E in higher plants seems clear since the vitamin acts in several circumstances, and always in very low concentration, on growth and flowering (Table 2). This is the principal fact demonstrated.

However our results raise the question of the relations between the action of vitamin E and that of gibberellin.

They essentially show that:

1. in the *Fragaria*-test the action of GA_3 and that of vitamin E on the length of I and P are additive (the two products do not interact);
2. the action of GA_3 is unspecific, increasing the lengths of both I and P, while vitamin E "distinguishes" the two organs (as the mother plant does), increasing I and decreasing P correlatively.

The additivity of the effects of vitamin E and GA_3 in the *Fragaria* test seems to contradict the results of Stowe (1961) who found that vitamin E and other tested lipids "act hormonally, as synergists of the action of auxins,

Table 2. *The hormonal activity of vitamin E in literature* (+ indicates a positive modification of the growth or flowering patterns).

Author	Action of vitamin E on:		Nature of the test
	flowering	growth	
Sironval (1957)	+	not studied	<i>Fragaria</i> test
Stowe (1961) and Stowe and Obreiter (1962)	not studied	+	Pea stem sections
		(in the presence of auxin, or of auxin and gibberellin)	
Bruinsma (1963)	+	+	unvernalised Petkus winter rye plants
Sironval (1963)	not studied	+	<i>Fragaria</i> test

and in the presence of an auxin, of gibberellic acid". We must observe here that some auxin, some gibberellin and some vitamin E are already present in the isolated plantlets of the *Fragaria* test. The test is clearly not totally deprived of these substances. We only find that there is no interaction between GA₃ and vitamin E when added to the test. In these conditions, it is not possible to decide if vitamin E would act in the total absence of auxin or gibberellin.

However, the additivity of the effects of the added products indicates that in our test the sites of action of GA₃ and vitamin E could be in some way distinct from each other. This conclusion is stressed by the fact that GA₃ and vitamin E act differently on the length of P or on the growth of the bud B. How are we to understand that vitamin E decreases the length of P when GA₃ increases it, if it is admitted that the two substances have a single common site of action? In fact, the situation is probably more complicated in the *Fragaria* test.

Stowe and Obreiter (1962) suppose that the activity of vitamin E is linked with the presence of an isoprenoid moiety in the molecule. This view is derived from the results of Wever, Gloor and Wiss (1958) who found that a hydrocarbon chain of a certain length (for instance phytol, squalene or the isoprene sidechain of vitamin E and K) facilitates the operation of cytochrome-c-reductase in the mitochondrial cytochrome system. For this and some other reasons, Stowe (1961) has suggested "that lipid activation of the cytochrome system could explain the results observed", and specially the effects of vitamin E on growth processes. The cytochrome system is believed to produce "at least some of the energy for auxin and gibberellin action".

In this connection, it is interesting to point out that the photoperiodic induction modifies the haematin content of the leaves of some photo-periodically sensitive plants (Sironval 1962). Under induction, modifications seem to occur in the cytochrome systems of the leaves. Moreover, inductive conditions increase the vitamin E content of the leaves in *Fragaria vesca* (Sironval 1960). These facts argue in favour of the suggestion of Stowe and support the idea that the action of vitamin E on growth and flowering is linked with the functioning of the cytochromes. We should like to include here not only the mitochondrial, but also the chloroplast cytochromes.

It is also certainly necessary, when considering the mechanism of the hormonal action of vitamin E, to keep in mind the numerous biological functions which have been attributed to this molecule as a whole (see review by Boyer 1960). In particular, more or less direct actions on RNA and DNA metabolism, like those described in animals by Young and Dinning (1951), are to be considered. The same holds for the possible connections between the metabolism of the phytol moiety of the vitamin E molecules and the metabolism of some isoprenoid compounds, especially the carotenoids and plant sterols.

The recognition of the hormonal character of the action of vitamin E in higher plants is precisely most interesting if one considers the fact that some of the biological functions of this substance have been thoroughly studied in other systems than plants.

Summary

Very low concentrations of vitamin E can modify the growth pattern of the first flower truss of the "Fragaria test". The hormonal nature of this action is indicated. Vitamin E seems to act in some other way than gibberellic acid does.

Some possible intracellular sites of the action of vitamin E are discussed.

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