UNCLASSIFIED

AD NUMBER
AD839542
NEW LIMITATION CHANGE
TO Approved for public release, distribution unlimited
FROM Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; MAY 1964. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Release Branch/TID, Frederick, MD 21701.
AUTHORITY
Fort Detrick/AMXFD ltr dtd 9 Feb 1972

THIS PAGE IS UNCLASSIFIED

TRANSLATION NO. 1106

DATE: 15 May 1964

Best Available Copy

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

30

INFLUENCE OF ETHYLENE ON GROWTH-SUBSTANCE FORMATION IN AVENA AND VICIA

by

P. A. Van der Laan

Botanical Laboratory, Utrecht

Recueil des Travaux Botaniques
Neerlandais, Vol 31, 1934, pp 691-742

Contents

INTRODUCTION

When we wish to learn something about the physiological phenomena of plants, we usually adhere to the following method: One of the external factors to which the plants are sensitive is varied while all other factors are kept constant; the plants respond with particular reactions. A study of this type aims at following the course of these phenomena and drawing conclusions from the results regarding the essence of plant-physiological phenomena.

In the present study the external factor is fresh air, and it is varied by the addition of a small amount of ethylene gas.

Plants are extraordinarily sensitive to ethylene, the most harmful component of illuminating gas, and the changes brought about by this gas are highly varied. ~~We intend to examine, in detail,~~ two of these influences of ethylene [were examined in detail]:

a) The decrease of the longitudinal growth of sprouts of Avena sativa; and

b) The horizontal growth of the otherwise vertically growing sprouts of Vicia faba.

CHAPTER I

1. General Literature Review

On examining the literature relating to the damages inflicted on the growth of plants by illuminating gas or ethylene, we do not obtain a uniform picture. We find either the description of more or less strong inhibitions, or the injury is not a pronounced one, or the plants are stimulated. The sensitivity greatly fluctuates with the different species, with the stages of growth, and with the authors performing the experiments.

Agriculture was at first concerned with damages caused by illuminating gas; the earliest researchers (Girardin 1864; Virchow 1870; Kny 1871; Spaeth and Meyer 1873) investigated trees on the street. Kny already reported on the differing sensitivity of different species; Speath and Meyer reported that the influence of the gas is most damaging during the growth stage, leading at that time to a falling of leaves and the death of cambium.

Wiesner (1878) studied the influence of light and gravity on Phaseolus sprouts. As a light source he used a gas flame which he burned continuously. It turned out that the plantlets exhibit a different manner of bending to light, according to whether it is the front- or the back side that bends (the front side is where the seed of the sprouts is located). Thus the sides exhibit different growth capacities; the tendency exists to grow away from the seed: In the beginning the front side grows faster, then the back side catches up so that the sprout becomes erect, then the front side again grows faster; finally a wave-like, horizontal growth sets in which Wiesner called "undulating mutation." Already Sachs (Lehrbuch d. Botanik (Textbook of Botany), 4th Ed., 1874, pp 828-829) mentioned nutation in discussing this concept which was created by him. The phenomenon may be seen also in Vicia faba, Pisum sativum and other species. According to Wiesner the cause of this phenomena is to be attributed to unfavorable growth conditions.

Noljubow (1901; 1911) was able to show that this was not the case. He found that impure air, particularly small doses of illuminating gas, had a role to play; he did not believe in the existence of an autonomous nutation; according to his concepts illuminating gas is supposed to lead, in the plants, to a geotropic conversion whereby the negative geotropism changes into a transverse one. It is immaterial from which position of the plant one starts out: according to him the sprout always appears horizontally, thus behaves like a lateral root.

Richter (1903-1910) continues to build on Wiesner's ideas. An autonomous nutation supposedly appears when the gas weakens the negative geotropism. Under normal circumstances the geotropism suppresses the nutation. As a proof of his views against those of Neljubow, he brings up that the sprouts always grow away from the seeds, independently of their position. The same nutation occurs when Richter eliminates the negative geotropism in normal plants by means of a clinostat.

Knight and Crocker (1913) determined the gas concentration at which the above-mentioned phenomena still occur in Pisum. Ethylene is found to be the most effective gas; already 0.0001% is effective. They further investigated the damages caused by tobacco smoke on the basis of the experiments of Molisch (1911), and concluded that even in that case ethylene was probably the most harmful factor. The gas influence consists of three parts (" triple response "):

1) Inhibition of longitudinal growth;
2) Stimulation of transverse growth; and
3) Horizontal nutation in the growing zone.

Further we must mention the exhaustive but experimentally unsatisfactory work of Sorauer (1916); he placed his experimental plants into a room in which a gas flame is burned continuously. He then compared the great damages which set in with those which have been brought about by lack of oxygen. He found some agreement, and as Sorauer further believed to have been able to detect transpiration- and assimilation inhibitions which in his opinion appear as a consequence of oxygen shortage, he concluded that all damages caused by gas are to be attributed to phenomena which had been described as consequences of oxygen shortage. His study, however, refers to lasting, severe, hence complicated gas damages which cannot without further considerations be compared with the results of other studies.

Subsequently the work of Wehmer (1917-1918) was published, dealing with illuminating-gas damages in different groups of plants.

The earlier authors almost always observed more or less pronounced growth inhibitions. A stimulating influence of ethylene on growth is only discovered when working with quite definite gas concentrations; like many poisons, ethylene, too, acts as a stimulant when present in very low amounts.

A frequently occurring stimulation phenomenon is the epinastic bending of grown leaves under the influence of illuminating gas or ethylene. Wachter (1905) was the discoverer of this phenomenon. Molisch (1911) demonstrated its occurrence in plants exposed to the effect of tobacco smoke, and Doubt (1917) has shown it in the case of many plants under the influence of illuminating gas; Schwarz (1927) communicates, in addition, that the same phenomena set in after 3 hours' stay in water at a temperature of 35°C. Further, by placing marks on the leaf stalk she was able to ascertain that the epinasty is a growth- and not a variation movement.

The best investigation of epinasty caused by ethylene was carried out by Crocker, Zimmerman and Hitchcock (1932). Many plants were tested for their sensitivity; only 36% displayed a clearly pronounced epinasty. The phenomenon was filmed with a tomato plant; it was found that the nutations and sleep movements of the leaves ceased in ethylene; a certain rigidity set in. The extent to which geotropism participated in the epinasty was thoroughly studied;under clinastating around a horizontal axis only 40% of the epinasty remained, but it subsisted in each case. Apparently geotropism has a predominant part in the development of the phenomenon, but it is not the sole determining factor; internal factors, too, play a role. The same holds true also for the horizontal growth of sprouts.

A third series of phenomena has become known through the requirements of technology. In the US the extraordinary sensitivity of plants to ethylene is utilised in many ways. Since a good review was published a short time ago (Mack and Livingstone, 1933), it will suffice here to give only a brief resume. It was found that small amounts of ethylene are able to shorten the rest period of plants to a considerable extent, and that in ethylene the time necessary for the ripening of picked fruit is significantly shorter (Chace and Denny 1924; Denny 1924a: Wolfe 1931). To this must be added that breathing is always more intensive, and perhaps it may be concluded quite generally that an accelerated metabolism is taking place (E.M. Harvey [illegible]; Denny 1924b; Regeimbal, Vacha and R.B. Harvey, 1927; Mack 1927; Davis and Church 1931; Mack and Livingstone 1933)

As was already mentioned, ethylene shortens the rest periods; a quite particular case is the acceleration of the development of runners in potato tubers (Vacha and R.B. Harvey 1927). Ethylene was not always the most effective agent (Denny 1926); often C_2H_4 compounds showed better results. R.B. Harvey (1925) and Mack (1927) reported that celery becomes whiter in ethylene.

Very interesting is the exhaustive work of Rossi (1933). He found that a treatment with ethylene shortens the time necessary for the fermentation and drying of tobacco leaves; the amount by which the time was shortened was 40%. He assumed that the effect of ethylene is limited to a stimulation of the enzymatic processes. In his opinion in this process the cell plasma is stimulated. The quality of the tobacco remains completely unchanged.

On the basis of the phenomena known so far we are by no means able as yet to analyze the effect of ethylene on plants; I think, however, that there is at least one hint in this regard: many times there were observed, under the influence of ethylene, certain growth changes. Since F.W. Went (1928) has established the close relationship between growth and growth-substance content, it appeared interesting to investigate the influence of the gas from this point of view.

It is not necessary to go into the history of the discovery of the growth substance in detail, since many reviews have already been published on this subject (F.W. Went 1928; Kostytschew-Went 1931; Du Buy and Nuernbergk 1932).

Boysen Jensen (1913) and Paal (1919) detected the existence of a substance which is responsible for phototropic bending. This discovery was elaborated by Cholodny (1927), F.W. Went (1928) and Dolk (1930) to their well-known growth-substance theory of tropism.

In orthotropic plant parts there exists a basipetal, all-sided transport of growth substance that is unilaterally deflected by light or gravity (collectively referred to by the general term "stimulus"); more growth substance leads to a greater growth, thus there occurs a unilaterally increased growth causing the deflection.

We are indebted to F.W. Went for a nice quantitative method for the determination of the growth-substance content, which since has made possible many thorough investigations; Van der Wey (1931) has introduced into this method some useful improvements.

The studies of Went have been elaborated in different directions:

Heyn (1931) was able to show in Avena that under the influence of the growth material the plasticity of cell membranes (that is, the ability to be irreversibly elongated) is increased, accordingly the turgor overstretches those cell membranes. - The ability to be elastically stretched (that is, the ability for reversible length changes), however, also plays a role, since it increases during the growth, but falls during inhibition of growth, e.g. through decapitation. Heyn and Van Overbeek (1931) showed that the ability to be elastically stretched is also influenced by the growth substance.

Van der Wey (1932) investigated the growth-substance transport in living coleoptile cells of Avena. The transport takes place much faster than a pure diffusion, definitely polar, only in basipetal direction. The amount of growth substance supplied does not have any influence on the transport velocity; even the temperature does not effect the growth-substance transport in any other way than it does any other physiological process.

Uyldert (1931) worked with sprouts of Tradescantia fluminensis. He inhibited growth and the geotropical reactivity by decapitation; after a supply of growth substance both occurred again. She was able to detect growth-substance transport in young internodes. The normal position of a sprout of Tradescantia deviates from the vertical by about 20°. Vertically oriented sprouts cut in on the dorsal side no longer perform any epinastic bending; but those which were nicked on the ventral side do. In the case of geotropic stimulation only the dorsal side conducts the growth substance; the geotropic stimulation causes a polarization of the growth-substance transport.

Du Buy (1933) determined the growth-substance yield of Avena coleoptiles during the development period; it was found that it remains constant for a very long time. Further, he investigated the different growth-substance conditions in reference to aging: I will return to this in greater detail below.

Van Overbeek (1933) was able to detect growth substance in the case of sprouts of Raphanus and Lepidium only in the cotyledons and the young leaves. Thus in these species there is a center for the production of growth substance, as in the Avena, in the tip of the coleoptile. When, however, the cotyledons are removed, then it is the tip of the hypocotyl that begins to form growth substance. He ascertained the production of growth substance in green leaves under the influence of light. There is, here, perhaps a connection between growth-hormone production and assimilation.

On the other hand Dijkman (1934) extracted much growth substance from the hypocotyls of etiolated sprouts of Lupinus, while in this case the cotyledons yielded no active growth substance. The growth substance occurs distributed over the entire length of the hypocotyl; there does not exit here any [illegible]mscribed growth-substance production center. Each cell pr[illegible] own growth substance. The direction of gravity has no effect [illegible] of growth substance formed; there occurs, in the horizontal hypocotyl a unilateral distribution of the basipetal growth-substance stream, as was already shown by Dolk (1930) in Avena. Here the lower side receives as much more growth substance as the upper side receives less. After a calculation by Dijkman the geotropical bending may be completely explained quantitatively on the basis of this unilateral distribution; n[illegible] growth reaction participates in its occurrence. This agrees with the findings of Cholodny (1929, 1930) and Dolk (1930); neither could detect any geo-gr[illegible] reaction.

Schmitz (1933) investigated the growth-hormone content of grass condyles; they contain this substance, the internodes do not. Here, too, there occurs on geotropic stimulation a polarization of the growth substance with the lower side receiving most of it. As is known under clinostasis the grass condyles grow on all sides, and it turned out that then more growth substance, too, will form on all sides. In this case, in contrast to the case of Dijkman, there will be a renewed formation of growth substance, due to geotropic stimulation. Still we must consider the behavior of grass condyles rather than the conditions prevailing with the sprouts of Lupinus as the diverging case.

Van der Wey (1934) demonstrated the existence of polar-growth-substance transport in the case of the shrub Elaeagnus, and found growth substance also in Valonia.

Thus investigations have been carried out with regard to the role of the growth substance in plant life. It was found that this substance is distributed quite generally, and that everwhere it enhances the extension of cells. During the last years, however, it has been proved beyond a doubt that the growth substance inhibits the longitudinal growth of roots (Cholodny 1927; Boysen Jensen 1933); cf. also Gartner (1932). This phenomenon is difficult to reconcile with [illegible] concepts regarding the mechanism of growth-substance action.

Bonner (1933) attacks the matter from another direction; he measured the growth of Avena coleoptiles in 3 mm long growth-substance-free coleoptile cylinders. Whether in this case it is the normal growth that is detected remains unsettled. In one growth-substance solution these cylinders are elongated; this growth varies with the growth-substance concentrations (the growth substance originates from Rhizopus). We find an optimal concentration; high concentrations have an injurious effect. The elongation of the coleptile cylinders is completely inhibited by a 0.001N KCN solution, also by a 0.05% phenylurethane solution; similarly, no growth took place in a nitrogen atmosphere. The same inhibitions were found also when the author determined the respiration of plant portions treated in the same manner. Addition of a small amount of growth hormone increased respiration; larger amounts disturbed it. From his studies Bonner concluded that an increase in the intensity of respiration is probably a necessary precondition for the action of the growth-substance causing growth.

2. The Gas. General Experimental Setup.

Ethylene, $H_2C=CH_2$, is a combustible, non-explosive, aromatic gas, difficultly soluble in water (0.25% at 0°C). Because of its double bond it is capable of undergoing addition reactions, e.g., with halogens, sulfuric acid, nitrogen oxides and metal salts. It is well absorbed by sulfuric acid. A review of the physical and chemical properties of ethylene is given by [illegible] and Egloff (1919).

The guiding principle in the search for an experiment methodology was as follows: It was necessary to prepare an experimental setup in which only one factor is changed: ethylene or no ethylene; otherwise all other experimental conditions are to remain completely constant.

Preparation of Ethylene: 25 g 96% ethanol, 150 g concentrated H_2SO_4 and 30 g sand are heated on the oil bath to 230°C. When the evolution of ethylene starts, a 1:2 mixture of alcohol and H_2SO_4 is added from a separating funnel. The gas is freed from impurities in the washing bottle with lye and H_2SO_4, and taken up under water; then it is collected in a gas reservoir (Erlenmeyer and Bunte 1874; Aberhalden's Handb. d. Physiol. Arb. Meth. (Handbook of Physiological Experimental Methods) I, 4, 36).

The Gas Box: The experimental plants are exposed to the influence of the gas in the sheet-metal box (22.4 x 12.5 x 16 cm) shown in Fig.1.

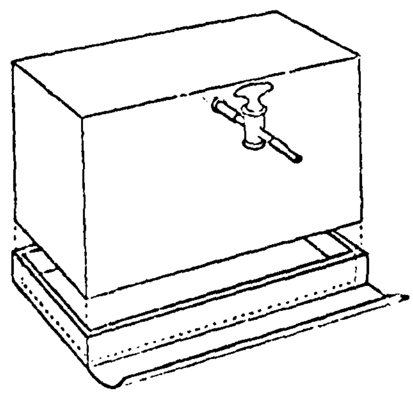


Fig.1. The Gas Box

A little ethylene gas is taken up, under water, by means of a gas pipette *) and pressed into the box through a glass stopcock; in order to be able to create a greater pressure, a rubber ball is fastened to the pipette. Always there was a second box without gas right next to the experimental box for control purposes.

Control experiments showed the following: Within given ethylene concentration limits (0.005-0.0005%) the effect is unchanged; hence it is not necessary to work always with an exactly determined concentration. This makes the method of operation significantly easier. The amount of gas used was within these limits in all experiments. Further, it was found that after a 24-hour stay in the control box no damages were caused by the lack of oxygen; in the gas box, however, marked changes were noted during this period; in this way it is established that Sorauer's findings are to be considered incorrect; thus the old experiments of Wieler (1883) and Jaccard (1893) are verified.

All experiments were carried out at a temperature of 22°C; in addition, those in the dark room were performed at a humidity of 92%.

*) Jordan and Hirsch 1927

Chapter II: INVESTIGATIONS ON AVENA SATIVA

3. General

In view of the fact that Avena sativa (Svabv's "Triumph Oat" (Siegeshafer)) lends itself to the most exact growth-substance determinations I chose this plant as the first experimental object. The plants were grown in the usual manner in water in glass containers (Went, 1928); they also stayed in the same darkroom.

After 3 1/2 days the plants were placed into the sheet-metal boxes and one half provided with a little ethylene; afterwards they were placed in a thermostat at the same temperature as that of the darkroom; the "noxious" gas should not get into the darkroom! On the next day the experiments were begun.

For the transport experiments (c.f. Van der Wey, 1932) it is necessary to carry out a very large number of growth-substance analyses on a single day; when one investigates, e.g., the transport during five different periods, it is necessary to use for the analysis of the agar blocks 5 x 12 = 60 Avena plants. When one determines the content of the lower and upper plates, then it is necessary to take double that number; and comparative experiments in gas and pure air again double the number of plants tested. Decapitation of 20 x 12 Avena sprouts, extraction of the primary leaves and setting up the agar blocks take up at least 100 minutes. Between the setting up of the agar on the first and last plant the time elapsed is 100 minutes, and this can cause a considerable error. Kogl and Haagen Smit were able to show that the ability of the Avena sprouts to react to a given amount of auxin fluctuates every hour by several tens of a percent (Kogl 1933).

In order to attain well-comparable experiments, the analyses should be carried out as contemporaneously as possible. This could be attained by means of an altered experimental technique. I did not employ, as in the previous case, a series of 12 plants for one experimental series, but used the sequences of each series one after another, so that each plant of a sequence belongs to another experimental series. The following experiment justifies this technique:

Table I
Comparison of Two Series of Differently Pretreated Test Plants in the Auxin Test

Series a) The small agar blocks of each experimental sequence were placed one after the other on two culture stands each

Series b) The plants of a culture stand were alternately provided with an agar block of each experimental sequence.

---

1) In this work the term "growth-substance" refers to untreated product extracted from plants; by contrast, the term "auxin" is employed for the product concentrated from urine, well-known chemically as "a-auxin" (cf. Kogl, Haagen Smit and Erxleben, 1932).

a.				b.			
1.	8,6° ± 0,5	19 Pfl.		1.	7,0° ± 0,5	20 Pfl.	
2.	7,6° ± 0,5	16 Pfl.		2.	8,1° ± 0,4	17 Pfl.	
3.	7,2° ± 0,6	17 Pfl.		3.	7,0° ± 0,5	16 Pfl.	
4.	5,8° ± 0,7	19 Pfl.		4.	7,6° ± 0,4	20 Pfl.	
1) Grösster Unterschied: 2,8°.				1) Grösster Unterschied: 1,1°.			

1) Greatest Difference.   Pfl. = plants.

4. Method of Growth Determination

The growth of sprouts must be determined contemporaneously under the same conditions both in ethylene and in pure air. To this end one of the above-described sheet-metal boxes is provided with a fully airtight separating wall, as well as with windows on the front- and back side (Fig.2). In this way two compartments are obtained, into one of which small amount of gas may be introduced. Each compartment contains 5-7 Avena sprouts.

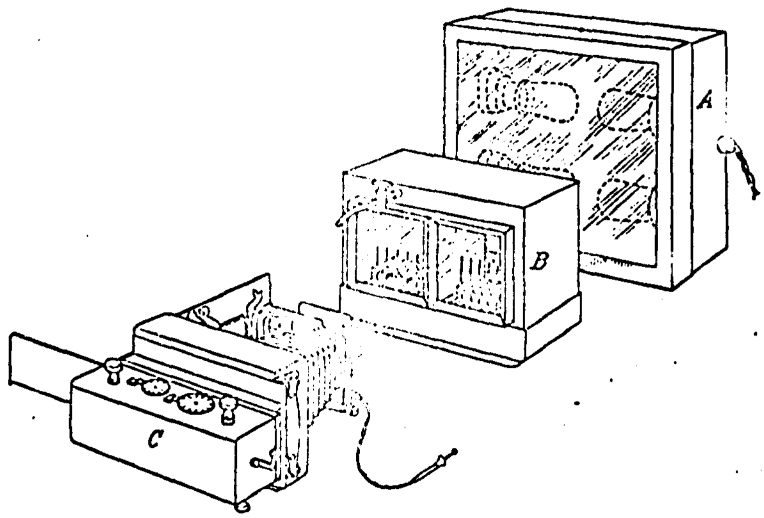


Fig.2. Explanation in Text.

Now it is verified whether the box is airtight. For this purpose I placed next to the first box a second one containing plants of the same age. After 12 hours the plants were exactly as long as those in the air-filled compartment, while the plants in the ethylene-gas containing compartment were much shorter. I measured this growth inhibition as follows:

A Zeiss photographic apparatus (C) is fastened to the same table on which the box (B) is situated, and focussed on the plants in the two compartments. The camera contains a magazine with panchromatic film which is advanced after each exposure. The red illumination lamp (A) behind the box is lit only during the time of exposure (Fig.2).

In this way, [illegible] obtained each time a double picture of 2 x 6 plants, half of which were exposed to the influence of the gas. The whole apparatus stood in the dark room with constant temperature and humidity, already described by F.W. Went (1928).

Following the suggestion of Du Buy and Nuernbergk, tin-foil marks were pasted on the plants. I placed the small glass holders of the coleoptiles very close to one another so as to be able to photograph as many plants together as possible. Pictures were taken every 30 or 60 minutes.

The films were finally projected on a screen and measured by the apparatus of Du Buy (1933, p 818).

5. Method of Determining Growth-Substance Production

According to the data of F.W. Went, nine Avena tips were placed each time on an agar plate for one hour.

The plants were then brought to the darkroom in the usual manner and then placed, in two series, in the sheet-metal boxes: one series received a small amount of ethylene.

Table 2. Influence of the Duration of Gas Action on Growth-Substance Production. The Analyses were carried out on a single day.

Length of Stay in Experimental Box	14 hours	18 hours	33 hours
In gas	4.6°	4.1°	4.0°
In pure air	6.8°	7.2°	6.4°

Table 2 shows that the growth-substance production in gas is less, but that on the contrary the length of stay in ethylene does not influence the growth-substance production. More on this limitation will be found in Section 9, p 13.

6. Method of Determination of Growth-Substance Transport

For the transport experiments the plants were set up exactly the same way as for the production experiments. The coleoptiles of the "gassed" plants were 3 1/2 - 3 cm long, those of the normal plants 4 - 4 1/2 cm. The two were of the same age.

I used the method of Van der Wey (1932) and cut coleoptile cylinders with the apparatus described on p 397 of his paper; after some practice [illegible] succeeded in bringing the cylinders into good contact with the agar plates without using his transport apparatus (p. 401).

7. Method of Determination of the Reactivity to Growth Substance

We investigated, by two different methods, the manner in which ethylene influences the reactivity of the sprouts to a given amount of auxin; first, we measured the magnitude of the growth-substance bending after a unilateral placement of auxin agar; second, we determined the magnitude of growth when auxin agar is placed on decapitated Avena coleoptiles on all sides (1).

1) Normal and "gassed" plants, grown in the usual manner, are provided according to Went's method with agar blocks containing the same amount of auxin. Thereupon they are again placed in the boxes and after 110 minutes a silhouette of the bending which has resulted in the meanwhile is recorded; this is then measured (Went 1928, p 26).

2) Coleoptiles about 3 cm long are decapitated; the primary leaf is simply extracted and then auxin agar applied on all sides. The plants are then placed in the same box with the separating wall as described above. The growth is likewise measured in the same manner (Fig. 2, p 10).

8. Determination of Growth (See Fig.3)

After staying in an atmosphere containing ethylene for one hour, the uninjured plants exhibit a 70% inhibition of growth. However, the growth was never completely inhibited. At the same time it is worth mentioning that the "gassed" plants are considerably thicker than the normal plants. On sections we see that the cells are much shorter and wider and the walls thicker than in the case of usual coleoptiles. The microscopic measurements of coleoptile cells of normal plants and gas plants gave:

Table 3

	"Gas" Plants	Normal Plants
Length	66.8 µ	112.0 µ
Width	19.5 µ	16.0 µ

All figures are average values of 50 measurements each.

Growth of Zones: The time of the beginning of growth inhibition is not the same for all zones. In the most strongly growing zone, the middle zone (7-15 mm from the tip), the absolute inhibition is maximum; thus, there exists a link particularly between the longitudinal growth and the effect of gas. It is to be expected that ethylene gas weakens the influence of the growth substance without which cell elongation is not possible.

Thus, several possibilities exist:

---

(1) I thank Professors Dr F. Kogl and Dr A.J. Haagen Smit for supplying me with the amount of a-auxin needed for my experiments.

1) Ethylene influences the growth-substance production at the tip;

2) Ethylene influences the growth-substance transport from the tip to the base;

3) Ethylene influences the ability of coleoptile cells to respond to growth substance; and

4) A combination of the above-mentioned factors.

We shall examine each of these possibilities in succession.

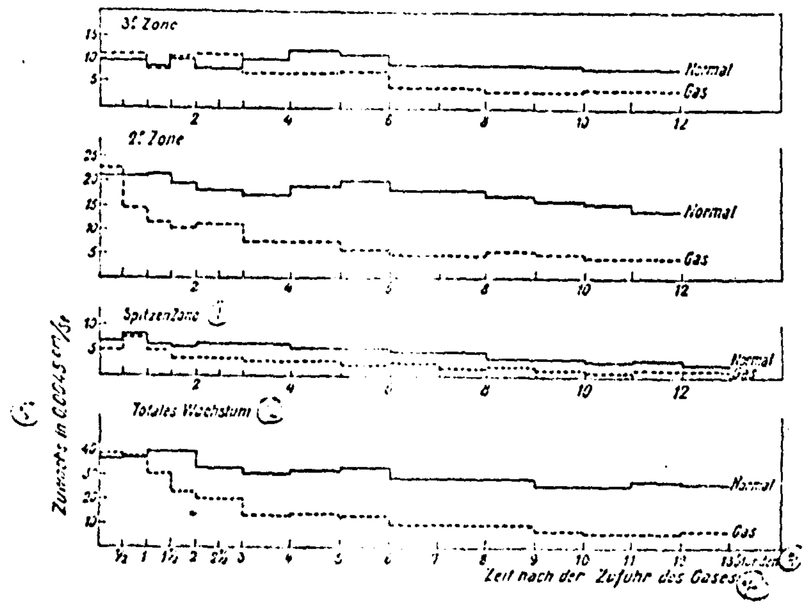


Fig. 9. Total Growth and Growth of Zones in Avena. Average Values of Five Experiments on 26 Jun, 7 Jul, 11 Jul, 12 Jul and 13 Jul 1933. 1) Tip Zone 2) Total Growth 3) Hours 4) Time after supply of Gas 5) Growth in 0.0045 cm/hour

a. Determination of Growth-Substance Production

It may be seen from Table 4 that ethylene has a marked influence on growth-substance production: the latter is reduced by about 1/3. These changes are similar to the phenomena arising when the tip of the coleoptile is injured or cut off. Then, too, the longitudinal growth is stopped due to lack of growth substance; the cells become thicker and stay short.

Hence in Avena ethylene injures the growth-substance-production center in the tip.

The first possibility mentioned in the previous section was verified, but percentually the growth is reduced to a much greater extent than the yield of active growth substance from the tip of the coleoptile on the agar plate. Nevertheless it does not seem proved whether the yield of active growth substance and the reduction of longitudinal growth are percentually comparable; all we know is that the amount of growth substance and the growth substance bending are proportional to each other within certain limits (F.W. Went 1928; Dijkman 1934). We do not know, however,

1) How much growth substance is necessary for a given growth;
2) Whether all growth substance obtained from a plant is used for the growth and vice versa; and
3) Whether we obtain, with our present methodology, all the active growth substance from the plant.

In recent times (Dijkman 1934; v. Overbeek, 1933) attempts have been made to deepen the theoretical insights through quantitative clues based on growth-substance determinations; as long, however, as the above questions remain unsettled we must take these theories with the greatest caution; in my opinion they rush ahead of the experiments.

Table 4. Growth-Substance Production in Avena in Gas and in Pure Air.

Datum (1)	Wuchsstoffproduktion in (2)		Verbleib in Gas in Stunden. (4)
	Gas	reiner Luft (3)	
18.11.32	4,9°	7,0°	± 20
21.11.	5,1°	6,7°	21½
25.11.	4,4°	8,3°	33
26.11.	5,1°	6,5°	12
28.11.	5,8°	8,9°	13
2.12.	4,6°	6,8°	14
3.12.	4,1°	7,2°	18
3.12.	4,0°	6,4°	35
7. 1.33	7,5°	9,2°	31½
15. 1.	1,3°	4,9°	15
20. 1.	2,9°	4,1°	35
20. 1.	3,5°	5,5°	35
20. 1.	3,8°	5,8°	35
Total	57,0 = 65 %	87,3 = 100 %	

1) Date 2) Growth-Substance Production in 3) Pure air 4) Length of Stay in the Gas, Hours

10. Determination of Growth-Substance Transport

The transport was determined by two methods:

A) Short cylinders (2 mm), high initial concentration (100°), short transport period;

b) Long cylinders (6 mm), low initial concentration (20-30°), long transport period ([illegible] hours).

When the transport is determined in the last-mentioned manner, it is possible to simultaneously determine the consumption of growth substance. Here it is naturally assumed that the initial concentration and, at the end of the experiment, the growth-substance concentration are determined both in the upper and lower plates (Van der Wey 1932, p 43[illegible]). In these experiments the transport takes place always in the gas, while the experiments with short transport periods were carried out during the after-effect period of the gas. I then placed the cylinders after cutting in the gas for a few hours, after which they were used in the transport experiments.

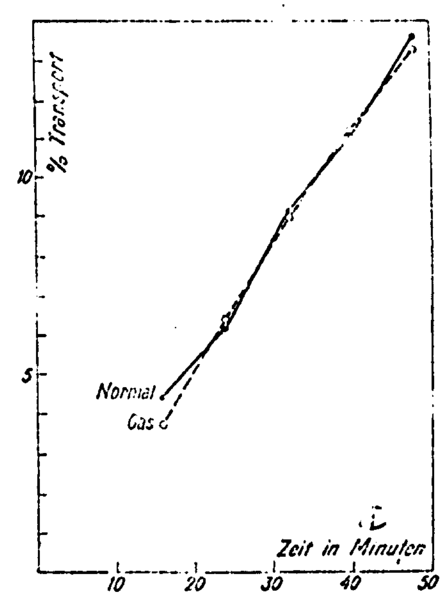


Fig.4. Velocity of Growth-Hormone Transport in Avena in the Case of Short Transport Period. 14 Feb 1939. Initial concentration 100°; Length of cylinder 2 mm. Additional Experiments may be found in Table 5. 1) Time in Min

Results: [a] From eight experiments with many different transport periods it was found that the transport was fully identical in normal and in gas-treated plants (Fig.4).

b. Also in the case of these 7 experiments I was unable to detect any differences either in growth-substance consumption or in long-lasting transport (Fig.5).

This proves unequivocally that ethylene has no influence in Avena on the growth-substance transport and growth-substance consumption.

Table 5. Growth-Hormone Transport in Avena, in Gas and in Pure Air Initial Concentration 100 %, Length of Cylinder 2 mm.

(1) Datum 1933		Transportzeit (3) 16 Min. %	24 Min. %	32 Min. %	40 Min. %	48 Min. %	Grenzwinkel (4)
7.2.	Gas	3,1	5,1	7,7	8,7	—	20,9°
	Normal	4,2	6,0	8,0	9,3	—	
8.2.	Gas	3,3	6,0	8,8	12,2	20,8	26,8°
	Normal	2,3	7,5	9,5	12,5	23,4	
13.2.	Gas	2,4	5,1	5,4	8,0	—	22,6°
	Normal	4,4	5,2	7,25	9,4	—	
14.2.	Gas	3,7	6,4	9,0	11,2	•13,3	23,4°
(2) Abb. 4	Normal	4,4	6,2	9,1	11,1	13,6	
15.2.	Gas	2,3	3,6	6,5	8,0	—	22,1°
	Normal	3,8	5,1	7,8	7,5	—	
17.2.	Gas	6,0	7,1	8,1	10,2	—	21,5°
	Normal	2,4	5,2	7,75	10,1	—	
20.2.	Gas	3,3	7,1	8,8	10,0	13,4	15,7°
	Normal	2,9	6,1	8,9	9,9	14,5	
21.2.	Gas	6,6	10,0	12,7	15,1	18,1	21,2°
	Normal	5,6	7,9	12,4	15,6	19,9	

1) Date 2) Fig.4 3) Transport Period 4) Critical Angle

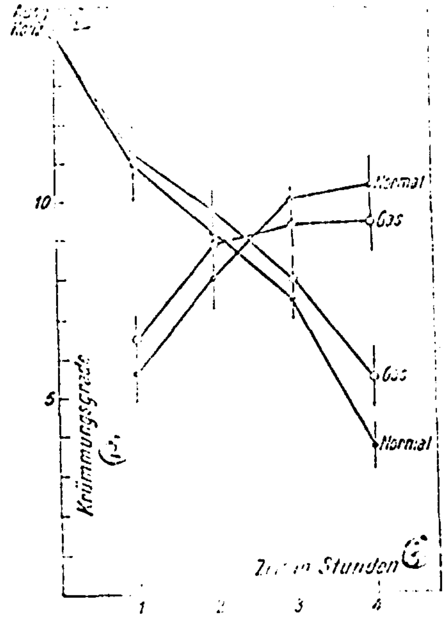
Hence the second possibility is not realistic.

The growth-substance transport is also independent of the amount of growth-substance supplied (Van der Woy, 1932), of the effect of the longitudinal component of gravity (Pfaeltzer 1934), and in addition, the effect of temperature is the same as in the case of other life processes. (Van der Wey 1932).

The growth-substance is thus very resistant to external factors, even when the latter have an effect on growth. Apparently it is one of the factors having a primary determining effect on the life phenomena of the plant. If one desired to determine the place occupied by the growth-substance mechanism in the series of plant-physiological processes, it is necessary in my opinion that he undertake first of all a thorough investigation of the connection between growth-hormone transport and metabolic processes, first of all respiration.

11. Determination of the Reactivity to Growth Substance

Experiments on this reactivity are not easy since it varies greatly, so that unequivocal experiments are hard to obtain (cf. Kogl 1933, Pfaeltzer 1934).



Key: 1) Initial Concentration 2) Degree of Bending
3) Time in Hours

Fig. 5. [illegible] of Growth-Hormone Transport and Growth-Hormone Consumption [illegible] Avena in the Case of Long Transport Period. Experiment of 3 Mar 1933. [illegible] of Cylinder 6 mm. Additional experiments are to be found in Table 6.

Table 6. Transport and Consumption of Growth Hormone in Avena. Initial Concentration 20-30°. Cylinders 6 mm. 1) Date 2) Transport Values in Degrees 3) After 1 hour 4) Initial Concentration 5) Maximum Consumption 6) Critical angle 7) Lower 8) Upper

Datum 1933		Transportwerte in Graden: Nach 1 St. unten	Nach 1 St. oben	1½ St. unten	1½ St. oben	2 St. unten	2 St. oben	2½ St. unten	2½ St. oben	3 St. unten	3 St. oben	4 St. unten	4 St. oben	Ausg. Konz.	Maxim. Verbrauch	Grenzwinkel
23.2	Gas	6,0	14,1	7,0	13,6	9,7	11,1	10,4	10,4	—	—	—	—	25,2°	4,9°	15,7°
	Norm.	5,75	14,3	6,9	13,6	8,7	12,1	10,6	9,8	—	—	—	—		4,5°	
23.2	Gas	—	—	7,25	9,0	—	—	7,2	6,0	—	—	—	—	19,5°	6,3°	—
	Norm.	—	—	7,3	9,2	—	—	8,5	5,8	—	—	—	—		5,2°	
1.3	Gas	5,3	11,7	—	—	7,3	6,25	—	—	9,25	4,8	—	—	24,7°	11,2°	—
	Norm.	8,4	9,2	—	—	7,4	8,4	—	—	11,25	5,0	—	—		8,5°	
3.3	Gas	6,5	11,25	—	—	8,9	9,8	—	—	9,4	8,0	9,5	5,5	20,2°	5,2°	18,8°
Abb. 5	Norm.	5,6	11,0	—	—	8,0	9,2	—	—	10,0	7,5	10,4	3,7		6,1°	
5.3	Gas	6,9	9,8	—	—	10,5	8,2	—	—	12,6	5,6	12,0	3,1	20,0°	4,9°	20,7°
	Norm.	6,7	9,0	—	—	9,7	8,0	—	—	10,4	3,6	11,4	2,0		6,7°	
14.3	Gas	—	—	3,3	8,2	7,0	6,8	9,0	4,7	—	—	—	—	20,2°	6,3°	20,3°
	Norm.	—	—	3,7	8,4	6,8	6,7	9,0	5,0	—	—	—	—		6,0°	
22.2	Gas	—	—	—	—	10,7	17,4	—	—	15,6	7,1	—	—	29,1°	6,4°	22,7°
	Norm.	—	—	—	—	11,8	16,0	—	—	16,8	8,8	—	—		3,5°	

First Method (see page 12): It was not possible to obtain a unif[illegible] pi[illegible] in this manner. The "gassed" plants often reacted more strongly[illegible] times, however, more weakly than the normal plants, or there was no difference at all. During these experiments I got the impression that the time of exposure to gas governs the reaction.

Second Method: With this experimental setup I was able to observe the influence of the gas during the onset of the reaction. Here it was clearly seen how matters stood: at first an increased growth set in, whereupon the reaction was more or less the same, and at the end the reaction of the gas-treated plants fell behind that of the normal plants (Fig.6).

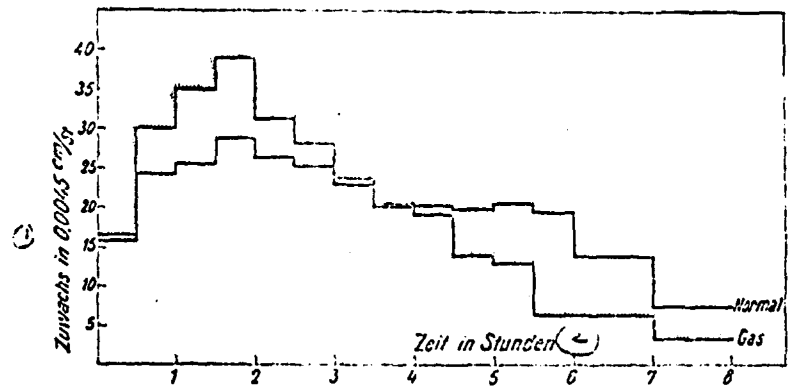


Fig.6. Ability to React to Growth Substance in Avena. Average Values of Five Experiments Performed on 24 Jul, 25 Jul, 27 Jul, 28 Jul and 31 Jul 33
1) Additional Growth in 0.0045 cm/hour; 2) Time in Hours

How is this increased growth to be explained? As a result of the gas effect the cells received, over a prolonged period, too little active growth substance, while on the other hand the supply of building materials continued. When the coleoptiles in this state are supplied a large amount of auxin, the latter initiates the longitudinal growth at an accelerated rate.

[illegible] role of growth substance as the initiating factor in cell growth is seen very clearly.

Summary of Experiments with Avena: Ethylene has a marked inhibitory effect on the growth of Avena coleoptiles. The elongation zone is inhibited to the greatest extent. The growth-substance production in gas-treated plants amounts to only 66% of that of normal plants. Ethylene injures the growth-substance production center in the tip.

The transport of growth substance as well as its consumption are completely uninfluenced.

The ability to react to a given amount of growth substance is temporarily enhanced; since, however, this factor is always subject to other con[illegible] (Kogl 19[illegible] Pfaeltzer 1934), I do not at this point wish to go i[illegible] more deeply.

[illegible] (1933) finds the following situation upon aging of Avena coleoptiles: The growth-substance yield remains the same, the transport increases, the growth-substance consumption increases, the reactivity decreases. Thus, these phenomena are precisely the opposite of those which I observed.

CHAPTER III

12. Studies with Different Dicotyledonous Sprouts

The following were planted in sawdust in the dark at 20°: Seeds of Phaseolus vulgaris, Vicia faba var. minor, Pisum sativum, Lepidium sativum, Lupinus angustifolius, Raphanus sativus.

After [illegible] days I placed the plants into similar sheet-metal boxes as those used in the Avena experiments, only their surface area was greater. Again one half of the plants were placed in the compartment with gas, the other half without gas. The compartments were aired daily, and fresh gas was given.

A considerable gas influence was noted throughout; still there were differences. The phenomena may be divided into several categories:

1) Horizontal nutation: A flat, straight growth tending slightly upwards, a smaller longitudinal growth, increased transverse growth. Here, too, we found the "triple response" in the sense of Knight and Crocker: epicotyls of Vicia faba minor and Pisum sativum.

2) The hypocotyl exhibits a strong thickening and becomes coiled like a pig's tail; at times the sprout again grows in the ground. They are short, abnormally developed plantlets: hypocotyls of Phaseolus vulgaris and Raphanus sativus.

3) The hypocotyl likewise thickens, the sprout nevertheless grows normally straight upwards and is only thicker and shorter: hypocotyls of Lupinus angustifolius and Lepidium sativum. Avena sativa behaves in the same manner.

All control plants which were also placed into sheet-metal boxes exhibited the well-known, stepped up long vertical growth of etiolated [illegible].

Thus at all times the longitudinal growth is injured and transverse growth is enhanced.

CHAPTER IV. EXPERIMENTS WITH VICIA FABA

13. General

After the determinations of Dijkman in hypocotyls of Lupinus, I tested the growth-substance conditions in epicotyls of Vicia. I always worked with etiolated plants whose anatomic details in comparison with normal pl... ... exhaustively described by Priestley (1926).

In the last few years Snow (1925-1933) has thoroughly investigated, in these sprouts, the influence of the tip on the sprouting of axillary buds. He observed the influence of a substance, very likely a growth substance, which inhibits the sprouting of buds in intact sprouts; when the growth substance supply was destroyed by decapitation, the buds burgeoning out quickly. Thus in this case the growth substance had an inhibitory effect.

Thimann and Skoog (1934) determined, by means of Went's method, the growth-substance content of plantules of Vicia faba. Especially in the end bud is there a large amount of growth substance. The resting axillary buds contain almost none; the budding ones, however, do contain growth substance. Thimann and Skoog succeeded in blocking the burgeoning of axillary buds by supplying the decapitated plants with artificial auxin (obtained from Rhizopus). Thus in this case the growth substance blocks gr... ... for the time being there is still the possibility that a role is played by an inhibitory substance.

We know today, thanks to the studies of Cholodny (1927) and Boysen Jensen (1933), that the growth hormone inhibits growth also in roots (cf. also Gorter 1932). Thus the growth substance is a material with a strongly regulatory effect on cell elongation, both in a positive and a negative sense. The above-described work by Heyn, relating exclusively to Avena, cannot as yet be considered as having a general validity. The question is still open how the growth-substance effects the plasticity of the root membranes. A more intensive study would be very desirable in this respect (see, however, Heyn, 1934).

In this work, however, I would like to limit myself to the influence of ethylene. For my studies I chose the epicotyls of Vicia faba var. minor (Mansholt's broad beans). In gas the sprouts exhibit the noteworthy behavior of not rising through the surface but boring through the sawdust and remaining horizontal (Fig.7). I have attempted to subject this phenomenon to analysis.

The seeds were softened under water for about 36 hours and then placed in wet sawdust in earthen vessels. Seven-day-old plants were best suited for the experiments. They are grown in the dark chamber under constant conditions, but already after two days the vessels were placed in two large boxes, one of which was always supplied with some ethylene. As already stated, the boxes were situated not in a room with constant temperature but in a thermostat.

14. Method of Growth Determination

One day before the beginning of the experiment etiolated normal sprouts were planted in one row in zinc vessels with sawdust, and provided with tin-foil labels. Two zinc boxes were provided with a glass window on the front and back; into each of these boxes were placed 4 plants.

Then I fastened the boxes firmly to the table of the cathotometer already employed by [illegible] verbeck (1933; Figs. 3 and 4, pp 555-556). The method of reading may be found in the latter reference.

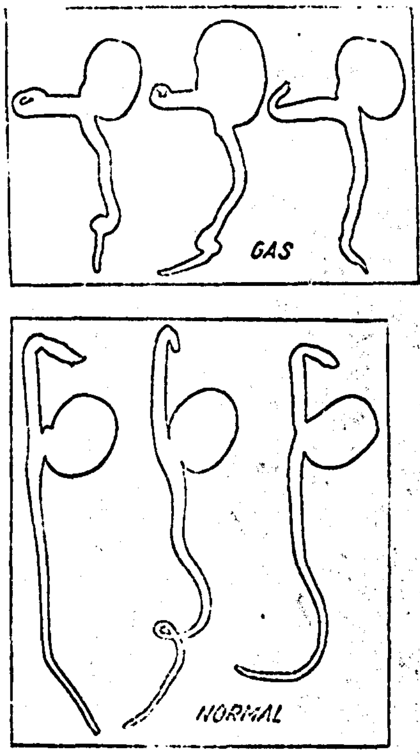


Fig.7. Horizontal Nutation in Vicia Faba

One of the boxes was supplied with a little ethylene, so that it was again possible to determine simultaneously the growth in gas and in pure air.

19. Method of Determination of Growth-Substance Production

It was investigated whether the epicotyls contain growth substance. For this purpose I cut 7 mm long pieces from the epicotyls, placed them for a few minutes on wet filter paper, and with their [illegible] side down on an agar block (2x2x0.9 mm). Each experimental series contains at least 18 plants, with a view to the variability of the material.

[illegible] elimination of the epicotyl pieces the agar blocks were placed, with a [illegible] of water, unilaterally on decapitated Avena coleoptiles. [illegible] of the coleoptiles gave the value of the growth-hormone content of the epicotyl piece.

16. Method of Determination of Growth-Substance Distribution

As in the preceding experiments 7 mm long epicotyl cylinders were cut out of 7-day old etiolated plants. According to Dijkman (1934, p 407), I placed them on a horizontally placed razor.

Since it had been found in the experiments on growth-hormone production (see section 18) that the epicotyls contain very much growth substance, it was necessary -- in contrast to Dijkman's procedure -- to apply artificial auxin at the free end of the cylinders. Besides I could determine the growth-substance content of the agar blocks already after a quarter of an hour, and so I could investigate whether a unilateral distribution in the sense of Dolk had set in. After this first quarter hour the agar blocks were placed quickly into a moist chamber until the performance of the growth-substance determination, and the epicotyl cylinder was provided with new, pure agar blocks. These, too, stood for 1/4 hour on the epicotyl pieces and then again replaced by new ones. This procedure was repeated several times until I acquired an idea regarding the time in which the unequal distribution set in. It was possible in this way to determine the geotropical presentation time.

At the same time the presentation time is determined in the usual manner, i.e., the time in which a bending takes place which is visible to the naked eye. For this purpose I planted nine plantules in a [illegible] and placed them horizontally in front of a screen covered with millimeter graph paper. Their position was read every 1/4 hour.

17. [illegible] of Growth (Fig.8)

The growth could be determined only every 30 minutes; the inhibition, however, certainly set in only during the second half hour of the influence of the gas; the growth of the gas-treated plants thereafter remains uniformly small for several hours, but does not completely disappear. The similarity with the curves given by Avena (Fig.3) are striking.

A measurement of the growth of the zones did not reveal anything particular. The inhibition of the growth occurred everywhere simultaneously and to the same extent.

At decapitation the epicotyls grow less strongly, but it takes quite long until the growth ceases entirely. The long-lasting growth may perhaps be explained by the large growth-substance stock of the epicotyl.

18. Determination of Growth-Substance Production

Already Thimann and Skoog (1934) showed that the epicotyls of Vicia Faba contain an extraordinary amount of growth substance. It was immaterial from which zone (Fig.9) I cut the cylinders out of 7-day plants; I always obtained a measurable amount of growth substance already after 1/4 hours

Table 7: 29 Mar 1933

Extraktionszeit: (1)			Anz. Pfl.	½ Stunde (2)	Anz. Pfl. (3)
Zone	1	[illegible] ± 0,8	14	13,7 ± 0,9	15
	2	[illegible] ± 0,7	18	14,3 ± 1,3	14
(4) Siehe Abb. 9	3	6,0 ± 0,9	16	11,5 ± 1,2	14
	4	6,0 ± 1,0	11	12,6 ± 0,9	15

1) [illegible] of Extraction 2) Hour 3) No of Plants 4) See Fig. 9.

However, [illegible] week old plants display in the basal regions, a decrease of growth-substance content:

Table 8; 10 Oct 1933

	Pfl. 6 Tage (5)	Anz. (6)	Pfl. 10 Tage.	Anz. (6)
Spitzenzone ... (1)	14,4 ± 1,3	14	16,5 ± 1,0	13
Mittlere Zone . (2)	14,7 ± 1,1	16	10,3 ± 0,8	15
Basale Zone .... (3)	13,9 ± 1,1	17	5,8 ± 1,3	11
Extr. Zeit ½ Stunde (4)				

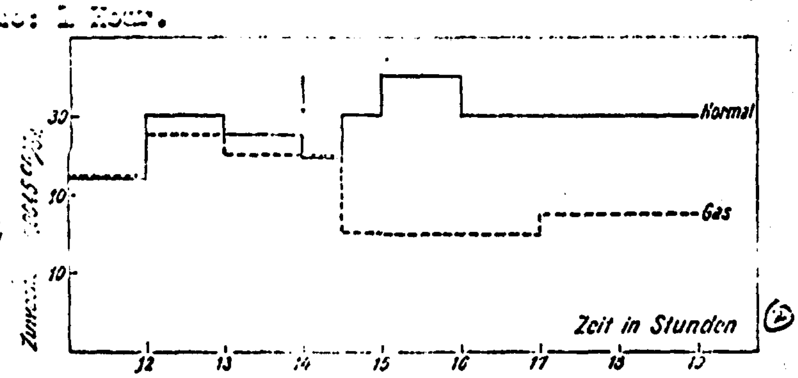
1) Tip [illegible] 2) Middle Zone 3) Basal Zone 4) Extraction Time 1/2 hour 5) Plan[illegible] 6 Days 6) Number

When the cylinder is placed inversely, then the yield of growth hormone is strongly decreased:

Table 9; 6 Oct 1933

Inverse	4.8 ± 0.6	18 plants
Normal	20.7 ± 1.5	15 plants (Critical Angle)

Extraction Time: 1 Hour.



[illegible] Growth in Vicia. Arrow indicates that at 14 Hours Gas was [illegible] in 0.0045 cm/hour; 2) Time in Hours

Fig. 9

In the case of Vicia faba, too, there apparently occurs only a basipetal transport.

The occurrence and distribution of the growth hormone of Vicia faba is fully comparable with the data of Dijkman in the hypocotyl of Lupinus albus (1933, p 112). Here, too, there is no firmly circumscribed growth-hormone center. However, Vicia furnishes 2-3 times more active growth substance than Lupinus.

The influence of the seasons is significant also in the darkroom: in winter the growth-substance production attains only one half of the summer production:

Table 10
Differences in Growth-Hormone Production in Summer- and Winter Plants

Date	Extraction Time	Growth-Hormone Content	Number of Test Plants
26 Sep 1933	1/2 hour	15.2 ± 0.5°	12
12 Dec 1933	1/2 hour	7.5 ± 0.8°	12

What is the situation of growth-substance production in ethylene? The yield of active growth substance is very strongly decreased by 0.0005% ethylene. Only a very small amount remains (Table 11).

When the "gassed" plants are afterward placed in pure air, a partial regeneration takes place; 8 Dec 1933: "gassed" plants, for 24 hours again in fresh air: ext. time 1 hour: 4.1 ± 0.9°; 18 plants.

Table 11. Growth-Hormone Production in Gas and in Pure Air in Vicia Faba

Datum 1933 (1)	Production (2)			(3) Anz. Pfl.	Extraktionszeit in Stunden (4)
	in Gas	Anz. Pfl.	Normal		
23.10	1,4 ± 0,2	35	12,5 ± 0,6	18	1
7.11	2,9 ± 0,3	36	22,7 (Grenzw.) (5)	32	5
8.11	6,5 ± 0,6	48	19,6 (Grenzw.)	45	4
14.11	9,8 ± 0,9	31	25,1 (Grenzw.)	28	4
15.11	7,2 ± 0,8	48	20,7 (Grenzw.)	42	4
24.11	6,4 ± 0,9	16	21,2 (Grenzw.)	15	4

1) Date 2) Production 3) No of plants 4) Extraction Time in Hours 5) Critical Angle

Even more significant is the following experiment:

Table 12: 12 to 15 Dec 1933: Extr. Time 1 Hour

(1) Normale Pfl.	15,0 ± 1,6	12 Pfl.
(2) Nach 24 St. im Gas	4,0 ± 0,8	17 „
„ 48 „ „ „	4,6 ± 1,0	17 „
(3) Gaspfl.	4,0 ± 0,4	12 Pfl.
(4) Nach 24 St. im reiner Luft	10,0 ± 0,9	12 „
„ 48 „ „ „ „	13,6 ± 1,1	14 „

1) Normal Plants 2) After 24 hours in gas 3) "Gassed" Plants 4) After 24 hours in Pure Air Pfl = Plants

The standard (Kogl 1933) of the reactivity of Avena coleoptiles is determined every day; the bendings occurring in the above experiment on different days are then recalculated for this standard.

When does the marked inhibition in growth-substance occur in the gas? A large number of sprouts are grown simultaneously; one quarter of them are analysed for their growth-substance content after 5 days, the other quarter are placed in the gas for 3, 5 and 9 hours. All analyses were carried out simultaneously:

Table 13: 18 Dec 1933

1.	Extraction Time 1 Hour:	7.6 ± 0.8	15 plants
2.	" " 1 Hour:	2.0 ± 0.4	17 " (3 Hours in Gas)
3.	" " 1 Hour:	2.0 ± 0.5	15 " (6 " " ")
4.	" " 1 Hour:	2.2 ± 0.4	18 " (9 " " ")

Thus after three hours the inhibition is already complete; it remains the same during the next hours. The next experiment shows that the maximal inhibition is attained already after the first hour:

Table 14. 20 Dec 1933

1. Extraction Time: 1 Hour	6.7 ± 0.8	11 plants		
2. " " 1 Hour	1.8 ± 0.6	12 "	(1 Hour in Gas)
3. " " 1 Hour	1.2 ± 0.4	12 "	(2 Hours " ")
4. " " 1 Hour	1.8 ± 0.6	13 "	(3 Hours " ")

I did not succeed in establishing the time at which the inhibition sets in with an even greater accuracy. These results, however, are fully comparable with the growth curves: growth and growth-substance content go hand in hand even here. All these conclusions, however, are only qualitative, since the growth inhibition by the gas is relatively much smaller than the decrease (almost an elimination) of the growth-substance content.

19. Determination of Transport and Distribution of Growth Substance

Already Dijkman (1934, p 420) described a few experiments on the connection between the time during which the effect of gravity is exerted and the distribution of growth substance. He determined, after one-half, 1, 2 and 3 hours, the differences between the amount of growth substance in the upper and lower halves of the horizontally placed hypocotyl cylinders of Lupinus albus. It was found that the difference in the first half hour still lay within the limits of error, but that after one hour a unilateral distribution sets in most clearly. The values of Dijkman, however, are not very regular, and have been obtained with very different numbers of plants; hence they need a more thorough confirmation. Thanks to the high growth-substance content proper to my material I was able to make this confirmation.

I determined the difference in growth-substance content in the upper and lower side every 15 minutes. It may be seen from Table 15 and the graphical representation (Fig.10) that the unilateral distribution of the growth hormone sets in in the third quarter hour after horizontal placement; the percentual distribution remains the same during the next hours; this was the conclusion reached already by Dijkman on the basis of his figures. If we measure the geotropic presentation time in the usual manner then the figures obtained are the same; here, too, I observed the first differences in growth between upper- and lower side in the third quarter hour. After this period the angle of deflection still increases, but the amount of increase in deflection per quarter hour remains approximately the same during the next hours; it may be compared with the curve of growth-substance content after the onset of the unequal growth-substance distribution. This then is a further support of the growth-hormone theory of geotropism, as originated by Cholodny-Dolk (1927-1930) and expanded by Dijkman.

But let us return to the discussion of the influence of gas. A determination of the growth-substance content of the upper and lower half of the cylinder, in the case of gas-treated plants, was difficult due to their

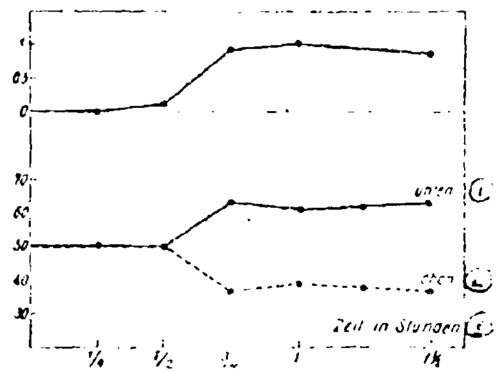


Fig.10. Relationship between unequal growth-hormone distribution and the increase in bending of geotropically stimulated epicotyls of Vicia Faba. The upper curve shows the increase in bending in degrees; the lower the growth-hormone content of the upper- and lower side in percent. 1) Upper 2) Lower 3) Time in Hours

Table 15. Appearance of Unilateral Growth-Hormone Distribution after Horizontal Placement. Only the Percentage Figures of the Growth-Hormone Values are shown. In each determination 14-18 plants were used. O = Upper side; U = Lower side. 1) Date 2) Average Value; Nach = After.

(1) Datum 1933	Nach 15 Min.		Nach 30 Min.		Nach 45 Min.		Nach 60 Min.		Nach 75 Min.		Nach 90 Min.	
	U	O	U	O	U	O	U	O	U	O	U	O
6.10.1.	52	48	46	54	62	38	—	—	—	—	—	—
II.	—	—	48	52	—	—	59	41	—	—	61	39
9.10.	—	—	50	50	60	40	63	37	61	39	—	—
11.10.	50	50	—	—	63	37	58	42	67	33	—	—
17.10.	—	—	49	5	—	—	56	44	60	40	—	—
23.10.	—	—	—	—	—	—	69	31	—	—	—	—
27.10.	48	52	57	43	67	33	—	—	59	41	65	35
(2) Mittelwerte:	50	50	50	50	63	37	61	39	62	38	63	37

small growth-hormone content. Nevertheless I obtained the following data:

Table 16. Unilateral Distribution of Growth Substance in Gas-Treated Plants in the Horizontal Position

Datum 1933 [1]	Wuchsstoffverteilung [2] Unterseite [5]	Wuchsstoffverteilung [2] Oberseite [6]	Zahl der Pflanzen [3] Unterseite	Zahl der Pflanzen Oberseite	Extraktionszeit in Stunden [4]
20.10.	1,4 ± 0,4	4,1° ± 0,7	18	16	2
7.11.	0,9 ± 0,3	2,0 ± 0,3	16	20	5
8.11.	2,5 ± 0,5	4,0 ± 0,6	23	25	4
14.11.	4,1 ± 0,7	5,9 ± 1,1	18	13	4
15.11.	3,1 ± 0,6	4,1 ± 0,6	25	23	4
Total	12,0	20,1	100	97	

1) Date 2) Growth-Hormone Distribution 3) Number of Plants 4) Extraction Time in Hours 5) Lower Side 6) Upper Side.

Thus the upper half always contains most of the growth substance. The value of the absolute difference should not be taken too high, since it is perhaps still a little too large due to the very small angle of bending. However, I believe nevertheless to have detected a difference.

20. Influence of Gravity on the Horizontal Growth of Sprouts of Vicia

Neljubow (1911) believed to have been able to show that the horizontally grown gas-treated plants always seek the horizontal position when exposed to gravity. In this way he comes to the assumption that the horizontal nutation -- in the sense of the term used by Wiesner -- is not autonomous but that there occurs a transverse geotropism.

It appeared to me fitting to undertake another study of the geotropic behavior of Vicia plants in gas.

To this end I planted Vicia faba seeds in the zinc containers used otherwise for growing Avena coleoptiles, and exposed one half of the plantules to gas; the plants were again divided into 3 series:

1) Horizontal stand;
2) Vertical stand: the side one which the sprout appears is directed upwards;
3) Same as 2, except this side is directed downwards.

The plants received, respectively, fresh air and fre gas every day. After 8 days the plants were taken from the sheet-metal boxes, stuck on photographic plates by means of needles and a silhouette was prepared. Figs 11a and 11b show the drawings prepared after these silhouettes were taken. An effect of geotropism on horizontal nutation is not observable.

Results of Experiments with Vicia Faba. The growth of Vicia faba plantules is strongly reduced by ethylene. The inhibition extends evenly to all zones.

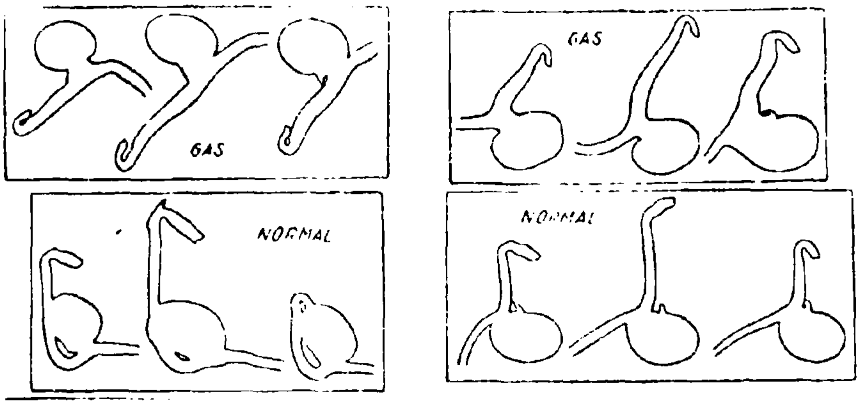


Fig.11a Fig.11b

Influence of Direction of Gravity on Horizontal Nutation

The epicotyls contain a very large amount of growth substance; in young plants the same amounts in all zones; in older plants less in the lower zones. In ethylene the yield of growth substance is very greatly reduced, so that only a very small amount remains.

The unilateral distribution of the growth hormone and the geotropic bending begin at the same time, in the third quarter hour after the beginning of the geotropic stimulation.

Under the influence of ethylene the growth substance remaining in the gas-treated plants is distributed unilaterally; the upper half contains most of the growth substance.

CHAPTER V

21. Summary of Results and Conclusions to be Drawn from Them; Theory

Briefly summarised, the results are to be formulated as follows:

A. Avena

1. Ethylene reduces longitudinal growth (section 8)
2. Ethylene reduces growth-substance production (section 9)
3. Ethylene enhances transverse growth (section 8)
4. Ethylene has no influence either on the growth-substance trans port or on the growth-substance consumption (section 10)
5. Ethylene has a transitory promoting effect on the ability to react to growth substance (section 11)

B. Vicia

6. Ethylene causes a horizontal growth uninfluenced by gravity (sections 17 and 20)

7. Ethylene very sharply reduces the yield of growth substance (section 18)
8. Ethylene causes a distribution of the remaining growth substance in such a manner that the upper side receives the greatest amount (section 19).

C. General

9. Ethylene does not change the least the effectiveness of a directly applied auxin solution (see p 32).

What conclusions can we draw from these facts? In regard to (1) and (2): It was found that there sets in, simultaneously with the already well-known strong inhibition of longitudinal growth also a significant reduction of the yield of active growth substance. Already F.W. Went (1928) has established that without growth substance no longitudinal growth is possible. I say "longitudinal" intentionally, since as can be seen from point 3, there is observed a very great transverse growth. This holds true also for gas-treated Vicia plantules which contain only very small amounts of growth substance. Apparently the transverse growth is much more independent of the growth-substance content than is longitudinal growth.

How do the cells change when this transverse growth occurs? We note, on sections (see Section 8, above) wide, short cells with thick, layered walls; apparently no increase in plasticity has taken place (see Heyn 1931). However, there did occur an increase in the substance of the cell walls (through apposition or intussusception).

This confirms also the view of Heyn that on action of growth substance on cell growth the primary change is in the plasticity of the cell wall, and only later does the overextended membrane get fixed through intussusception of new particles. On the contrary, Soding (1934) assumes on the basis of his experiments with stretching organs that in cell stretching there sets in immediately an intussusception growth. Since, however, it is seen from the present experiments that in ethylene no elongation sets in as a result of the damage to the growth-substance production, but that there does occur a transverse growth through intussusception, Soding's view appears to me doubtful at the least.

The supply of new building materials from the roots to the sprout cells is apparently not too much disturbed by ethylene; this is proved by the fact that gas-treated plants exhibit a strong guttation and display no particular transpiration disturbances. Because of the increase in the substance of the cell walls the sprouts become thicker, and this way the ability for elastic stretching and the elastic stretching itself are decreased (see Heyn 1931). The cell stretching which normally renders the walls longer and thinner, practically does not set in, due to the strongly reduced amount of active growth substance, a condition brought about by the ethylene. The cells remain short and thick; the position is stabilized and a subsequent supply of growth substance does not bring about much change in these cells. Nevertheless I was able to detect a tendency toward equalization. The transitory enhancing effect of ethylene on the ability to react to growth substance (point 5) shows that the reduced cells react to the administration of artificial growth substance with an exaggerated cell elongation.

This reaction may be interpreted as an attempt to bring about normal conditions again.

Thus in the case of Avena the damages brought about by ethylene gas may be attributed to an inhibition of growth-hormone formation.

Ethylene strongly restricts the effect of growth hormone. Under these very strongly altered conditions the plantule of Vicia faba grows horizontally and no longer reacts to the effect of gravity (point 6).

The sprout of plantules of Vicia faba is formed, in principle, dorsiventrally, but it behaves orthotropically (Benecke-Jost, Pfl. Phys. (Plant Physiology), 4th Edition, 1923, Vol 2, p 284). The dorsiventrality bendings are completely eliminated by the strongly negative geotropism. Because of the influence of gas the geotropic sensitivity (point 7) disappears simultaneously with the greater part of the active growth substance. Without a large amount of growth substance no geotropism is possible here. Now the dorsiventrality which up to then was suppressed, manifests itself, and the horizontal nutation sets in. The remaining growth hormone is unilaterally distributed in the horizontal sprouts (point 8), and the dorsal side receives most of it.

This state may be best interpreted by the following picture: At high water a river bed is completely filled up; we see a uniform, even water surface. Nevertheless there is a sand bank on the botton. When the water in the river bed is considerably reduced, the water will flow only on one side of the river bed; the sand bank will remain dry. It is the same way in the case of the horizontal nutation of our plantules. The one-sided growth-substance flow sets in only when the main current is dammed up on account of the influence of the gas.

Thus we may consider the horizontal nutation — in agreement with the older views of Wiesner — as an autonomous epinasty which is a priori determined by an inherent dorsiventrailty (see, among others, Rawitscher 1925, 1932).

Von Guttenberg (in Fortschr. d. Bot. 1932) and his students Hennings and Freytag (1931) give the following explanation for the so-called autonomous or inherent epinasty of dorsiventral plant parts. After discussing the work of Miss Uyldert, he says (quoted from p 241):

"The explanation seems to lie in the fact that the dorsiventrality of these organs is not only external but also internal (plasmatic), since on its part one of the dorsal sides allows the passage of growth substance exclusively or predominantly. The epinasty must then always occur when there is an absence of induced polarity, which is produced e.g. by gravity light, and it is eliminated the moment when polarization is produced from the outside."

The behavior of the sprouts of Vicia faba under the influence of ethylene fits very well into this scheme. By means of ethylene it is possible to investigate the autonomous, inherent epinasty which otherwise is hidden.

In Vicia the very small amount (0.0005%) of ethylene eliminates the effectiveness of the growth substance almost completely; the growth substance is present only in traces; the possibility that the ethylene might decompose the growth substance in a purely chemical manner comes to mind, but this is nonsensical from the chemical point of view. Nevertheless I passed ethylene through one half of an auxin solution for three hours; the other half remained untreated for control purposes. The result was:

Ethylene platelets	8.7 ± 0.4	19 plants;
Controls	9.1 ± 0.6	18 plants.

Hence a direct influence of ethylene on auxin was not detectable. This was to be expected after the experiments in section 11: there, too, the gas does not cause any damage to the auxin in the block.

Is it possible to make a uniform representation of the e[illegible]t of ethylene on plants? The effect on the longitudinal growth is to be ascribed in any event to the effect on the yield of active growth substance. Further, on the basis of my findings even the marked horizontal growth becomes understandable. However it was mentioned in the Introduction that there exist other, as yet uninvestigated gas effects. Nevertheless I can say a little more about this than was possible in the Introduction.

The epinasty of grown leaves in ethylene (Crocker, Zimmerman and Hitchcock 1932) is to be ascribed to the same causes as the horizontal nutation; the latter, in fact, is based on an epinasty. It was shown already by Schwarz (1927) that here we were dealing with an increased growth of the upper side of the leaf stalk, and Crocker et al confirmed this. The experiences acquired with plantules have shown me that there the gas damages are the consequence of a lack of growth substance. If it should turn out that in the case of grown leaves in ethylene the phenomenon of epinasty is likewise determined by a lack of growth substance -- and as long as no inhibitory substance has been detected, I consider this very probable -- then here a stronger growth should occur upon reduction of the growth-substance reserves; in other words, under normal circumstances the growth-substance keeps a tight rein on leaf epinasty. Then there would exist here a similar situation as in the axillary buds of Vicia (Snow, Thimann and Skoog): the growth substance is not only the "growth-initiating stimulus" as has been thought before, but this plant hormone often has a regulatory function: it keeps the various plant-physiological processes in equilibrium.

The rest of the ethylene effects are more difficult to interpret with our current knowledge. The shortening of the rest periods and the accelerated fruit ripening are effects produced on enzymatic processes whose growth-substance conditions -- provided they exist at all -- are as yet completely unknown.

In my opinion the following points are of importance in regard to the problem of the formation of growth substance in the cells:

1) Very small amounts of ethylene very considerably inhibit, in epicotyls of Vicia faba, the yield of active growth hormone. These strong inhibitions caused by traces of a poison often take place in connection with enzymatic processes. I mention, e.g., the complete inhibition of the effect of respiratory enzymes by traces of HCN, H_2S, CO and other, so-called specific inhibitory substances (Warburg 1921, 1925). Since the property of ethylene absorption is also known (Nord and Franke 1927), perhaps we should undertake a comparison of inhibitions produced in this way and the effects on growth-hormone production described in this work.

2) Bonner (1933) showed that these Warburg inhibitions take place also in coleoptile cylinders which elongate under the influence of growth substance. According to his data, the effect of the growth substance on growth is determined, among other things, by an increase in respiration.

3) Very small amounts of ethylene act, apart from the yield of growth substance, also very strongly on the ripening of fruit and the length of rest periods; both are enzymatic processes. The same holds true for the fermentation of tobacco leaves, which likewise is accelerated by ethylene (Rossi 1933).

4) Ethylene forms complex compounds with inorganic catalysts, namely some heavy metals.

In my opinion all these points indicate the fact that the formation of growth substance in the cells is based on an enzymatic process.

SUMMARY

A review of the factual matter established in this work may be found on p 29. From this we can conclude that in the case of Avena the effect of ethylene hits particularly the growth-substance production, and hence the longitudinal growth.

The horizontal nutation in Vicia faba is caused by an autonomous epinasty which is manifested only when the geotropic sensitivity is weakened, due to the effect of the gas. A similar phenomenon takes place, perhaps, in the epinasty of grown leaves.

The hypothesis is expressed that the formation of growth substance is based on an enzymatic process.

I cannot end this paper without expressing my most heartfelt thanks to my highly esteemed teacher, Prof Dr F.A.F.C. Went, for his outstanding guidance in this study.

BIBLIOGRAPHY

Abderhalden, E., Handbuch der physiologischen Arbeitsmethoden (Handbook of Physiological Work Methods) I, 4, 36.

Benecke-Jost, Pflanzenphysiologie (Plant Physiology), 4th Edition, Vol II, p 284. (1923)

Bonner, James (1933): Studies on the Growth Hormone of Plants, IV. On the Mechanism of the Action. Proc. Nat. Ac. Scie. 19, No 7, 717.
--- (1933): The Action of the Plant Growth Hormone. Journ. Gen. Phys. 17, 63.

Boysen Jensen, P., (1913): "Conduction of Phototropic Stimulation in Avena Coleoptiles." Berichte d. deutsch. Bot. Ges. (Bulletin of the German Botanical Society) 31, 559.

--- (1933): "Detection of Growth Substance in Roots." Planta 19.

Buy, H.G. du, (1933): "Growth and Phototropism in Avena Sativa." Rec. Trav. Bot. Neer. 30, 798.

--- and Nuernbergk, E., (1932): "Phototropism and Growth of Plants." Part I. Supplement. Biol. 9, 358.

Chace, E.M., and Denny, F.E., (1924): Use of Ethylene in the Coloring of Citrus Fruit. Ind. Eng. Chem. 16, 339.

Cholodny, N., (1927): "Growth Substances and Tropisms in Plants." Biol. Zentr. Bl. 47, 604.

--- (1929): "Growth of the Vertically and Horizontally Oriented Stem." Planta 7, 702.

--- (1930): "Microphotometric Studies on the Growth and Tropisms of Coleoptiles of Avena Sativa." Jahrb. fur wiss. Bot. (Yearbook of Botany) 73, 720.

--- (1931): "Physiology of Plant Growth Hormone." Planta 14.

Crocker, W., Zimmermann, P.W., and Hitchcock, A.E., (1932): Ethylene-Induced Epinasty of Leaves and the Relation of Gravity to It. Contrib. Boyce Thomps. Inst. 4, 177.

Davis, W.B. and Church, C.G., (1931): The Effect of Ethylene on the Chemical Composition and the Respiration of the Ripening Japanese Persimmon. Journ. Agric. Res. 42, 165.

Denny, F.E., (1924a): Hastening of Coloration of Lemons. Journ. Agric. Res. 27, 757.

--- (1924b): Effect of Ethylene upon Respiration of Lemons. Bot. Gaz. 77, 322.

--- (1926): Hastening the Sprouting of Dormant Potato Tubers. Americ. Journ. of Bot. 13, 118.

Dijkman, M.J., (1934): "Growth Substance and Geotropic Bending in Lupinus." Rec. Trav. Bot. Neerl. 31, 391.

Dolk, H.E., (1930): "Geotropism and Growth Hormone" Dissertation, Utrecht.

Doubt, Sarah L., (1917): The Response of Plants to Illuminating Gas. Bot. Gaz. 63, 209.

Erlenmeyer and Bunte (1873): Liebig's Annalen d. Chem. 168, 64.

Freytag, H. (1931): "Studies on the Plagiotropism of Tropaeolum Leaves." Planta 12, 267.

Girardin, J.P.L., (1864): "Influence of Illuminating Gas on Trees in Promenades and Streets." Jahresber. Agrik. Chem (Yearbook of Agricultural Chemistry) 7, 199.

Gorter, C.J., (1932): "Problems of Growth Substance in Roots." Dissertation, Utrecht.

Guttenberg, H. von. (1932) in "Fortschritte der Botanik" (Advances of Botany) published by F. von Wettstein, Berlin.

Harvey, E.M., (1915): Some Effects of Ethylene on the Metabolism of Plants. Bot. Gaz. 60, 193.

Harvey, R.B., (1925): Blanching Celery. Minn. Agric. Exp. Sta. Bull. 222.

Hennings, F., (1931): "Studies on the Plagiotropism of Coleus Axillary Buds." Planta 12, 239.

Heyn, A.N.J., (1931): "Mechanism of Cell Elongation." Rec. Trav. Bot. Neerl. 28, 113.

--- (1934): "Plasticity of Cell Membranes under the Influence of Growth Substance." Proc. Kon. Akad. Wet. Amst. (Proceedings of the Royal Academy of Sciences, Amsterdam) 37, 180.

--- and Overbeck, J. van (1931): "Further Experiments on the Plastic and Elastic Expandability of the Cell Membrane." Proc. Kon. Akad. Wet. Amst. 34, 1190.

Jaccard, P., (1893): "Influence of Gas Pressure on the Development of Plants." Rev. Generale de Bot. (General Review of Botany) 5, 289.

Jordan, H.J., and Hirsch, G.C., (1927): Ubungen aus der vergleichenden Physiologie (Experiments in Comparative Physiology). Berlin.

Knight, L.J., and Crocker, W. (1913): Toxicity of Smoke. Bot. Gaz. 55, 337.

Kny, L., (1871): "Influence of Illuminating Gas on Tree Vegetation, and its Testing." Bot. Zeit. 29, 852; 867.

Kogl, F., (1933): "On Auxins." Zeitschr. f. angew. Chem. 46, 469.

----, Haagen Smit, A.J., and Erxleben, Hanni (1932): "On a Plant Hormone of Cell Elongation. Preparation of Pure Auxin from Human Urine." Ztschr. f. physiol. Chem. (Journal of Physiological Chemistry), 214, 241.

Kostyschew-Went, (1931): Lehrbuch der Pflanzenphysiologie (Textbook of of Plant Physiology), Vol II, Berlin.

Mack, W.B. (1927): The Action of Ethylene in Accelerating the Blanching of Celery. Plant Physiology 2, 103.

---- and Livingstone, B.E. (1933): Relation of Oxygen Pressure and Temperature to the Influence of Ethylene on Carbon Dioxide Production and on Shoot Elongation in very young Wheat Seedlings. Bot. Gaz. 94, 625.

Malisoff, W., and Egloff, G., (1919): Ethylene. Journ. of Physical Chem. 23, 65.

Molisch, H., (1911): "Influence of Tobacco Smoke on Plants." Sitz. Ber. K. u. K. Akad. Wien (Proceedings of the Imperial and Royal Academy of Vienna) 120, I, 3, 813.

Neljubow, D., (1901): "On Horizontal Nutation of the Stem of Pisum Sativum and a Few Other Plants." Beih. Bot. Centralbl. 10, 128.

---- , (1911): "Geotropism in the Laboratory Air." Ber. deutsch. Bot. Ges. 29, 97.

Nord, F.F., and Franke, K.W., (1927): On the Mechanism of Enzyme Action: II. Further Evidence Confirming the Observation that Ethylene Increases the Permeability of Cells and Acts as a Protector. Journ. Biol. Chem. 79, 27.

Overbeek, J. van, (1933): "Growth Stuff, Growth Reaction to Light and Phototropism in Raphanus." Rec. Trav. Bot. Neerl. 30, 539.

Paal, A. (1919): "On Phototropic Stimulus Conduction." Jahrb. f. wiss. Bot. 58, 406.

Pfaltzer, J.W., (1934): "Longitudinal Energy, Growth Substance and Growth in Avena Sativa Coleoptiles." Dissertation, Utrecht.

Priestly, J.H., (1926): Light and Growth. New Phytol. 25, 145.

Rawitscher, F., (1925): "Contributions to the Theory of Plagiotropism." Zeitschr. f. Bot. 17, 212.

--- (1932). Der Geotropismus der Pflanzen. (Plant Geotropism), Jena.

Rocoimbal, R.O., Vacha, G.A., and Harvey, R.B., (1927): The Effect of Ethylene on the Respiration of Bananas During Ripening. Plant. Physiol. 2, 357.

Richter, O., (1903): "Plant Growth and Labora ory Air." Ber. d. deutsch. Bot. Ges. 21, 180.

--- (1906): "On the Effect of Impure Air on Heliotropism and Geotropism." Sitz. Ber. K. u. K. Akad. Wien, 115, I. 1.

--- (1910): "Horizontal Nutation." Idem, 119, I. 1

Rossi, U., (1933): "The Ethylenation of Tobacco." Bollet. Tecn. Ist. Sper. "Leonardo Angeloni" Scafati (Bulletin of the "Leonardo Angeloni" Technical Institute for Research) 30, 223. (Resume in French).

Sachs, J., (1874): Lehrbuch der Botanik (Textbook of Botany), 4th Edition, pp 828-829.

Schmitz, H. (1933): "On Growth Substance and Geotropism in Grasses." Planta 19, 614.

Schwartz, Hanna (1927): "Influencing of Growth by Gaseous and Liquid Stimulus Substances." Flora, New Series, 22, 76.

Snow, R. (1925): The Correlative Inhibition of the Growth of Axillary Buds Ann. of Bot. 39, 841

--- (1931-32): Experimentation on Growth and Inhibition, I-III. Proc. Roy. Soc. London, B 108, 209; B 111, 86.

--- (1932): Growth Regulators in Plants. New Phytol. 31.

--- (1933): The Nature of the Cambial Stimulus. New Phytol. 32, 288.

Soding, H. (1934): "On Growth Mechanism of Oat Coleoptiles." Jahrb. f. Wiss. Bot. 79, 231.

Sorauer, P., (1916): "Studies on Injuries Caused by Illuminating Gas." Zeitschr. f. Pflanzenkrankh. (Journal of Plant Diseases), 26, 129.

Spath and Meyer (1873):"Observation of the Influence of Illuminating Gas on Tree Vegetation." Land. Versuchsstat. (Agricultural Experiment Station) 16, 336.

Thimann, K.V., and Skoog, F. (1933): Studies on the Growth Hormone of Plants, III. The Inhibiting Action of the Growth Substance on Bud Development. Proc. Nat. Acad. of Sci. 19, No 7, 714.

Uyldert, Ina E., (1931): "Influence of Growth Substance on Plants with Intercalary Growth." Dissertation, Utrecht.

Vacha, G.A., and Harvey, R.B., (1927): The Use of Ethylene, Propylene and Similar Compounds in Breaking the Rest Period of Tubers, Bulbs, Cuttings and Seeds. Plant Physiol. 2, 187.

Virchow, R., (1874): "Influence of Illuminating Gas on Tree Vegetation." Jahresb. Agrik. Chem. 13-15, 237.

Wachter, W., (1905): "Chemonastic Movements of Calisia Repens Leaves." Ber. d. deutsch. bot. Ges. 23, 379.

Warburg, O., (1921): Biochem. Zeitschr. 119, 161.

—— (1925): "Effect of HCN on Alcoholic Fermentation." Biochem. Zeitschr. 165, 196.

—— (1928): "Catalytic Effects of Living Substance."(Die Katalytischen Wirkungen der Lebendigen Substanz), Berlin.

Wehmer, C., (1917-8): "Effect of Illuminating Gas on Plants, Parts 1-5." Ber. d. deutsch. Bot. Ges. 35, 135; 318; 403; 38, 140; 460.

Went, F.W., (1928): "Growth Substance and Growth." Rec. Trav. Bot. Neerl. 25, 1.

Wey, H.G. van der (1931): "Quantitative Methods with Growth Substance." Proc. Kon. Akad. Amst. 34, 875.

—— (1932): "Mechanism of Growth-Substance Transport." Rec. Trav. Bot. Neerl. 29, 279.

—— (1934): On the Occurrence of Growth Substance in Marine Algae. Proc. Kon. Akad. Wet. Amst. 36, 759.

—— (1934): "Growth Substance in Elaeagnus Angustifolius." Ibid. 36, 761.

Wieler, A., (1883): "Influencing of Growth by Reduced Oxygen Partial Pressure." Unters. aus d. Bot. Inst. Tubingen (Studies from the Tubingen Botanical Institute) I, 206.

Wiesner, J., (1878): "Undulating Nutation of Internodes." Sitz. Ber. K. u. K. Ak. Wien 77, 15.

Wolfe, H.S., (1931): Effect of Ethylene on the Ripening of Bananas. Bot. Gaz. 92, 337.