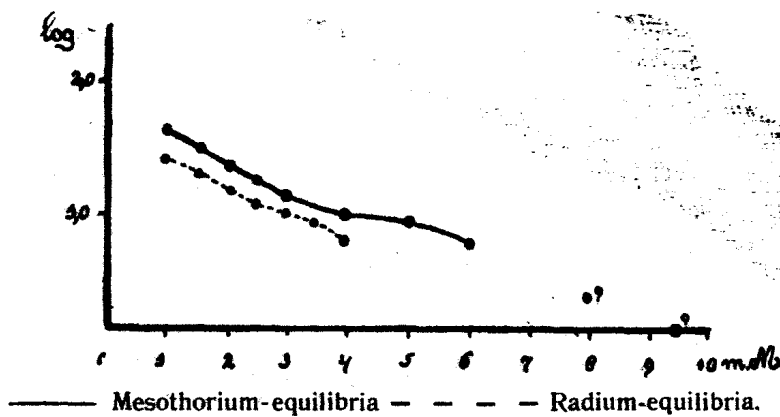


Citation:

Ameijden, U.P. van, The influence of light- and gravitational stimuli on the seedings of *avena sativa*, when free oxygen is wholly or partially removed, in:
KNAW, Proceedings, 19 II, 1917, Amsterdam, 1917, pp. 1165-1173

Distance between radium and heart (mm.)	Uratyl-nitrate added to the primary mixture (mgrms.)
4	5
3,5	6,5
3	9
2,5	11,5
2	15
1,5	18
1	22



O a method may be found to determine the biological value of a radio-active preparation (in a recovering sense). But it is out of place here to deal with this practical application.

Botany. — “The influence of light- and gravitational stimuli on the seedlings of *Avena sativa*, when free oxygen is wholly or partially removed”. By Dr. U. P. v. AMEIJDEN. (Communicated by Prof. F. A. F. C. WENT).

(Communicated at the meeting of February 24, 1917).

§ 1. Introduction.

Our conception of the influence of oxygen removal on geotropism and phototropism is mainly due to CORRENS¹⁾. His method of working

¹⁾ C. CORRENS. Ueber die Abhängigkeit der Reizerscheinungen höherer Pflanzen von der Gegenwart freien Sauerstoffes. *Flora* 75. 1892.

consisted in placing the experimental plants in complete or partial vacuum, and was that employed by most investigators before him. The dependence of the geotropic stimulation process on oxygen was examined by partially exhausting the vessel containing the seedlings and then placing them in a horizontal position. The seedlings were then observed for 6—12 hours, to see whether curvature took place. Thus he found for instance that seedlings of *Helianthus* were still capable of reaction, when the oxygen was reduced to traces; *Sinapis* seedlings on the other hand required at least 4—5% of oxygen to develop a curvature. When no curvature took place, there was also no after effect in ordinary air. CORRENS concludes from these experiments that oxygen is necessary for the execution of a geotropic process.

His heliotropic experiments were so arranged that the seedlings were continuously exposed in the receiver to unilateral day light, and he concludes that oxygen is necessary also for heliotropic stimulation. The quantities of oxygen which just permit of heliotropic curvature differ, however from the minimum quantities allowing a geotropic reaction. Thus for a phototropic reaction of *Sinapis* seedlings the oxygen could not be reduced below 6 percent.

Geotropic curvatures are therefore, according to CORRENS, executed by the same objects at a lower pressure than phototropic ones. In my opinion CORRENS is not justified in drawing this conclusion from his experiments since he compares stimulation intensities, the result of which perhaps are curvatures of very different degree. Geotropic and phototropic stimuli can only be compared, if we employ stimuli of such intensity that they produce maximal curvatures of the same strength.

ÁRPÁD PAÁL¹⁾ published a paper dealing exclusively with the influence of rarefaction of the air on the geotropic stimulation process and therein considered separately the perception and the reaction, the former by determining the presentation-times under normal pressure and after evacuation to various extents, the latter by causing perception to take place at normal pressure and allowing the reaction to occur under reduced pressure. His experiments led him to the view that with diminution of pressure the presentation-times and the reaction-times are lengthened.

It should be noted in this connection that he of course still adhered to the old conception of presentation- and reaction-times and

¹⁾ ÁRPÁD PAÁL. Analyse des geotropischen Reizvorgangs mittels Luftverdünnung. Jahrb. f. wiss. Bot. L. pag. 1. 1912.

that he did not yet attach to them the meaning which was afterwards given them by ARISZ¹⁾. I have always used both terms in the sense in which ARISZ uses them, hence the apparent contradiction between the results of ÁRPÁD PAÁL and my own.

§ 2 *Methods.*

The experiments were all carried out at the same temperature by placing the boxes with seedlings in an electrically heated thermostat, in which the temperature was kept constant by means of a thermoregulator. In the middle of the back wall of the thermostat there was an opening through which the axle of the clinostat passed, enclosed in an oil packing, so that no air could enter from outside; this also secured the easy rotation of the axle. The end of the axle in the thermostat could be screwed into the clamp intended for holding the boxes with seedlings. The latter remained in the thermostat throughout the duration of the experiment and could in this way be stimulated geotropically as well as phototropically since the front and side walls were of glass, so that the plants could be rotated on the horizontal clinostat axis immediately after stimulation. All experiments were carried out with a single box of seedlings. The clamp was arranged for and held two boxes, but the second merely acted as a counterpoise in order to obtain as far as possible a uniform rotation of the clinostat.

All experiments were carried out under a total pressure of one atmosphere and therefore the air in the thermostat was gradually replaced by nitrogen diffusing in from the commercial metal cylinders. Since the latter contain 4–5 percent of oxygen, the gas was first passed through washing bottles containing alkaline pyrogallol, in order to remove the oxygen. Since, however, not all the oxygen was absorbed and CO was moreover formed, the gas was passed through a red-hot tube containing copper, in order to absorb the rest of the oxygen, and a little CuO, in order to oxidize the CO formed to CO₂. The gas treated in this manner was allowed to enter the thermostat and the air contained in the latter was thus gradually driven out through an exit. It took 1½ to 2 hours to wash out all traces of oxygen, as was shown by estimations with a phosphorus pipette.

In order to trace the influence of oxygen deprivation on the geotropic and phototropic stimulation processes I first carried out a

¹⁾ W. H. ARISZ. Untersuchungen über den Phototropismus. Recueil des Travaux Botaniques Néerlandais. Vol. XII, 1915.

large number of experiments in air in order to obtain a standard, from which possible deviations might be measured. In these experiments I used stimuli of arbitrary intensity. Thus the geotropic stimulation consisted in placing the seedlings during a quarter of an hour in a horizontal position, which therefore means an intensity of stimulus of 900 mgs. Phototropic stimulation took place by exposure during eight seconds to a lamp, placed at a metre's distance from the middle of the box, with the seedlings arranged so that intensity of the light falling on them was 5 metre candles. This therefore corresponds to a stimulus of 40 metre-candle-seconds. I now determined the maximal curvatures corresponding to the two stimuli and the intervals of time between the beginning of stimulation and the attainment of maximum curvature, i.e. the reaction times. The result is, that for both stimuli the maximal curvature is 2 mm. and that the geotropic reaction time is 65 minutes, the phototropic 75 minutes.

§ 3. *Influence of oxygen deprivation on perception.*

In order to see whether *Avena* seedlings are able to perceive a stimulus in an oxygen-free atmosphere, I first left them for some time in the thermostat, while a current of nitrogen was passing.

When the objects had in this way been deprived of oxygen for some time the stimulus was administered, when they were still in a nitrogen atmosphere; immediately afterwards the nitrogen current was stopped and ordinary air was sucked through the thermostat by means of an aspirator. In considering the length of the preliminary sojourn (fore-period) in nitrogen, given below, it must be remembered that this includes the $1\frac{1}{2}$ —2 hours, necessary to free the thermostat completely from oxygen. During the reaction time the seedlings were therefore in air; when this time was up the seedlings were removed from the thermostat and their curvatures were measured.

I. Geotropic experiments.

Two or three hours of preliminary sojourn in nitrogen had not the slightest effect. A subsequent stimulus of 900 mgs. expressed itself by a maximal reaction of 2 mm. in the air. A fore-period in nitrogen of 5 hours was clearly evident by a diminished response, and after 6 hours there was no reaction at all.

TABLE 1.

Strength of stimulus 900 mgs. Temperature 20° C. Reaction time 65 minutes.

Fore-period in nitrogen	Number of seedlings	Amount of curvatures in mm.
5 hours	5	1 1 ½ 0 0
	7	1 1 1 ½ 0 0 0
	5	1 ½ ½ ½ 0
	4	½ ½ 0 0
6 hours	8	all without curvature
	5	id.
	5	id.

2. Phototropic experiments.

In these experiments also a preliminary stay of three hours was without the slightest effect on the curvatures, even six hours in nitrogen were not quite sufficient to prevent the reaction entirely, but an eight hours stay in nitrogen before stimulation was enough.

TABLE 2.

Strength of stimulus 40 M.C.S. Temperature 20° C. Reaction time 75 minutes.

Fore-period in nitrogen	Number of seedlings	Amount of curvature in mm.
6 hours	5	1½ 1 ½ ½ ½
	8	1½ ½ ½ ½ 0 0 0 0
	7	1 1 ½ ½ 0 0 0
	4	½ ½ ½ ½
	6	1 ½ ½ ½ 0 0
8 hours	6	all without curvature
	6	id.
	8	id.

It is evident therefore, that the seedlings must be deprived of oxygen for a long time in order to lose their irritability altogether.

Since the possibility existed, that especially in the space-between the cotyl and the first leaf, sufficient oxygen remained for a long time to account for the prolonged irritability, I repeated the experiments with seedlings of *Sinapis alba* and obtained with them mutatis mutandis, the same results; we must therefore assume that the seedlings, as a result of intramolecular respiration, have sufficient energy at their disposal to perceive stimuli for a considerable time, albeit in lessening degree. We may not, however, conclude at once that no perception of stimulus can occur in the absence of oxygen, for it might quite well be that the stimulus is indeed perceived, but that the processes in the plant, which cause the reaction, have already been so influenced by the want of oxygen, that no curvature was possible. For these reasons I carried out geotropic experiments, in which the objects had a six hours' fore-period in nitrogen and phototropic ones, in which this period was 8 hours; in both cases perception took place in the air, this being therefore the sole point of difference from the previous set of experiments.

1. Geotropic experiments.

TABLE 3.

Strength of stimulus 900 mgs. Temperature 20° C. Reaction time 65 minutes.

Fore-period in oxygen	Number of seedlings	Amount of curvatures in mm.									
6 hours	8	2	1½	1	1	1	1	1	1	½	½
	7	1½	1½	1	1	1	1	½			
	7	2	1	1	1	1	1	½			
	8	1½	1½	1½	1	1	1	½	½		

2. Phototropic experiments.

TABLE 4.

Strength of stimulus 40 M.C.S. Temperature 20° C. Reaction time 75 minutes.

Fore-period in oxygen	Number of seedlings	Amount of curvatures in mm.									
8 hours	9	1½	1½	1	1	¼	¼	½	½	½	0
	8	1½	1½	1	1	½	½	½	0		
	6	1	1	½	½	½	½				
	8	1½	1½	1	1	1	½	½	0		

On comparison of the above experiments with those in tables 1 and 2, it at once follows that since in those of tables 3 and 4 there were curvatures, and not in those of tables 1 and 2, seedlings are unable to perceive a geotropic or phototropic stimulus in the absence of oxygen.

The fact that the curvatures obtained in the later experiments are smaller than those obtainable under normal conditions proves, that the seedlings have undergone a harmful influence from the prolonged want of oxygen, which still makes itself felt after the normal conditions have been reestablished.

§ 4. *The influence of oxygen deprivation on the reaction.*

In order to study the influence of an oxygen-free atmosphere on the reaction, I gave the seedlings a preliminary stay of 3 hours in nitrogen, administered the stimulus in this gas, and left them without oxygen also during the reaction time. The earlier experiments had shown that after a fore-period of 3 hours in nitrogen, the stimulus is still perceived normally in this gas. In a few experiments I watched the seedlings for a considerable further time in nitrogen in order to see whether a curvature occurred later. In that case we should have to postulate a lengthening of the reaction time owing to absence of oxygen. In the other experiments I admitted oxygen at once after the normal reaction time had elapsed, in order to see whether there was any after effect in this gas.

1. Geotropic experiments.

The plants remain the whole time in nitrogen.

TABLE 5.
Strength of stimulus 900 mgs. Temperature 20° C.

Fore-period in nitrogen	Time elapsed since beginning of stimulation in minutes	Number of seedlings	Amount of curvatures in mm.
3 hours	65	7	all without curvature
	100		id.
	125		id.
	150		id.
	65	8	id.
	100		id.
	130		id.
	65	8	7 without curvature, 1 with asymm. apex
	100		id.
	130		id.
	65	7	all without curvature

2. Phototropic experiments.

The seedlings remain the whole time in nitrogen.

TABLE 6.
Strength of stimulus 40 M. C. S. Temperature 20° C.

Fore-period in nitrogen	Time elapsed since beginning of stimulation in minutes	Number of seedlings	Amount of curvature in mm.
3 hours	75	7	all without curvature
	100		id.
	140		id.
	170		id.
	75	8	id.
	105		id.
	130		id.
	75	8	id.
	100		id.
	135		id.
	75	9	id.

If I replaced the nitrogen by air after 65 minutes, or respectively after 75 minutes there was always a slight after effect, which was plainly visible about one hour after air had begun to be sucked through. This is further evidence, that the stimulus had indeed been perceived, but that without oxygen no reaction could show itself. That these curvatures were so slight, is a proof that the stimulus was already passing off, and therefore we cannot speak of a lengthening of the reaction time as a result of the absence of oxygen. These experiments show, that a perceived geotropic or phototropic stimulus is unable to give a reaction in the absence of oxygen; further that there are no indications in favour of a lengthening of the reaction time.

§ 5. *Influence of an atmosphere with low oxygen content.*

By passing the gas from the nitrogen cylinder straight into the thermostat, without passing it first through the washing bottles with pyrogallol and the tube with red-hot copper, the plants were in an atmosphere containing 4—5 % of oxygen. I only investigated the influence on the perception, by giving the seedlings a fore-period in

this mixture and allowing perception also to take place in it, and then letting any possible reaction occur in ordinary air.

1. Geotropic experiments.

TABLE 7.

Strength of stimulus 900 mgr. Temperature 20° C. Reaction time 65 minutes

Fore-period in 4.3 % oxygen.	Number of seedlings.	Amount of curvature in mm.									
6 hours	9	2½	2	2	2	2	1½	1½	1½	1	
8 "	9	3	2½	2	2	2	2	1½	1½	1½	
24 "	8	2	1½	1	1	1	1	½	½		

2. Phototropic experiments.

TABLE 8.

Strength of stimulus 40 M. C. S. Temperature 20° C. Reaction time 75 minutes.

Fore-period in 4.3 % oxygen.	Number of seedlings.	Amount of curvature in mm.									
10 hours	9	2½	2	2	2	2	1½	1½	1½	0	
24 "	9	2	1½	1½	1½	1	1	1	0	0	

After a stay of 24 hours in the mixture of nitrogen and oxygen an influence on the perception is noticeable in both cases. The seedlings therefore remain able for a long time to perceive a geotropic or phototropic stimulus in an atmosphere containing a relatively low amount of oxygen. Here also there is no indication of a difference in the reaction to these two kinds of stimuli, contrary therefore to the opinion of CORRENS, according to which a geotropic curvature can be executed in a lower percentage of oxygen than a phototropic one.

Utrecht, February 1917.

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