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TOXICITY OF SMOKE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 171

LEE I. KNIGHT AND WM. CROCKER

I. Introduction

(WITH FOUR FIGURES)

Extensive unpublished experiments have been conducted in this laboratory to determine the reliability of the etiolated epicotyl of the sweet pea as a test for the presence of traces of heavy hydrocarbons in the atmosphere. As an outgrowth of this work, we have had occasion to study the response of this organ to smoke produced by the burning of various carbon-bearing substances, with the idea of discovering the constituent or constituents that produce the response.

The complete statement of the response of this seedling to gaseous impurities will involve two additional papers, one under the title "Is methane toxic?" and the other "The sweet pea epicotyl as a delicate test for heavy hydrocarbons." Both of these papers will be published shortly.

NELJUBOW (**25**) has shown that the etiolated epicotyl of the pea seedling has an abnormal growth in "laboratory" or "impure" air. We may speak of the abnormality as a triple response: change of negative geotropism to diageotropism, increased growth in thickness, and reduced rate of growth in length. We tested about 20 varieties each of garden peas and sweet peas, and found the sweet peas in

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general considerably the more sensitive. Two varieties of these, under the trade names Earl Cromer and Gladys Unwin (Vaughan's Seed Store), were especially sensitive.

II. Methods

GROWING THE SEEDLINGS

Gladys Unwin and Earl Cromer are both hard-coated varieties. After 24 hours' soaking in water, about 10 per cent swell, and 10 days are required for all to swell. Consequently, in order to get uniform growth in the seedlings, the coats are scratched with a file and the seeds soaked for 15 hours in distilled water. They are then thoroughly shaken up with several changes of distilled water and placed in a thin layer between sterile wet filter papers and allowed to germinate. When the longest hypocotyls have reached a length of 5 mm., the seeds are transferred to wet sterile absorbent cotton in large petri dishes and allowed to grow in total darkness at 20°–24° C. until the epicotyls have an average height of 2–3 cm. This method gives cultures fungus-free and of far more nearly uniform growth than can be obtained by less careful methods. It also gives cultures bearing only the more vigorous seedlings. This is especially important, for sensitiveness to atmospheric impurities rises with the vigor of the seedlings. With all these precautions there is considerable irregularity in the rate of growth of the various epicotyls in a culture. The entire process must be carried on in what the German workers have called pure air, which means air practically free from the heavy hydrocarbons, especially ethylene. A quantity of this substance equaling one part in ten million of atmosphere interferes with the growth of the seedling.

In case the gas to be tested is very soluble in water, it is necessary to protect it against contact with the moist cotton bearing the roots. This is done by covering the substratum with low-melting paraffin mixed with pure paraffin oil. In many cases it was also found desirable to have a desiccating agent in the experimental chamber to keep its walls free from water given off by transpiration. This especially holds for gases like SO_2 and NH_3.

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METHOD OF EXPOSING TO GASES AND VAPORS[1]

The exposure to gas or vapor was made in galvanized iron cans of the type shown in fig. 1, and of three capacities, 10, 50, and 100 liters. Each can consists of two pieces, the can proper and the bottom. The can proper has at *a* a side tube bearing a no. 3 two-holed rubber stopper. The stopper is equipped with glass tubes, rubber tubes, and screw clamps. The lower edge of the can has an out-turned brim, 1 cm. wide, at right angles to the sides of the can; this gives the can close continuous contact with the bottom. The bottom is a galvanized iron disk with 3 mm. of the edge turned up at right angles to the plane of the disk. The brim of the can fits closely inside the upturned margin of the bottom. To seal a culture in the can, it is placed on the bottom piece, the can set in position, and the gutter above the brim of the can carefully sealed with molding clay mixed with vaseline in such proportions as to give the desirable consistency. The seal will hold against a very considerable unequal pressure of gas. Vaseline was chosen as the mixing medium because it does not give off any fumes injurious to the seedlings and makes a material that maintains the same consistency after years of use. If the gas studied required the use of only a few cc., it was forced in through one of the tubes at *a* under a small head of mercury or water. Mercury was used in case the gas was very soluble in water, otherwise water was used. If a larger volume of the gas was required, suction was applied to one of the tubes at *a*,

FIG. 1.—Apparatus for exposure to gases and vapors: explained in text.

[1] The description of methods here will cover the whole field of work, thereby avoiding redescription in later papers. For that reason many matters mentioned here do not apply to the work on smoke.

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after which it was clamped. This suction served as the force for drawing in the gas through the second tube.

In cases where very large quantities of the gas were required, as with methane, the can was replaced by a bell jar and a water seal used. The whole apparatus was then placed in a dark chamber. To make sure of the reliability of the can, this method was also frequently used as a check in case of gases demanding only low concentration and not readily absorbed by water. If the vapor of a very volatile liquid, like ethyl ether, was to be used, a measured quantity of it was forced from a pipette into the upturned end of one of the glass tubes at *a*. A glass dish bearing absorbent cotton was attached just under the inner down-turned end of the glass tube. The liquid volatilized from the cotton and its vapor was distributed throughout the can. Applying the gases and vapors in this way, at the most distant point from the seedlings, insured that they would not at any time receive higher than the finally distributed concentration. In case the vapor of a slightly volatile liquid, like propyl alcohol, was to be applied, the desired amount was dropped on absorbent cotton and quickly sealed in the can with the cultures. In this case, of course, the clamps at *a* are screwed down or the hole provided with a solid cork.

The cultures are always subjected to the influence of the gas for three days, the same period used in our experiments with the carnation. At the close of an experiment the epicotyls in any culture of controls vary in height from 5 to 13 cm., while the cultures subjected to injurious concentrations of gases show less growth.

In this method of experimentation, as is seen, the epicotyls are not subjected to the same concentration during the entire three days, for the gas is applied only at the beginning of the experiment. The concentration must fall to a degree varying with the different gases, due to absorption by the plant and substratum, and to some extent due to a slow diffusion outward through the seal. If the gas is one that is readily absorbed by the plant, as SO_2, the method probably more nearly determines the lethal dose. In the case of the carbon-bearing gases (acetylene, ethylene, propylene, methane, and carbon monoxide), with the exception of acetylene, which is

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rather soluble in water, the method certainly gives a very close approximation to the constant concentration necessary to give a response. The object of the work was to determine the nature of the response given by the several gases and vapors and to approximate the concentration necessary to produce it. For this purpose the method is adequate.

III. Historical

1. RESPONSE OF THE PEA EPICOTYL

a) Horizontal nutation

As has been stated, the epicotyl of the pea seedling grows prostrate or horizontal in "laboratory" air. NELJUBOW (**25**) has spoken of this response as a *horizontal nutation*, and mentions ethylene and acetylene, both constituents of illuminating gas, as especially effective in producing it. He has shown (**26**), also, that the response is not limited to the pea epicotyl, but appears in the etiolated epicotyls of *Ervum lens*, *Lathyrus odoratus*, *Vicia sativa*, and *Tropaeolum*. SINGER (**42**) has observed the same for the potato stem. NELJUBOW has shown that the response in the pea epicotyl in an atmosphere containing ethylene varies with the concentration of ethylene. Beginning with the higher concentrations and passing to the lower, he mentions the following grades of response: (1) no growth, death; (2) no elongation, a swollen knob; (3) elongation slow, swelling, diageotropism; (4) elongation faster, little swelling, diageotropism; (5) like (4) except obliquely placed; (6) erect, but growth reduced by half.

The horizontal nutation has been a subject of no little investigation. With mere mention we can pass over WIESNER'S (**43**) conception of undulating nutation induced by darkness, and RIMMER'S (**39**) autonomic nutation induced by dryness, for at that time there were not sufficient data available for a rational interpretation of the response. MOLISCH (**20**) and KÖRNICKE (**17**) concluded that impurities of laboratory air affect geotropic and heliotropic sensibility in opposite ways, weakening the former and strengthening the latter. MOLISCH (**21,** pp. 170, 171) has apparently abandoned the idea of increased heliotropic sensibility.

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RICHTER (32, 33, 34) speaks of the impurities weakening negative geotropism and has continuously maintained that they increase heliotropic sensibility. GUTTENBURG (13) has spoken of the horizontal nutation as due to weakened negative geotropism and vigorously denied increased heliotropic sensibility. He attributes the conclusion of other workers on the latter point to inaccuracies in experimentation, especially the failure to use the clinostat, thereby eliminating the antagonistic action of gravity.

NELJUBOW (26) has maintained since 1901 that the response is induced diageotropism. The evidence set forth in his last paper seems conclusive, marked as it is by excellence of experimentation. He criticizes the other workers for using "laboratory" air in which the quantity of effective impurity must vary from hour to hour, and in which the amount of impurity cannot be measured at any time. He used mixtures of pure air with 1–3 ppm. of ethylene. To maintain constant concentration the mixture was renewed daily. In this atmosphere on a horizontal clinostat the etiolated pea epicotyls showed great reduction in rate of elongation, also swelling but not bending. When grown in the same atmosphere off the clinostat, they showed the same characters with the addition of horizontal nutation. If in such an atmosphere the epicotyls were raised or lowered out of the horizontal position, they again assumed it. NELJUBOW maintains that diageotropism is the only assumption that can explain this behavior. He emphasizes that there is no autonomic nutation determining the direction in which the epicotyl turns to assume the horizontal position, but that it is entirely determined by its position in relation to the pull of gravity. RICHTER (34), working with seedlings of *Vicia sativa*, *V. villosa*, and *Pisum sativum*, shows that such a statement can hold only for epicotyls more than 1 cm. tall. On a clinostat or in "impure" air which "weakens" negative geotropism, the young etiolated epicotyls turn "backward" (in the direction in which the closed side of the curved tip faces) and crowd themselves closely against the substratum. This is an autonomic nutation incapable of manifesting itself off the clinostat in pure air on account of the counteraction of negative geotropism. Judging from the work of both NELJUBOW and RICHTER, the horizontal nutation of the etiolated

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epicotyl of the pea, if less than 1 cm. tall, is due to the joint action of autonomic nutation and diageotropism; but, if taller than 1 cm., it is due to diageotropism alone. It should be mentioned in this connection that NELJUBOW worked mainly with taller epicotyls, which probably accounts for the autonomic nutations escaping detection by his careful methods.

b) The swelling

The increased diameter or swelling of a plant organ in the presence of poisons is apparently a rather commonly observed phenomenon. COOK and TAUBENHAUS (5) observed that in proper concentration of tannin the mycelia of various fungi tend to become short, thick, and much septate. GROTTIAN (12) finds that anaesthetics, especially chloroform, produce a swelling in the root just back of the tip, with constrictions above and below. NĚMEC (27) finds that chloral, ether, benzine, benzene, and alcohol vapors have a similar effect.

The anatomical structures of the swollen zone, along with the physiological condition causing the peculiar structure, has been a subject of some comment. As RICHTER (35) has stated, the swollen region shows an abundant development of collenchyma, also numerous rifts more or less lined with cork. RICHTER (36) has related the swollen condition and the development of rifts to excessive osmotic pressure induced by the poisons. So far as his cited articles are concerned, there is no evidence that he has made any measurements of osmotic pressures. His conclusions are based on two lines of indirect evidence. First, many observers have found an increase in osmotically active substances in plants grown in an atmosphere bearing poisons. JOHANNSEN has shown that soluble sugars increase in plants in the presence of ether and other anaesthetics. PRIANISCHNIKOW (29) has shown that etiolated lupine seedlings grown in laboratory air have a much greater amount of asparagin than those grown in pure air. The following table (p. 344) gives the difference, in percentage, wet weight, of asparagin in 12-day seedlings in pure air and laboratory air.

GRAFE (10) has shown that soluble sugars accumulate in plant organs at the expense of starch in an atmosphere bearing formalde-

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hyde. GRAFE and RICHTER (11) have lately demonstrated that an atmosphere bearing acetylene or carbon monoxide changes radically the course of metabolism in various plant organs. In seedlings of *Vicia villosa* and *V. sativa* and stems and tubers of the potato, carbon monoxide (0.038 to 0.29 volume per cent in air) causes an increase in the sugar and amino content. In fatty seeds (squash and mustard) there is a decrease in sugar and amino compounds and an increase in glycerine and fatty acids. Acetylene interferes with the synthesis of sugar from glycerine and condensation of glycerine and fatty acids to fats.

Pure air		Laboratory air	
Cotyledon	Epicotyl	Cotyledon	Epicotyl
0.140	0.289	0.348	0.625

The second evidence that RICHTER offers for poisons causing increased osmotic pressures is the fact that they often produce proliferation of sublenticular tissue similar to the substomatal proliferations or intumescences caused by high humidity. Very often, also, the lenticular protrusions, as well as other tissues, show guttation in the presence of poisons, which RICHTER interprets as indicating high osmotic pressure. Measurements of osmotic pressure would certainly be more to the point in the case of pea seedlings, though they might give less ground for philosophy and even a reverse conclusion. Aside from known relations to osmotic pressure, and only indirectly bearing on the point under discussion, it is well known that poisons, especially atmospheric impurities, produce considerable alteration in the structure and form of plants. The late work of GATIN (**8, 9**) on the effect of tarred roads on vegetation furnishes excellent examples of this. He finds that the fumes of tar bring about the disappearance of endodermis, alteration in the size and number of layers of cells in the cortex and other regions of the stem, and the transformation of doubly compound leaves into singly compound ones. Of less fundamental importance, perhaps, is the disappearance of starch and the formation of cork on leaf organs and young stems. RICHTER (**37**) has lately

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summarized the effects of poisons upon the development of plant structures. Under modifications due to increased cell pressure, he mentions inhibition of growth in length, favoring of growth in diameter, splitting of the tissues with formation of rifts, lenticular and intumescence formation, and maceration in the living body. Other modifications that he relates to the rise of turgor are collenchyma formation, thickening of the epidermis, vacuolization of the nucleus, and fusion of the nuclei.

These two lines of general evidence are very interesting in connection with the response of the pea seedling, but after all they leave essentially untouched the real solution of the physiology of the response. This becomes more evident when it is remembered that our work shows three distinct types of response of this seedling to atmospheric impurities: (1) that produced by ethylene, acetylene, propylene, illuminating gas, various sorts of smoke, and possibly methane, and characterized by decreased rate of elongation, swelling, and diageotropism; (2) that produced by ether, chloroform, benzol, toluol, thiophene, xylol, cumene, and other substances, and characterized by decreased rate of elongation and swelling but not diageotropism; (3) that produced by ethyl alcohol, propyl alcohol, pyridine, hydrogen sulphide, hydrogen chloride, and other substances, and characterized by decreased rate of elongation, but neither swelling nor diageotropism. A careful detailed study of osmotic pressure, permeability, and metabolic (enzymatic, acid, respiratory, etc.) behavior of the seedlings, in each group of poisons might throw much light on the internal intimate physiology of the three types of response. A detailed study in this line might also relate diageotropism more closely with physical and chemical characters of the organ. This organ furnishes especially desirable material for such a study because of the ease with which it is changed from a negative to a diageotropic organ.

2. EFFECT OF TOBACCO SMOKE

MOLISCH has shown that tobacco smoke is extremely toxic to many plants. In his first paper (**22**) he reports the effect of tobacco smoke on various seedlings and microorganisms, and in a second paper (**23**) the effect upon adult plants. A third paper (**24**)

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brings together all the main conclusions of the work, and emphasizes the bearing of the findings upon the growth of plants in dwellings, laboratories, etc.

Seedlings (*Vicia sativa*, *Pisum sativum*, *Cucurbita Pepo*, *Phaseolus vulgaris*, and others) are very sensitive to tobacco smoke. In its presence *Vicia sativa* epicotyls show what we have termed the triple response, also they fail to develop anthocyanin, a feature RICHTER (**38**) has observed for many plants grown in laboratory air. MOLISCH states that one to three whiffs of cigar or cigarette smoke in a 4.3-liter container caused the triple response. Smoke of paper, straw, and wood has effects similar to tobacco smoke, while fumes of nicotine have little influence on the seedlings. Carbon monoxide, pyridine, and hydrogen sulphide, in considerable dilution in the atmosphere, produce effects similar to smoke. MOLISCH quotes PONTAG as showing that cigar and cigarette smoke bear considerable quantities of carbon monoxide. On the basis of these data, MOLISCH concludes that carbon monoxide is probably the constituent determining the toxic limit of tobacco smoke for seedlings. Acquaintance with the work of NELJUBOW (**25**), CROCKER and KNIGHT (**6**), and LEHMANN (**18**) should have led him to recognize the high toxicity of ethylene for epicotyls of seedlings and other plant organs, the rather low toxicity of carbon monoxide, and the universal presence of ethylene in tobacco smoke. This immediately suggests the probability of ethylene being the constituent determining the toxicity for seedlings. MOLISCH'S "whiff" methods are poorly adapted for matching the amount of carbon monoxide in the applied smoke against the amount demanded to produce the response.

Many microorganisms are likewise remarkably sensitive to tobacco smoke. A whiff of tobacco smoke blown across a culture of *Pseudomonas lucifera* "puts it out" in 0.5–1 minute. It readily recovers its power to phosphoresce when returned to sea water. *Chromatium vinosum*, *Beggiatoa*, *Spirillum* sp., *Amoeba*, *Vorticella*, *Paramoecium*, *Didymium nigripes*, and *Gymnodimium fucorum* are very sensitive to tobacco smoke, while *Pinnularia* and *Phycomyces nitens* are rather resistant.

Adult vascular plants behave variously toward the impurity.

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Tradescantia guianensis, *Selaginella Martensii*, *Tolmiaea Menziesii*, *Eupatorium adenophorum*, and various echeverias are not interfered with at all by low concentrations of smoke in the air, and only slightly inhibited in their growth by great concentrations. The mature plants that are sensitive to tobacco smoke manifest it in three ways: (1) by chemotactic movements of the leaves; the leaves of *Boehmeria utilis* and *Splitgerbera biloba* showed epinastic movements when in a glass chamber of 4.5–7 liters with 1–3 whiffs of cigar or cigarette smoke; in *Boehmeria* the movement continues beyond the vertical position, with the formation of spirals in the petiole; (2) by lenticular protuberances; the development of lenticular protuberances occurred in sprouts of the potato and stems of *Boehmeria polystachya* and *Goldfussia glomerata* in the presence of tobacco smoke; while in *Salix rubra* and *Sambucus nigra* such protuberances develop in moist air, the process is greatly hastened by tobacco smoke; guttation commonly occurs in this lenticular tissue, due, as MOLISCH assumes, to high osmotic pressure; (3) by fall of leaves; tobacco smoke causes leaf fall in many plants; this is especially true of leguminous forms; 24–48 hours of exposure to tobacco smoke causes almost complete loss of leaves in *Mimosa pudica*, *Caragana arborescens*, *Robinia pseudacacia*, *Halimodendron argenteum*, and others; paper and wood smoke were likewise very effective in producing leaf fall, and nicotine very weakly so. MOLISCH states that in the mature plants he was not able to determine the constituent of tobacco smoke that does the injury.

IV. Observation and experimentation

AN OBSERVATION

A mishap in the greenhouses of this laboratory has some of the virtues of a real experiment. An attempt to kill the insects by the common method of burning tobacco stems resulted in the acute poisoning of many of the plants. This application of a high concentration of smoke for a short time did not, of course, show responses in the nature of nasties, swellings, abscissions, and nulled tropisms, as observed by MOLISCH with the application of lower concentrations

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for long periods. The injury in this case must be stated in terms of the region and extent of killing. The records were taken five days after the mishap.

The following showed no injury: BRYOPHYTES, *Marchantia polymorpha*, *Conocephalus conicus* (not under spray); PTERIDOPHYTES, *Cyrtomium falcatum*, *Azolla caroliniana*, *Salvinia natans;* SPERMATOPHYTES, cycads, when not actively growing (*Dioon edule*, *D. spinulosum*, *Zamia floridana*, *Macrozamia Miquelii*, *Encephalartos cycadifolia*, *E. Lehmanii*, *E. Altensteinii*, *E. horridus*, *E. caffer*, and *Ceratozamia mexicana*), *Hibiscus rosa-sinensis*, *Zebrina pendula*, *Begonia semperflorus* (flowers and foliage), *Pelargonium zonale*, *Ficus elastica*, *F. lyria*, *Sagittaria variabilis*, and *Lemna trisulca*.

The following forms showed evident injury: BRYOPHYTES, *Riccia* (in pots on benches, all killed), *Conocephalus conicus* (growing under spray, all killed); PTERIDOPHYTES, *Lygodium* sp. (leaves all killed at tips and margins and many halfway back), *Pteris longifolia cristata* (tips and margins of leaves killed, brown spots on leaves), *Nephrolepis bostoniensis* (slight injury), *Aspidium longifolium* and *A. nidus* (some of the plants killed to the ground), *Alsophila denticulata* (badly injured); SPERMATOPHYTES, *Impatiens Balsamina* (all the older leaves brown-spotted), tomato (leaves entirely killed in most cases, but only at the tip in some), *Persia gratissima* (older leaves all killed), *Stevia serrulata* (all but youngest leaves killed), *Vinca alba* (ends and margins of leaves slightly injured), *Coleus* spp. (all older leaves fallen, young plants showed less injury). It is of interest that *Conocephalus conicus* growing on the benches was not injured at all, while a vigorous culture that had been growing for a long period under a spray was completely killed. It is possible that good water supply caused the development of a loose, poorly protected structure. It is likewise possible that the great surface of the spray water kept it nearly saturated with the poison of the smoke. From the data given above, one can see that in general the better cutinized forms are more resistant. Some will be interested in comparing these injuries with those from illuminating gas observed in a greenhouse by WILCOX (44). There is evidence to indicate that both injuries are due to a common substance, as we will see later. In the case reported by WILCOX, the poisoning

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was more acute, due to longer application and possibly to greater concentration of the toxic material.

EXPERIMENTS

We will publish only a type experiment under each head, but in every case the experiment has been repeated several times to make sure the type experiment tells the truth. As has already been stated, we used the etiolated epicotyl of the sweet pea as the plant organ for testing the toxicity of smoke and its constituents, for we learned from our earlier experiments its behavior toward a great number of gaseous impurities, including the main constituents of smoke. This renders the determination of the constituents fixing the toxicity of smoke a simpler matter. Since the responses of the seedling to certain smokes and the variation of response with concentration is similar to the behavior toward ethylene, it is well to have in mind NELJUBOW'S (**26**) statement of the six types of response, varying with the concentration of the ethylene. They will furnish the data for an interpretation of the experimental results given below. Beginning with the higher concentrations, the responses are: (1) no elongation, death; (2) no elongation, vertical position, a knoblike swelling; (3) considerable elongation, swelling, horizontal position of the growing swollen part; (4) elongation greater, little swelling, horizontal position of part grown in ethylene-containing atmosphere; (5) like (4) except obliquely placed; (6) erect, but elongation rate reduced by half. Our failure to get a strictly horizontal position in many cases where NELJUBOW obtained it may be due to the fact that he changed the gas every day, thus maintaining an essentially constant concentration, while we applied the gas once and allowed it to stand for three days. The gradual absorption of the gas by plant, substratum, etc., may dilute it so that the epicotyl partly recovers its upright position. For our purpose, however, one application of the gas is adequate.

Experiment I.—Effect of unwashed smoke

When the experiment was set up, the etiolated epicotyls (Gladys Unwin) were 2–3 cm. tall, slim and vertical. The duration of exposure was three days. The following data show the sorts and

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concentrations of the smokes used, along with condition of the epicotyls at the close of the experiment:

1. Check (in duplicate) in 10-liter chamber; epicotyls 6–11 cm. tall, vertical and slim.

2. Lighted cigarette sealed in 10-liter chamber; epicotyls 2.5–3.5 cm. long; swelling 0.5–1 cm. long; shorter swelling vertical and longer ones declined as much as 90°.

3. Lighted pine splinter in 50-liter chamber; epicotyls 3–5 cm. long; swelling 1–2 cm.; declination 50°–90°.

4. Lighted piece of linen paper (not giving lignin test) in 10-liter chamber; epicotyls 3–3.5 cm. long; swelling knoblike; no declination.

5. A lighted sheet of ashless filter paper (6 cm. diameter) in 10-liter chamber; epicotyls 1–2 cm. long; swelling 1–2 cm.; declination 60°–90°.

6. Two lighted straws (12 cm. long) partly burned in 10-liter chamber; epicotyls 3–3.5 cm. long; swelling knoblike, to 1 cm. long; declination 0°–90°.

7. Three whiffs of cigarette smoke in 10-liter chamber; reaction almost identical with 6.

8. 0.13-gm. linen paper burned as open sheet in 10-liter chamber; epicotyls 3–5 cm. long; swelling 1.5–2.5 cm.; declination 60°–90°.

9. 0.06-gm. linen paper burned in 10-liter chamber; epicotyls 4–7 cm. long; no swelling; diameter everywhere greater than in checks; declination 0°–15°.

10. 0.09-gm. linen paper burned in 10-liter chamber; reaction about the same as 9.

11. 1.45-gm. linen paper burned in 100-liter chamber; epicotyls 2.5–3.5 cm. long; swelling 0.5–0.75 cm.; slight declination.

12. 1.45-gm. linen paper burned in 50-liter chamber; reaction about as 11.

13. 0.36-gm. linen paper in 100-liter chamber; epicotyls 4–6.5 cm. long; no swelling, but larger diameter than checks; declination 20°–40°.

From this experiment it is evident that the smoke from the cellulose and lignin compounds is rather toxic. In agreement with Molisch, it shows that the toxic effect of tobacco smoke is not due to substances peculiar to tobacco, but is as marked for the smoke of pure cellulose. The unwashed smoke from 0.13 gm. of cellulose in 10 liters gives a response lying between Neljubow's responses 3 and 4.

Experiment II.—Effect of washed smoke

In preparing the smoke, the cigar or cigarette (tobacco or paper) was smoked by suction and the smoke washed through two special wash bottles, one containing 15 per cent H_2SO_4, the other 40 per cent NaOH. According to Lehmann (**18**), the first removes all

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NH_3, nicotine, tar, and solids, while of course the latter removes CO_2 and H_2S. There results a mixture of colorless gases which is stored over water. This method of storage is adequate, since the most toxic constituent of the smoke is very slightly soluble in water, as shown by the slight fall in toxicity when thus stored, also by evidence given later in the paper.

To obtain the cigarette smoke used in the following experiments, 7 Murad cigarettes, weighing 1.3 gm. each, were two-thirds consumed, with a yield of 10 liters of gas. Each liter of smoke, therefore, results from the smoking of 0.6 gm. of cigarette; 20 liters of cigar smoke were produced by 15 gm. of cigars; each liter of smoke required 0.75 gm. of cigar. The paper smoke was derived from a cigarette of bond paper not giving lignin tests; 4 liters of smoke were produced from 1.4 gm. of paper; each liter was derived from 0.35 gm. of paper. So far as we know, the tobacco and paper smoke thus washed will contain, in common, nitrogen of the air drawn through in smoking, more or less unused oxygen, certain dry distillation gases of the carbon compounds involved (methane, ethylene, acetylene, and carbon monoxide), and perhaps higher homologues of methane, ethylene, and acetylene. The tobacco smoke will contain in addition traces of pyridine and perhaps other compounds. In this experiment the epicotyls in each culture at the beginning of the exposure varied from 2 to 3 cm. in height. The seedlings were subjected to the smoke for three days in total darkness at 20° to 24° C. The following data give the various concentrations and varieties of smoke used, along with the condition of the epicotyls at the close of the experiment.

1. Check culture in 50-liter chamber; epicotyls 6–12 cm. tall, vertical and very slim.

2.[2] 25 cc. of cigar smoke (output of 0.0188 gm. of cigar) in 50-liter chamber; epicotyls 4–7 cm. tall; greatest declination 30°; no swelling; greater diameter than check.

3. 50 cc. cigar smoke (output of 0.0375 gm. of cigar) in 50-liter chamber; epicotyls 3.5–4.5 cm. long; swollen; declined portion 1.5–2.5 long, with a declination of 75°–90°.

[2] In all the work the smoke was measured under existing atmospheric pressure. No corrections for barometric pressure and temperature were deemed necessary, for a doubling of the concentration was required to give noticeable differences in response, hence the errors of this method were far beyond detection by the seedling.

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4. 100 cc. cigar smoke (output of 0.075 gm. of cigar) in 50-liter chamber; epicotyls 3.5–4 cm. long, with swollen regions 0.75–1.5 cm. long; swollen zones varying from upright to horizontal; the shorter swollen upright zones indicate that there is no growth in length.

5. 130 cc. cigar smoke (output of 0.097 gm. of cigar) in 50-liter chamber; many epicotyls showed knoblike swellings; no growth in length and no declination; many others were dead.

6. 200 cc. cigar smoke (output of 0.15 gm. of cigar) in 50-liter chamber; epicotyls mostly dead; living ones vertical, with a knoblike swelling; no growth in length.

7. 330 cc. of cigar smoke (output of 0.25 gm. of cigar) in 100-liter chamber; condition of epicotyls between that of 5 and 6.

8. 40 cc. of cigarette smoke (output of 0.024 gm. of cigarette) in 10-liter chamber; epicotyls 3–4.5 cm. long; swollen zone 1–2 cm. long; declination of swollen portion 80°–90°.

9. 20 cc. of cigarette smoke (output of 0.012 gm. of cigarette) in 10-liter chamber; epicotyls 3–6 cm. long; swollen portion 2–3 cm. long; declination of swollen part 70°–90°.

10. 10 cc. cigarette smoke (output of 0.006 gm. of cigarette) in 10-liter chamber; epicotyls 4–9 cm. long; little declination; no swelling; diameter of epicotyls greater than that of checks.

11. 20 cc. paper smoke (output of 0.007 gm. of paper) in 10-liter chamber; epicotyls 3–4.5 cm. long; swelling 0.75–1.5 cm. long; swollen portion vertical to horizontal.

12. 10 cc. paper smoke (output of 0.0035 gm. of paper) in 10-liter chamber; epicotyls 4–5 cm. long; swelling 1–2.5 cm. long and declined 70°–90°.

Several things are evident from these experiments. The smoke of paper and tobacco cigarettes is still very toxic after thorough washing with 15 per cent H_2SO_4 and 40 per cent NaOH. Later data will show that the constituents thus washed out have a comparatively low magnitude of toxicity. It becomes probable then that the high toxicity of paper and tobacco smoke is determined by the dry distillation carbon-bearing gases. Figuring on the basis of 10-liter containers, 10 cc. of cigar smoke (output of 0.0075 gm.), 40 cc. of cigarette smoke (output of 0.024 gm.), and 10 cc. paper cigarette smoke (output of 0.0035 gm.) give the third response mentioned by NELJUBOW (reduced rate of elongation, swelling, and horizontal position of the swollen part).

On the basis of the amount of washed smoke necessary to give the response, the cigar and paper cigarette smokes are about equally effective, and the tobacco cigarette smoke about one-fourth as

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effective; while on the basis of the dry weight necessary to produce the smoke, the paper is more than twice and the cigarette only about one-third as effective as the cigar. The toxicity of the several smokes undoubtedly depends in part on the oxygen supply during the smoking. This will determine to some degree the amount of dry distillation gases escaping oxidation, although much of these will escape oxidation under any condition, for the heat is sufficient beyond the ignited portion of the cigar or cigarette to cause dry distillation, and here no burning of the dry distillation gases can occur. When the same paper used in making the paper cigarettes was burned as an open sheet in the 10-liter container, it required 0.14–0.21 gm. to give NELJUBOW's third response. When burned in this way, the paper smoke is approximately 0.02 as toxic for this seedling. Burning the open sheet insures a more complete oxidation of the dry distillation carbon-bearing gases, both because of better oxygen supply and because of surer contact with the flame. It should be stated that the paper cigarettes used, though rolled only to moderate tightness, were difficult to smoke in the machine. They required considerable more suction than the cigarette and probably had low oxygen supply, as later analyses will indicate.

Experiment III.—Effect of washed smoke and chemical analyses

Two paper cigarettes, one loosely rolled and the other tightly, were smoked and washed with 15 per cent H_2SO_4 and 40 per cent NaOH and stored separately. The analyses of this sort of smoke according to the methods described by HEMPEL (**14**) are as follows:

Loosely wrapped paper cigarette; 4.78 gm. of paper smoked, with a yield of 8384 cc. of washed smoke.

	A	B
Volume of smoke taken for analysis	99.6 cc.	99.9 cc.
Volume after absorption with 40 per cent NaOH	99.6	99.9
Volume after absorption with bromine	99.5	99.9
Volume after absorption with phosphorus	99.5	99.9
Volume after absorption with ammoniacal cuprous chloride	84.4	85.0
Volume of CO	15.1	14.9

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This analysis gives a trace of heavy hydrocarbons (bromine-absorbed gases) and approximately 15 per cent of carbon monoxide. The figures show that each gram of paper produced 263 cc. of CO. This yield is very high when compared with the yield from tobacco in cigarettes, cigars, and pipe as reported by LEHMANN (**18**). In these it varies from 15 to 101 cc. of CO per gram of tobacco. Our figures may be a little high, due to incomplete drying of the stub before weighing.

Tightly wrapped paper cigarette. In this case 3.41 gm. of paper produced 7357 cc. of washed smoke.

Analysis	A	B
Volume of smoke taken for analysis	97.6 cc.	92.4 cc.
Volume after absorption with 40 per cent NaOH.	95.8	90.6
Volume after absorption with phosphorus	95.8	90.6
Volume after absorption with bromine water	95.8	90.6
Volume after absorption with ammoniacal cuprous chloride	81.1	77.0
Volume of CO	14.7	13.6

In this analysis considerable CO_2 appears. On the basis of the CO_2-free smoke the CO constitutes 15+ per cent, approximately the same percentage shown in the analysis of the loosely rolled cigarette. There is not a measurable amount of heavy hydrocarbons, but in an analysis like this, where no corrections are made for temperature changes, it is possible to leave undetected 0.2 cc. In this case each gram of paper produced 327 cc. of CO, an even higher yield than given by the loosely rolled cigarette. This too is probably a little high, due to insufficient drying before weighing the stub back.

Effect on the seedlings.—At the beginning of the experiment the epicotyls were 2–3 cm. tall. The cultures were sealed in 10-liter cans and subjected to the smoke for 3 days. The following data show the nature and concentration of the smoke used, along with the condition of the seedlings at the close of the experiment.

1. Check; epicotyls 5–13 cm. tall, vertical and slim.

Washed smoke from loosely rolled paper cigarette

2. 10 cc.; epicotyls 3.5–5 cm. long; swollen portion 1–2 cm. long, with declination of 75°–90°.

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3. 20 cc.; epicotyls 3–4.5 cm. long; swollen portion 1–1.5 cm. long, with declination of 75°–90°.

4. 50 cc.; epicotyls 3–4 cm. long; swollen portion 0.5–0.75 cm. long, with little or no declination.

Washed smoke from tightly rolled paper cigarette

5. 10 cc.; epicotyls 3–4.5 cm. long; swollen portion 1–1.5 cm. long, with declination of 70°–90°.

6. 20 cc.; epicotyls 3–4.5 cm. long; swollen portion 0.75–1.5 cm. long, with declination of 75°–90°.

7. 50 cc.; epicotyls 3–4 cm. long; swollen portion 0.5–0.75 cm. long, with little or no declination.

It appears from these experiments that the tightly rolled cigarette gives slightly the more toxic smoke, though not markedly

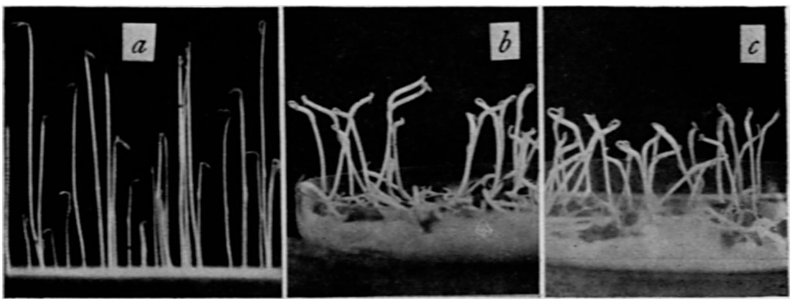

FIG. 2.—Responses to paper smoke: *a*, check; *b*, response to 10 cc. of smoke from loosely rolled paper cigarette in 10-liter chamber; *c*, response to 20 cc. of smoke.

so. In both sorts of smoke 10–20 cc. in 10 liters gives NELJUBOW'S third response, while 50 cc. in 10 liters gives his second response. It is apparent that considerable variation in concentration is necessary before a noticeable difference in response is shown. Even doubling the concentration may modify the response only slightly. This condition holds for ethylene, carbon monoxide, and a number of other gases, as our later paper will show. On the other hand, with pyridine, ethyl and propyl alcohol, acetylene, and others, rather slight variation in concentration is evidenced by very noticeable differences in the response. A series of photographs will give a more vivid idea of the response to smoke. Figs. 2 and 3 show the response to various concentrations of paper smoke.

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Experiment IV.—Effect of bromine absorption on the toxicity of paper smoke

In this experiment the washed smoke from the loosely rolled paper cigarette was absorbed with bromine by the gas analysis method described by HEMPEL. All traces of bromine were later absorbed by washing with 40 per cent NaOH. As is well known, bromine absorbs the heavy hydrocarbons (acetylene, ethylene, etc., with their higher homologues). It does not absorb methane or carbon monoxide. If the substance determining the toxicity of this smoke is one of the heavy hydrocarbons, this treatment should reduce the toxicity. If it is carbon monoxide or methane, it should not greatly modify the toxicity. The cultures were sealed in 10-liter chambers and subjected to the various sorts and concentrations of smoke for three days. The data show the condition of the epicotyls at the close of the experiment.

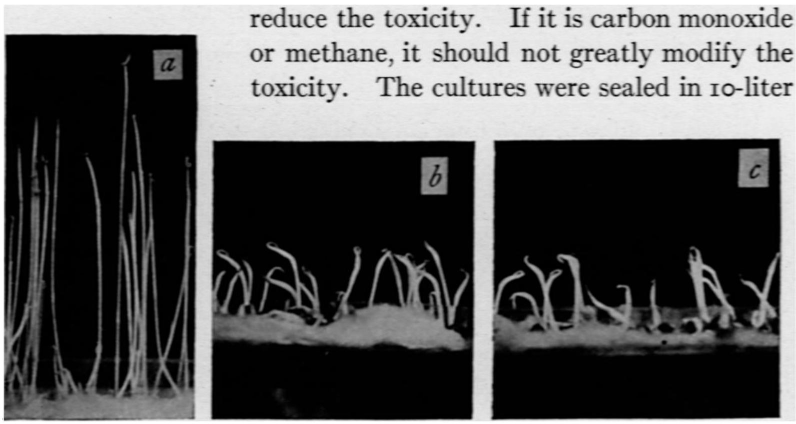

FIG. 3.—Responses to paper smoke: *a*, check; *b*, response to 20 cc. of smoke from tightly rolled paper cigarette in 10-liter chamber; *c*, response to 50 cc. of smoke.

1. Check; epicotyls 5–11 cm. tall, vertical and slim.

2. 20 cc. washed smoke not absorbed with bromine; epicotyls 3–4.5 cm. long; swollen portion 1–1.5 cm. long, with declination of 75°–90°.

3. 25 cc. (duplicates) washed smoke absorbed with bromine; epicotyls 6–12 cm. tall, vertical and slim.

4. 85 cc. washed smoke absorbed with bromine; epicotyls 4–9 cm. tall, vertical and slim.

5. 69 cc. washed smoke absorbed with bromine; epicotyls 4–9 cm. tall, slim and straight.

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6. 92 cc. washed smoke absorbed with bromine; epicotyls 3–7 cm. long; no swelling and little declination.

7. 550 cc. washed smoke absorbed with bromine; epicotyls 3–5 cm. long; swelling 2–3 cm.; declination 10°–60°.

It is evident from this series of cultures that absorption with bromine greatly reduces the toxicity of paper smoke and shows that substances belonging to the heavy hydrocarbons are the ones determining the toxic limit. The response given with 550 cc. of bromine-washed smoke is probably due to the CO, for that amount of smoke contains about 82 cc. of CO.

Experiment V.—The effect of coal smoke

The smoke was withdrawn from the furnace of a large flat building on a cold day, when large volumes of soot-free air were pouring from the chimney. The smoke showed the following analysis.

Volume analyzed	93.6 cc.	99.3 cc.
After NaOH	92.2	97.6
Difference = CO_2+SO_2	1.4	1.7
	92.2	97.6
After phosphorous	75.8	80.2
Difference = O_2	16.4	17.4
	75.8	80.2
After ammoniacal cuprous chloride	75.6	79.9
Difference = CO	0.2	0.3

This smoke contained about 1.6 per cent of CO_2 and SO_2 together; long storage over water had probably reduced somewhat the percentage of these gases. The oxygen was reduced to a little less than 18 per cent, while only a trace of CO was present.

The following data report the results from exposing the test seedlings to various concentrations of this smoke. Part of these cultures were run in water-sealed bell jars and part of them in cans. At the beginning of the experiment the epicotyls were 2–3 cm. long, and after every exposure were slim and straight.

1. Control; epicotyls 6–12 cm. tall.
2. 20 cc. in 10-liter chamber; epicotyls 6–13 cm. tall.

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3. 50 cc. in 10-liter chamber; epicotyls 6–13 cm. tall.
4. 500 cc. in 10-liter chamber; epicotyls 5–10 cm. tall.
5. One liter in 10-liter chamber; epicotyls 4–9 cm. tall.
6. Three liters in 6 liters; epicotyls 3–7 cm. tall.
7. Two liters in 6 liters; epicotyls 3–7 cm. tall.

It is evident that the chimney smoke is very slightly toxic. In one-half an atmosphere of this smoke the epicotyl is less inhibited in growth than in one part in 1000 of the smoke from the loosely rolled paper cigarette. This shows that the latter smoke is more than 500 times as toxic as the coal smoke used. In commercial furnaces it is customary to supply just enough air to oxidize completely all gases. Any considerable excess adds to the volume of heated air passing out of the chimney and to an economic loss from this source. It is in this that the flat-owner can be criticized rather than the point in question, the addition of poisonous carbon-bearing gases to the air, for his furnace was receiving about 10 times the volume of air necessary to give complete combustion. High oxygen supply probably accounts for the small amount of reduced carbon-bearing gases and for the low toxicity of the smoke. It is an open question in commercial furnaces, where there is little excess of oxygen, whether there is a sufficient amount of these gases to play any part in the injury of vegetation, as SEARLE (**41**) has suggested. In general, the injury from coal smoke has been attributed entirely to tars and the oxides of sulphur. It is certain, however, that carbon-bearing gases, especially ethylene, might be in sufficient concentration to do injury and still be in too small quantities for detection by chemical methods (**14**, p. 257). A full discussion of this point is given in the last section of this paper.

Effect of the various constituents of smoke

The experiments already recorded afford evidence that the heavy hydrocarbons determine the toxic limits of tobacco and paper smokes. It is desirable, however, to know the magnitude of toxicity of the several constituents, also the nature of the response produced in the epicotyl by each. Moreover, it is desirable to determine the particular hydrocarbon responsible. For paper smoke this will demand the study of ethylene, propylene, acetylene,

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methane, and carbon monoxide, and in tobacco smoke pyridine, ammonia, hydrocyanic acid, and nicotine in addition, leaving entirely out of consideration the tars which have a relatively low toxicity where only their vapors are involved, and probably no such a magnitude of toxicity under any conditions as several of the carbon-bearing gases. Of these substances we will give detailed experiments only on carbon monoxide, hydrocyanic acid, and nicotine. For the other substances it will suffice merely to cite a portion of a table to appear in one of our later papers, along with the details from which it is derived. We consider the details on carbon monoxide here because it is the more abundant toxic gas and because MOLISCH suggests that it may be the one rendering the smoke so toxic to seedlings.

Experiment VI.—Effect of carbon monoxide

It is first desirable to make sure that the carbon monoxide used is free from the noxious gases, or at least that the effect produced is due to the contained CO and not to some impurity. For this reason the carbon monoxide was generated by three different methods and the three products compared as to their effects. It is assumed that if equal amounts of the three sorts of gas produce equal effects, the effect is due to the CO and not to impurities. Since the heavy hydrocarbons are so toxic, it was thought well to see whether washing the CO in bromine would reduce the toxicity.

When oxalic acid was heated with several times its weight of concentrated H_2SO_4 and washed with 40 per cent NaOH, a gas resulted which gave (duplicate analysis) 99 per cent absorption with ammoniacal cuprous chloride. Potassium ferrocyanide was heated with 8–10 times its weight of concentrated H_2SO_4 and washed with 40 per cent NaOH. In duplicate this showed 96 per cent CO. Sodium formate was heated with concentrated H_2SO_4 and produced a gas giving 89 per cent absorption with ammoniacal cuprous chloride.

The epicotyls were 2.5–3.5 cm. tall at the beginning of the experiment and were inclosed in 10-liter cans. The following data show the sources and concentrations (correcting for impurities) of CO used, and the condition of the seedlings at the close of the experiment.

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1. Check; epicotyls 6–11.5 cm. tall, vertical and slim.

a) Potassium ferrocyanide-derived CO

2. 50 cc.; epicotyls 4–6.5 cm. long, with declination of 20°–45°; no swelling, but larger diameter than checks.

3. 100 cc.; epicotyls 3–5 cm. long; swelling 1–2 cm. long, with declination of 70°–90°.

4. 200 cc.; epicotyls 2.5–4.5 cm. long; swelling 1–1.5 cm. long, with declination 80°–90°.

5. 100 cc. washed in bromine; epicotyls 3–5.5 cm. long; swelling 1–2.5 cm. long; declination mostly 30°–60°.

6. 200 cc. washed in bromine; epicotyls 2.5–4 cm. long; swelling 1–2 cm. long; declination 60°–90°.

b) Oxalic acid-derived CO

7. 50 cc.; epicotyls 4–7.5 cm. tall; no swelling, but diameter larger than checks; declination 25°–35°.

8. 100 cc.; epicotyls 3–5.5 cm. long; swelling 1–2.5 cm. long; declination 60°–90°.

9. 200 cc.; epicotyls 3–5 cm. long; swollen zone 1–1.5, with declination 75°–90°.

c) Sodium formate-derived CO

10. 50 cc.; epicotyls 5–7 cm. long; no swelling, but declination 15°–40°.

11. 100 cc.; epicotyls 4.5–5.5 cm. long, with swelling 1.5–2.5 cm. long, and declination 60°–90°.

12. 200 cc.; epicotyls 3–5 cm. long; swollen zone 1–2 cm., with declination 70°–90°.

A series of photographs will show the response to CO (oxalic acid-derived) of various concentrations (fig. 4). It is evident that the responses obtained in this experiment are due to the CO contained in the gases and not to impurities. When the data and figures on CO are compared with those on smoke from paper cigarettes, it is seen that the smoke is about 10 times as toxic as pure CO; 10 cc. of the smoke give responses similar to 100 cc. of CO, and 20 cc. of the smoke similar to 200 of CO. Since the smoke is approximately 15 per cent CO, it contains only about $\frac{1}{60}$ enough CO to determine its toxicity. This helps to substantiate the conclusion that the heavy hydrocarbons determine the toxic limit of paper and tobacco smoke, so far as the plant organ studied is concerned.

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Various amounts of nicotine (1–30 drops) were placed on filter papers and sealed in 10-liter cans with cultures. Only the smaller amounts completely volatilized, but in none was any inhibition of growth noticeable. The test seedlings in culture cans of various sizes, bearing several grams of pulverized KCN, showed no inhibition of growth. In this connection it should be stated that HCN exists in such small quantities in tobacco smoke (0–60 mg. from 100 gm. of tobacco) that LEHMANN (18) does not take it into consideration as a toxic factor with the smoker. It is certainly much less to be considered in plants to which it is rather slightly toxic.

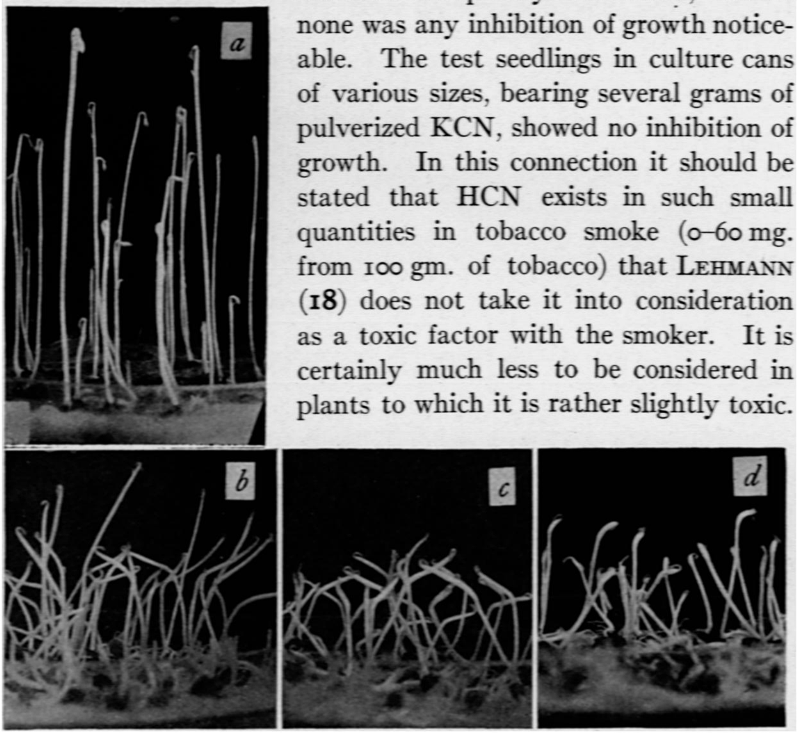

FIG. 4.—Responses to CO: *a*, check; *b*, 50 cc. of CO in 10 liters; *c*, 100 cc. of CO in 10 liters; *d*, 200 cc. of CO in 10 liters.

The following table (p. 362) shows the nature of the responses caused by other constituents of tobacco and paper smoke and the concentrations necessary to produce them.

In this table inhibition of growth corresponds to the sixth response of NELJUBOW, declination to the fifth, and horizontal nutation and swelling to the third. Only 4 of the 30 or more common gases and vapors whose effect on this organ we have studied certainly produce "declination" or "horizontal nutation and swelling." They are ethylene, acetylene, propylene, and carbon monoxide. This does not include the mixtures of gases (illuminating gas

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and various smokes) in which the response is quite certainly due to one of these constituents. It also omits methane, for which it is not yet certain whether the response produced by the very high concentration given above is due to methane or to impurities. This uncertainty exists because methane derived by three different methods gave extremely great differences in magnitude of toxicity, though analyses showed the three sorts to contain approximately the same percentages of methane. At most, methane is very slightly toxic, if indeed further experiments do not prove it entirely harmless.

Gas used	Parts per million of atmosphere to produce		
	Inhibition of growth	Declination	Horizontal nutation and swelling
Ethylene	0.1	0.2	0.4
Acetylene	100.0	250.0	500.0
Propylene	75.0	1000.0	1000.0
Carbon monoxide	5000.0	5000.0	10,000.0
Methane	60,000.0?	200,000.0?	500,000.0?
Pyridine	300.0	None	None
Hydrogen sulphide	500.0	None	None
Ammonia	3000.0	None	None

The nature of the response (triple response) of the seedling to paper and tobacco smoke shows that it must be caused by one of the four carbon-bearing gases mentioned above or by homologues of some of them. Before going into the probability as to which of these determine the effective limit of the smoke, let us consider the concentrations of other constituents in unwashed tobacco smoke and the chances that they may play some part, at least, in the inhibition of growth, in experiments such as reported in this paper or those performed by MOLISCH.

According to LEHMANN (**18**) 100 gm. of tobacco when smoked in a pipe or as a cigar produces about 16 cc. of H_2S. In experiment II (3) in which the smoke from 0.0375 gm. of cigar is placed in a 50-liter can, NELJUBOW's third response appears. The H_2S content in this experiment, if it had not been washed out, would be 1 part in 8 million of atmosphere, or about 0.0002 sufficient to reduce growth. According to the same worker, 100 gm. of tobacco when thus smoked produces 0.935 mg. of NH_3. In experiment II

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(3), according to these figures, if the smoke had not been washed, ammonia would have been present in the proportion of 10 parts per million of atmosphere, or in about 0.003 sufficient concentration to inhibit growth. Pyridine exists in very small quantities in tobacco smoke, certainly far below amounts that would inhibit growth in experiment II (3).

Both the nature of the response of the seedling and the concentration of the smoke necessary to produce it indicate that one of the four carbon-bearing gases mentioned above or homologues of some of them fix its toxic limit. We have shown that it cannot be carbon monoxide on account of insufficient quantities of that substance. In what percentages of the smoke must the others exist to determine its toxicity? Let us consider experiment III (2) of washed smoke of the loosely rolled paper cigarette, almost as toxic as any tested. In this case it required 10 cc. of the smoke in 10 liters to give NELJUBOW'S third response, or the response listed in the table above as "horizontal nutation and swelling." Using the figures in the table above as the basis for calculation, ethylene must be present in 0.04 per cent, acetylene 50 per cent, and propylene 100 per cent of the smoke, to determine its toxicity. In experiment III (2) under discussion, the heavy hydrocarbons were not in sufficient concentration to be detected by the gas analysis methods used, which should easily detect 0.2 per cent. If one of these three gases is responsible, it must be the ethylene. In short, the sweet pea seedling will give the triple response in concentrations of ethylene 0.001–0.002 sufficient to be detected by gas analysis methods, while it will respond by reduced growth in concentrations 0.0003 to 0.0005 sufficient to be thus detected.

It is possible that in tobacco burned in the open, as is done when using it as an insecticide in greenhouses, the ammonia is produced in larger quantities as compared with the heavy hydrocarbons, and that ammonia much more nearly approaches the toxic limit. We have already shown that paper burned as a cigarette produces smoke 50 times as toxic as when burned as an open sheet. This means a great fall in the production of heavy hydrocarbons under conditions of high oxygen supply. There is probably also less ammonia produced when the aeration is better, for LEHMANN

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(18) found that in cotton cigarettes impregnated with nitrates much of the nitrate nitrogen was reduced to ammonia nitrogen. Such reductions likely occur to a much slighter degree under better conditions of aeration. Similar conditions may hold for hydrogen sulphide.

V. General considerations

In the destructive distillation gases from carbon compounds, whether we consider smoke or illuminating gas, the preponderant toxicity of the heavy hydrocarbons, especially ethylene, is very interesting. The present paper shows this relation to hold for the sweet pea epicotyl, while a former paper pointed out the same situation for the carnation flower. Mr. E. M. HARVEY of this laboratory has shown that the ethylene in illuminating gas determines the toxic limit of that mixture to the roots of *Vicia Faba*, though in this case the magnitude of toxicity is much less than in the cases of the two plant organs mentioned above. In the light of the facts set forth in this paper, it becomes probable that the extreme toxicity of smoke for seedlings observed by MOLISCH (22) can be attributed to the heavy hydrocarbons. It is as yet unanswered whether the nocuous character of smoke to various micro-organisms, and to the organs of mature angiosperms as observed by this writer, is due to the same constituent. Whatever be the case, it is clear that some plants are quite resistant to the destructive distillation gases of carbon compounds, as MOLISCH states and as RICHARDS and MACDOUGAL (31) have found. To what degree the resistance is due to protective structure or permeability characters and to what degree to peculiarities of the plasma cannot be stated.

It is probable that production of the toxic materials from carbon compounds begins considerably before the lower temperature limit set for destructive distillation is reached. In soils it was found in this laboratory that heating but slightly above 90° C. for an hour liberated substances that produced the "triple response" in the pea epicotyl. Mr. HARVEY is now making a study of the gases liberated from soils when heated at various temperatures. It is evident that contact of hot steam pipes with soil in greenhouses

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may produce gases very toxic to plants. Whether they are likely to reach sufficient concentration to do injury is not determined.

Injuries from coal smoke are generally attributed to tars and oxides of sulphur (**3, 4, 7, 40,**), while reduced carbon-bearing gases have never been considered as a factor. According to HEMPEL (**14**, p. 257), these gases, especially the heavy hydrocarbons, exist in such small quantities, if at all, even when the oxygen supply is very little more than enough to produce complete oxidation, that they cannot be detected by gas analysis methods. This does not mean that they can be neglected as a source of injury to vegetation, for, as we have shown, growth rate is reduced in the pea epicotyl in 0.0003–0.0005, the least concentration of ethylene detectable by gas analysis methods. In short, while the gas analysis methods are quite adequate for guarding against considerable energy loss due to incomplete combustion of heavy hydrocarbons in furnaces, the only way to make sure that they are not in sufficient concentration to do injury to vegetation is to use a more delicate test, such as the pea epicotyl.

One factor that favors the effectiveness of the oxides of sulphur as plant poisons in the open as against heavy hydrocarbons is their great solubility in the plant cell, which would lead to their accumulation even under great variation in the atmospheric concentration, whereas the heavy hydrocarbons will accumulate to a far less degree, and variations in concentration greatly reduce their injurious effects. It is probable that smoke from the beehive coke oven is much richer in heavy hydrocarbons than furnace smoke, especially in the early firing (**2**). Part of the destruction of vegetation about these may be due to the carbon-bearing gases, though here, as in furnace smoke, there is an abundance of sulphur dioxide and tars. The economic loss through injury to vegetation is probably rather slight, because of the nature of the region in which this industry is carried on. It certainly is inconsiderable beside the $44,000,000 worth of products this wasteful method of coking is pouring into the atmosphere annually in the United States alone (**19**).

Artificial illuminating gas is a source of great economic loss through injury to plants. A large number of cases of injury to greenhouse stock in different parts of the country have been called

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to our attention. As we have already pointed out (6), these losses generally occur during cold periods in winter. This insures a frozen crust, promoting lateral diffusion of the gas from the faulty mains; it also prevents ventilation of the greenhouses. So far as evidence for the constituents that produce the injury goes, it suggests the heavy hydrocarbons, though it is not by any means proved that these are responsible for all such injuries. A source of greater loss from illuminating gas is injury to shade trees. This injury is through the roots, in contrast to the injury in greenhouses. We are unable to state as yet what constituents produce the injury, though Mr. HARVEY'S work in this laboratory indicates that ethylene determines the toxic limit of the gas for the roots of *Vicia Faba*. Even so, it is possible that the less volatile materials of the gas accumulating in the soil may really be the source of injury, the power of accumulation overbalancing the higher toxicity. The determination of the constituents producing the injury and the tenacity with which they adhere to the soil are of great importance. They determine how soon and under what conditions replacing of the dead trees by new ones can be carried out. Mr. HARVEY is now attempting to answer these questions. The odor-producing substances of illuminating gas are retained in the soil with great tenacity, but so far as the pea epicotyl is concerned, these substances are innocuous, at least in concentrations easily detected by smell. Natural gases are generally low in heavy hydrocarbons; in fact, those of the Appalachian system bear none so far as chemical tests indicate (1); they consist mainly of methane and ethane. This gas should be very low in its toxicity to plants. The Baku natural gas is said (30) to contain some olefines.

The few facts established in this field suggest the need of rationalizing various practices in vogue and summarily abolishing others; for instance, the practice of burning tobacco stems in greenhouses for killing insects. This is a matter of differential poisoning, applying a poison that will kill the insect without injuring the plants. The processes volatilize nicotine and set free carbon monoxide, ethylene, and other gases. So far as we know, it is not certain which is the insecticide. If it is nicotine, why not volatilize nicotine from an extract and avoid the deadly plant poison ethylene?

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If it is carbon monoxide, why not generate it chemically and thereby avoid ethylene? Again, it is a rather common practice to have the heating furnace in more or less open connection with the greenhouses. If one recognizes the probability of the dry distillation gases escaping from a furnace, along with the extreme toxicity of ethylene, he can see the need of abolishing this practice.

So far as known, the etiolated epicotyl of the sweet pea is the most sensitive plant organ to ethylene. As has been stated, it is inhibited in growth by 1 part in 10,000,000 of atmosphere. The open flower of the carnation is only a little less sensitive. In our original measurements (**6**), which were made on plants that had been bearing flowers for several months, 1 part in 2,000,000 of atmosphere "put the open flower to sleep" in 12 hours. Some later measurements with plants soon after they had begun to flower showed that 1 part in 3,000,000 of atmosphere caused the same response. While the open flowers on these younger plants were much more sensitive to ethylene, the buds proved much more resistant than the buds of plants longer in bearing.

If, in the few cases tested, two such sensitive plant organs have been found, it is probable that many more exist. So far as we know, there is in nature no special absorbent for ethylene, also no cycle for the gas, as there is for carbon dioxide and oxygen. Even if both existed, one doubts if 1 part in 10,000,000 would lead to a withdrawal. Processes of civilization are continually adding to the ethylene in the atmosphere, as burning of all carbohydrates, burning of coal (?), escaping of artificial illuminating gas, producing of gas in the beehive method of coking, escaping of certain sorts of natural gas, and probably other processes. Having no estimate of the total additions from these sources, one cannot calculate whether accumulation in the atmosphere up to a danger point is likely to occur.

The etiolated epicotyl of the sweet pea is a very delicate test for the heavy hydrocarbons, especially ethylene. One of the papers to be published later will show that under proper application it is also a very reliable test for this group of substances. It could be used to determine the presence or absence of this group of gases in coal smoke, gas from coal (**28**), and natural gas, where gas

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analysis methods are inadequate. To the experimenter in plant physiology it furnishes an excellent means of making sure that the laboratory air is sufficiently "pure" not to interfere with plant response, while to the practical greenhouse man it furnishes a means of determining the probability of injury from illuminating gas or other mixtures bearing ethylene.

Summary

1. The smoke from tobacco cigars and cigarettes which has been thoroughly washed in 15 per cent H_2SO_4 and 40 per cent NaOH is very toxic to the etiolated epicotyl of the sweet pea. In the case of cigar smoke thus treated, 1000 parts per million of atmosphere give a triple response: reduction of rate of elongation, swelling, and diageotropism of the portion growing in the impurity; 5000 parts per million of atmosphere completely stop elongation and produce a swollen knob, while the epicotyl remains vertical; still higher concentrations kill the epicotyl before any form change occurs.

2. On the basis of dry weight burned, the washed smoke from cellulose paper cigarettes is even more toxic. The characters of the responses produced are identical with those produced by smoke from tobacco cigars and cigarettes.

3. When smoke from equal amounts of cellulose paper, smoked as a cigarette on one hand, and burned as an open sheet on the other, are compared, it is found that the former is 50 times as toxic as the latter. Higher oxygen supply during burning greatly reduces the toxicity. A large part of the toxic gases are undoubtedly oxidized to CO_2 and H_2O.

4. In the cigarette smoke of cellulose paper the following gases are present: carbon dioxide, carbon monoxide, acetylene, ethylene, methane, and some higher homologues of the last three. Washing out the carbon dioxide does not reduce the toxicity of the smoke, nor will carbon dioxide produce the type of response produced by the smoke. Carbon monoxide, acetylene, ethylene, propylene, and perhaps methane produce the same type of response as smoke. Carbon monoxide is in 0.015 sufficient concentration to determine the effect of smoke. It is not certain that methane is toxic at all;

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if so, it is not in 0.00001 sufficient concentration to produce the response. The other three gases mentioned are not present in the smoke in sufficient quantities to be detected by ordinary gas analysis methods. Considering the magnitude of toxicity of acetylene and propylene, it is impossible that they play any part in the toxicity of paper smoke. The great toxicity of ethylene makes it probable that it determines the toxic limit. One part of ethylene in 10,000,000 of atmosphere inhibits elongation of the epicotyl, 4 parts in 10,000,000 produce the triple response. The toxicity of paper smoke is greatly reduced by washing with bromine, which is further evidence that ethylene or some other heavy hydrocarbon is the effective gas.

5. In addition to these gases, tobacco smoke bears hydrogen sulphide, ammonia, nicotine, hydrocyanic acid, and pyridine. None of these produces the type of response in the seedling caused by the smoke, and they exist in the smoke in concentrations far below that necessary to determine the toxic limit. The facts stated in this paper, along with the work of MOLISCH and others, show the hazard of using tobacco smoke as an insecticide for greenhouses.

6. The etiolated epicotyl of the sweet pea is a very delicate test for the heavy hydrocarbons (ethylene), exceeding many fold the delicacy of any chemical test.

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LITERATURE CITED

1. ALLEN, I. C., and BURRELL, G. A., Liquefied products from natural gas; their properties and uses. U.S. Bur. of Mines (technical paper) **10**:1–28. 1912.
2. BAUERMANN, HILARY, Coke in Encyclopaedia Britannica (11th ed.) **6**:655–657. 1910.
3. COHEN, J. B., and RUSTON, A. G., Smoke, a study of town air. pp. 1–88. Edward Arnold, London. 1912.
4. ———, The nature and extent of air pollution by smoke. Nature **81**:468–469. 1909.
5. COOK, M. T., and TAUBENHAUS, J. J., The relation of parasitic fungi to the contents of the cells of the host plant. I. The toxicity of tannin. Delaware Exp. Sta. Bull. **91**:1–77. 1911.
6. CROCKER, WM., and KNIGHT, L. I., Effect of illuminating gas and ethylene upon flowering carnations. BOT. GAZ. **46**:259–276. 1908.

This content downloaded from 130.133.008.114 on November 27, 2016 14:05:53 PM
All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-

7. CROWTHER, C., and RUSTON, A. G., Effect of atmospheric impurities near a large city. Journ. Agr. Sci. **4**:25–55. 1911.

8. GATIN, C. L., Die gegen die Abnutzung und den Staub der Strassen angewendeten Verfahren und ihre Wirkung auf die Vegetation. Zeitschr. Pflanzenkrank. **22**:193–204. 1912.

9. ———, Le goudronnage des routes et sou action sur la végétation avoisinante. Ann. Sci. Nat. Bot. **15**:165–252. 1912.

10. GRAFE, V., Untersuchungen über das Verhalten grüner Pflanzen zu gasformigen Formaldehyde. Ber. Deutsch. Bot. Gesells. **29**:19–26. 1911.

11. GRAFE, V., und RICHTER, O., Über den Einfluss der Narkotika auf die Anatomie und die chemische Zusammensetzung von Keimling. Anz. Kais. Akad. Wiss. (Wien) Math. Nat. Kl. **48**:536–538. 1911.

12. GROTTIAN, WALTER, Beiträge zur Kenntniss des Geotropismus über den Einfluss einiger anästhesierender Substanzen auf das Wachstum und den Geotropismus der Wurzeln. Beih. Bot. Centralbl. **24**:255–285. 1909.

13. GUTTENBERG, H. R. v., Über das Zusammenwirken von Geotropismus und Heliotropismus und die tropistische Empfindlichkeit in reiner und unreiner Luft. Jahrb. Wiss. Bot. **47**:462–492. 1910.

14. HEMPEL, WALTHER, Methods of gas analysis (translation from the third German edition by L. M. DENNIS). xix+490. New York: Macmillan. 1906.

15. KNIGHT, L. I., ROSE, R. C., and CROCKER, WM., A new method of detecting traces of illuminating gas. Science N.S. **31**:636. 1910.

16. ———, Effect of various gases and vapors upon the etiolated seedling of the sweet pea. Science N.S. **31**:635–636. 1910.

17. KÖRNICKE, M., Weitere Untersuchungen über die Wirkung von Röntgen- und Radiumstrahlen auf die Pflanzen. Ber. Deutsch. Bot. Gesells. **23**: 324–333. 1905.

18. LEHMANN, K. B., Chemische und toxikologische Studien über Tabak, Tabakrauch, und das Tabakrauchen. Archiv. für Hygiene **68**:319–420. 1908–1909.

19. MOLDENKE, RICHARD, The coke industry of the United States as related to the foundry. U.S. Bur. of Mines Bull. **3**:1–32. 1900.

20. MOLISCH, H., Über Heliotropismus im Bacterienlichte. Sitzungsber. Kais. Akad. Wiss. (Wien) Math. Nat. Kl. **111**:141–148. 1902.

21. ———, Leuchtende Pflanzen. viii+1908 (citation pp. 170–171). Jena: Gustav Fischer. 1912.

22. ———, Über den Einfluss des Tabakrauches auf die Pflanze. Teil I. Sitzungber. Kais Akad. Wiss. (Wien) Math. Nat. Kl. **120**:3–30. 1911.

23. ———, Über den Einfluss des Tabakrauches auf die Pflanze. Teil II. *Op. cit.* **120**:813–838. 1911.

24. ———, Die Pflanze und Tabakrauch. Umschau **15**:259–264. 1911.

25. NELJUBOW, D., Über die horizontale Nutation der Stengel von *Pisum sativum* und einiger anderen Pflanzen. Beih. Bot. Centralbl. **10**:128–139. 1901.

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All use subject to University of Chicago Press Terms and Conditions (http://www.journals.uchicago.edu/t-and-

26. ———, Geotropismus in der Laboratoriumsluft. Ber. Deutsch. Bot. Gesells. **29**:97–112. 1911.

27. Němec, B., Über die Einwirkung des Chloralhydrates auf die Zell- und Kernteilung. Jahrb. Wiss. Bot. **39**:645–730. 1903.

28. Porter, H. C., and Ovitz, F. K., The escape of gas from coal. U.S. Bur. of Mines (technical paper) **2**:1–14. 1911.

29. Prianischnikow, D., Zur Frage der Asparaginbildung. Ber. Deutsch. Bot. Gesells. **22**:35–43. 1904.

30. Redwood, Sir Boverton, Natural gas in Encyclopaedia Britannica **19**: 274. 1910.

31. Richards, H. M., and MacDougal, D. T., The influence of carbon monoxide and other gases upon plants. Bull. Torr. Bot. Club **31**:57–66. 1904.

32. Richter, O., Pflanzenwachstum und Laboratoriumsluft. Ber. Deutsch. Bot. Gesells. **21**:180–194. 1903.

33. ———, Über den Einfluss verunreinigter Luft auf Heliotropismus und Geotropismus. Siztungsber. Kais. Akad. Wiss. (Wien) Math. Nat. Kl. **115**:265–352. 1906.

34. ———, Die horizontale Nutation. *Op. cit.* **119**:1052–1084. 1910.

35. ———, und Grafe, V., Über den Einfluss der Narkotika auf die Anatomie und die chemische Zusammensetzung von Keimlingen. Verh. Gesells. Deutsch. Narutf. und Ärtze **80**:189. 1908.

36. Richter, O., Über Turgorsteigerung in der Atmosphäre von Narkotika. Lotos **56**:106–107. 1908.

37. ———, Neue Untersuchungen über Narkose im Pflanzenreiche. Mitt. Naturwiss. Ver. Univ. Wien **9**:14. 1911.

38. ———, Über Antocyanbildung in ihrer Abhängigkeit von äuseren Faktoren. Med. Klinik. **3**:1015–1020. 1907.

39. Rimmer, F., Über die Nutationen und Wachstumsrichtungen der Keimpflanzen. Sitzungsber. Kais. Akad. Wiss (Wien) Math. Nat. Kl. **89**:393–422. 1884.

40. Ruston, A. C., and Crowther, C., Impurities in the atmosphere of towns and their effects upon vegetation. Report Brit. Ass. Adv. Sci. 577–578. 1910.

41. Searle, J. M., Coal smoke and its practical abatement. Report Engineer's Soc. Pittsburgh. pp. 18–19 (meeting of June 18, 1912).

42. Singer, M., Über den Einfluss der Laboratoriumsluft auf das Wachstum der Kartoffelsprosse. Ber. Deutsch. Bot. Gesells. **21**:175–180. 1903.

43. Wiesner, J., Die undulierende Nutation der Internodien. Sitzungsber. Kais. Akad. Wiss. (Wien) Math. Nat. Kl. **77**:15–54. 1878.

44. Wilcox, E. M., Injurious effects of illuminating gas upon greenhouse plants. Ann. Report Neb. State Hort. Soc. pp. 278–285. 1911.

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