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REVIEW ARTICLE

Experimental plant research and the discovery of carbon dioxide-mediated global greening: a tribute to Wilhelm Pfeffer (1845–1920)

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Abstract

One century ago, the German chemist and botanist Wilhelm Pfeffer (1845–1920) died, shortly after finishing his last lecture at the University of Leipzig. Pfeffer was, together with Julius Sachs (1832–1897), the founder of modern plant physiology. In contrast to Sachs, Pfeffer's work was exclusively based on the principles of physics and chemistry, so that with his publications, notably the ca. 1.600 pages-long *Handbuch der Pflanzenphysiologie* (2. ed., Vol. I/II; 1897/1904), experimental plant research was founded. Here we summarize Pfeffer's life and work with special emphasis on his experiments on osmosis, plant growth in light vs. darkness, gravitropism, cell physiology, photosynthesis and leaf movements. We document that Pfeffer was the first to construct/establish constant temperature rooms (growth chambers) for seed plants. Moreover, he pioneered in outlining the carbon-cycle in the biosphere, and described the effect of carbon dioxide (CO_2)-enhancement on assimilation and plant productivity. Wilhelm Pfeffer pointed out that, at ca. 0.03 vol% CO_2 (in 1900), photosynthesis is sub-optimal. Accordingly, due to human activities, anthropogenic CO_2 released into the atmosphere promotes plant growth and crop yield. We have reproduced Pfeffer's classical experiments on the role of CO_2 with respect to plant development, and document that exhaled air of a human (ca. 4 vol% CO_2) strongly promotes growth. We conclude that Pfeffer not only acted as a key figure in the establishment of experimental plant physiology. He was also the discoverer of the phenomenon of CO_2-mediated global greening and promotion of crop productivity, today known as the "CO_2-fertilization-effect". These topics are discussed with reference to climate change and the most recent findings in this area of applied plant research.

Keywords Anthropogenic CO_2 · Carbon dioxide · Climate change · Global greening · Photosynthesis · Wilhelm Pfeffer

Abbreviations

CO_2	Carbon dioxide
O_2	Oxygen
H_2O	Water
Face	Free-air CO_2 enrichment
C3	Basic photosynthetic metabolism
NASA	National Aeronautics and Space Administration

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Introduction

Life on Earth depends on the photosynthetic activity of green plants, algae and cyanobacteria. These photoautotrophic producers enable the existence of heterotrophic organisms, such as fungi and animals, via sunlight-driven assimilation of carbon dioxide (CO_2), production of carbohydrates, and the release of molecular oxygen (O_2). As top-consumers in the food chain, humans strictly depend on primary agriculture, i.e., soil tilling, cultivation and growing/harvesting of tubers, seeds, fruits, etc. As pointed out in a recent *Review Article* published in this journal, secondary agriculture, i.e., the drying, sorting, preserving, storing and packing of fruits, seeds, vegetables etc. is of enormous economic importance (Yadav et al. 2020).

It is obvious that secondary agriculture depends on light-driven photosynthetic CO_2-assimilation in the green leaves of crop plants (Kutschera et al. 2010, 2020). Recently, an article entitled "Robust response of terrestrial plants to rising CO_2" (Cernusak et al. 2019) corroborated the hypothesis that "global greening", which is dominated by terrestrial ecosystems in China and India, is largely due to anthropogenic carbon dioxide emissions (Piao et al. 2020). However, in none of the articles published on "global photosynthesis (or greening)" thus far, the discovery of this phenomenon, which is of practical importance for primary agriculture, has been mentioned.

Five years ago, the life and work of Julius Sachs (1832–1897)—the founder of modern experimental plant physiology—was outlined in two articles (Kutschera 2015a, b). In these contributions, the achievements of Wilhelm Pfeffer (1845–1920) (Fig. 1), who died 100 years ago in Leipzig (Germany), were mentioned. In this context, the term "Sachs-Pfeffer-Principle of Experimental Plant Research" was coined, and Pfeffer's role as co-founder of plant physiology described. However, in contrast to the scientific work of Sachs, which has been analyzed extensively (Morton 1981; Kutschera and Niklas 2018a, b), the achievements of Pfeffer are much less known in the general scientific community. This is documented by the fact that on January 31, 2020, the 100th anniversary of Pfeffer's death, only one short pertinent paper was published (Goedecke 2020).

Over the past ten months, no other Pfeffer-obituary appeared in the scientific literature. Therefore, in this article, we recount the remarkable career and scientific contributions of this outstanding plant biologist/chemist, with special reference to Pfeffer's ignored work on photosynthesis, the CO_2-cycle in the biosphere, and global greening. The significance of Pfeffer's insights for plant physiology and biochemistry *today* is documented.

From pharmacist-chemist to the Herr Geheimrath Professor Pfeffer

Wilhelm Friedrich Philipp Pfeffer was born on March 9, 1845, in Grebenstein close to Kassel (Federal State of Hesse, Germany) as the son of a pharmacist. The typical old-time "Apotheker" used to be a curiosity-driven naturalist with an inborn tendency to study plants and animals. Accordingly, it is documented that "Willi Pfeffer" was educated by his father before he attended the gymnasium in Kassel, where he matured in the "Untersekunda" in 1860. Under the guidance of his parents, and a relative of his mother, Pfeffer started to collect and study plants at an early age. This interest in the flora of Germany (and the Swiss Alps) persisted throughout his life.

After finishing a first education as a pharmacist under the supervision of his father in Grebenstein, Pfeffer studied chemistry and botany at Göttingen, Marburg, Berlin and Würzburg. In 1865, when he was only 20 years old, Pfeffer earned his doctor's degree (Dr. phil. in chemistry) at the University of Göttingen (Pfeffer 1865). His first independent scientific publications were devoted to plant taxonomy, with a focus on mosses of the Swiss Alps. Over the subsequent two decades, Pfeffer was first an assistant in the laboratory of Julius Sachs at Würzburg (1870/71) and, after

Fig. 1 Portrait of Wilhelm Pfeffer (ca.1900) and title page of his *Handbuch*, vol. I, 2. ed. 1897. The stamp (Gregor Kraus) indicates that this issue of the book belonged to the successor of Julius Sachs, who was a Professor at Erlangen before he took over the chair of botany at Würzburg University. The hand-written signature reads "Prof. Dr. Pfeffer" (images taken from the private collection of U. Kutschera)

earning his habilitation, held university positions in Marburg (private lecturer), Bonn (assistant professor) and Basel (full professor). In 1878, he moved to the University of Tübingen, where he stayed for the next decade.

In 1887, the famous Professor Pfeffer accepted the position of "Ordinarius and Director of the Botanical Garden" at the university of Leipzig in the eastern part of Germany, where he continued his scientific career until his death. In 1904, he was awarded the title "Geheimrat", of which his wife was very proud. Over two decades, Pfeffer and the late Prof. Eduard Strasburger (1844–1912) of Bonn were the two most famous German botanists. Accordingly, over many years, they attracted to their respective Institutes numerous foreign students and visiting scientists, notably from the United States. In the "Pfeffer lab" they learned how to design experiments, generate data sets, and interpret these findings appropriately.

Like Julius Sachs, Pfeffer was a hard-working, dedicated scientist throughout his life. From early in the morning until late in the evening he was in his Institute, where he lectured, supervised students, took examinations, wrote scientific papers and books, and hosted guest scientists. In addition, in 1895, he took over, together with E. Strasburger, the Editorship of the *Jahrbücher für Wissenschaftliche Botanik* (Annual Books for Scientific Botany).

His final years during World War I (1914–1918) were characterized by financial and personal hardship, which resulted in health problems from which he did not recover. After Pfeffer's son (his only child) was killed in a WW I-battle on 15 September 1918 at the age of 34 years, he developed depressions. As detailed by Fitting (1920), the famous professor gave his last lecture on 30. January 1920, i.e., on the last day of the winter term. Since the University of Leipzig had just established a new law of mandatory retirement for all full professors at 75 years of age (Emeritierung), Pfeffer developed even more severe depressions, because science was his life. Accordingly, when he came back home from his lecture, he told his wife that it would be nice if he could die now. One day later, on 31 January 1920, at 5 p.m., Wilhelm Pfeffer died at home of a heart failure. He was survived by his widow, his daughter-in-law, and a grandson (Bünning 1975, 1977; Parker 2014).

The art of experimentation: Wilhelm Pfeffer's achievements

As noted above, Pfeffer earned his Ph.D. in chemistry and thereafter studied the systematics and biogeography of mosses (Pfeffer 1867). Fitting (1920) pointed out that his interest in the physiology of plants was elicited by his mentor J. Sachs, who offered him a position as an assistant in his laboratory at the University of Würzburg. Under the supervision of Sachs, Pfeffer studied the effect of light on photosynthesis. This work (Pfeffer 1871) was accepted at the University of Marburg as "Habilitationsschrift" (thesis for qualification as a lecturer). Accordingly, Pfeffer's academic career was strongly promoted by Sachs, a key figure in the plant sciences during the nineteenth century (Morton 1981; Kutschera and Niklas 2018a, b).

Although Sachs had developed numerous methods and machines for the study of basic physiological processes in plants (germination, growth in darkness vs. light, photosynthesis in aquatic plants etc., see Kutschera and Briggs 2009, 2012, 2013, 2016), it was the "chemical botanist" W. Pfeffer, who improved and perfected this research agenda, labelled as "Sachs-Pfeffer principle of experimental botany".

In his most famous, two-volume book *Pflanzenphysiologie. Ein Handbuch der Lehre vom Stoffwechsel und Kraftwechsel in der Pflanze*, Pfeffer pointed out that without physics and chemistry, we will never be able to understand the workings of living organisms. The first edition of the *Handbuch* was published in 1881. The second, much expanded version of this book (Fig. 1) appeared in two volumes (Pfeffer 1897/1904) and was a 620 plus 986, i.e., ca. 1,600 pages-long summary of all pertinent facts known at that time on the physiology of plants (inclusive of fungi and bacteria). The monograph was translated into French and English, and served as the most significant reference book on this subject over two decades.

As a basic tenet, Pfeffer (1897/1904) wrote that all living processes are based on physics and chemistry. Accordingly, Pfeffer strongly rejected the then-popular idea of a "Lebenskraft" (vital force). In a well-known sentence, he argued that the aborigines of Australia may assume that, in a modern watch, an unknown (supernatural) force may be the driver of this complex mechanical clock. By analogy, Pfeffer (1897/1904) concluded that the belief in a "vital force" is a misguided assumption or superstition.

Most importantly, Pfeffer developed methods for the analysis of osmotic pressure of solids, the continuous recording of plant growth via an electric motor-driven apparatus (Auxanometer), and the action of gravity on plant organ bending (Klinostat) (Fig. 2). These novel apparatuses enabled plant scientists of his time to perform quantitative experiments at an unprecedented accuracy, and provided novel insights into the physiology of green organisms, from aquatic plants via mosses to crop species (beans etc.). These studies were recently re-evaluated in a modern context (Kutschera and Khanna 2016).

However, Pfeffer's most important innovation was his idea to construct constant temperature rooms to raise populations of plants for experimental analysis. In a paper

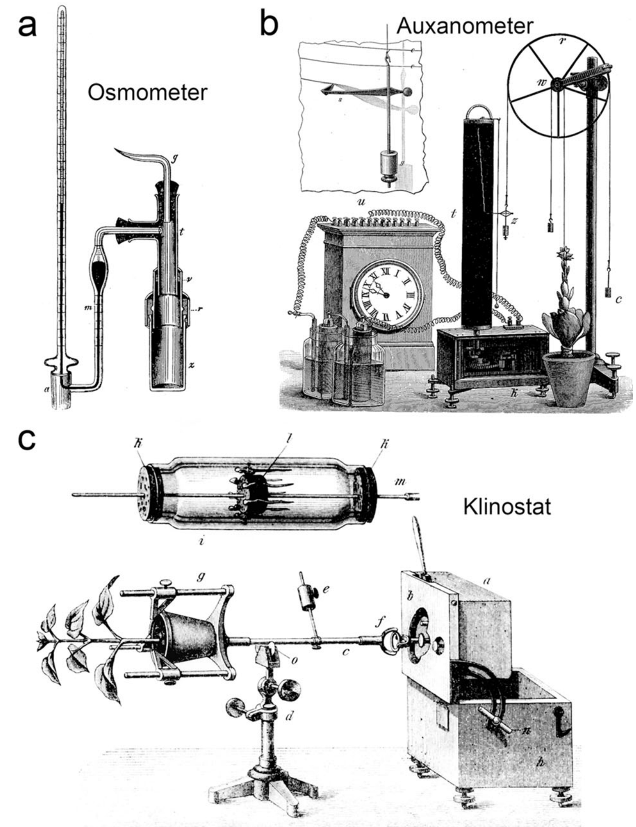


Fig. 2 Wilhelm Pfeffer's novel machines for the study of osmotic pressures of solutions and physiological processes in plants. Osmometer, which was comprised of a cylinder with an integrated semi-permeable membrane (a), growth recorder driven by an electric motor to analyze stem elongation (Auxanometer) (b), and Pfeffer's Klinostat, a machine for horizontal placement and rotation of green plants or juvenile seedlings (c) (adapted from Pfeffer, W.: Pflanzenphysiologie, Leipzig 1897/1904)

entitled "Ein Zimmer mit konstanten Temperaturen" (A room with constant temperatures), Pfeffer (1895) pioneered the development of "plant growth chambers". Eighty years later, Downs and Hellmers (1975) published their well-known monograph *Environment and the Experimental Control of Plant Growth*. In the historic part of their account, the pioneering work of Pfeffer (1895) is not mentioned. Instead, they refer to the book *Experimental Control of Plant Growth* of Fritz W. Went (1903–1990) who, in 1957, published this important monograph—again without a reference to Pfeffer (1895).

Finally, it should be noted that Pfeffer (1900) was the first to use projection apparatuses for the demonstration of physiological processes, and to publish—as a German botanist—in English (see, for instance, his articles on gravitropism of roots and the significance of plant metabolism in *Annals of Botany* and *Proceedings of the Royal Society London*; Pfeffer 1894, 1898).

Plant development in darkness vs. light

Although Sachs (1865, 1868, 1887) had described the process of plant growth in darkness (Etiolement) vs. light in many details, it was Pfeffer (1897/1904) who coined the key term "Photomorphotische Wirkung des Lichts" (photomorphotic action of light). His most famous drawing, showing two potato (*Solanum tuberosum*) plants taken from the same batch of tubers and raised either in the dark or in white light, illustrates the drastic photomorphotic action of solar radiation on shoot- and leaf development (Khanna and Kutschera 2020a, b). However, the German botanist performed many more of such experiments on the phenomenon of "etiolation". Figure 3 a shows the effect of darkening of the tip of a light-grown *Allium*-plant on flower development. Pfeffer (1897/1904) concluded that, for flower development to occur, not "only the photosynthetic provisioning of nutrients", but, in addition, a light-signal is required in order to establish these terminal organs. He also described hypocotyl development in dark-grown seedlings, such as Cucumber (*Cucurbita* sp.) (Fig. 3b). In this context, Pfeffer (1897/1904) argued that there must be "correlations" between the upper (expanding) and lower (mature) part of the developing hypocotyl. Today we know

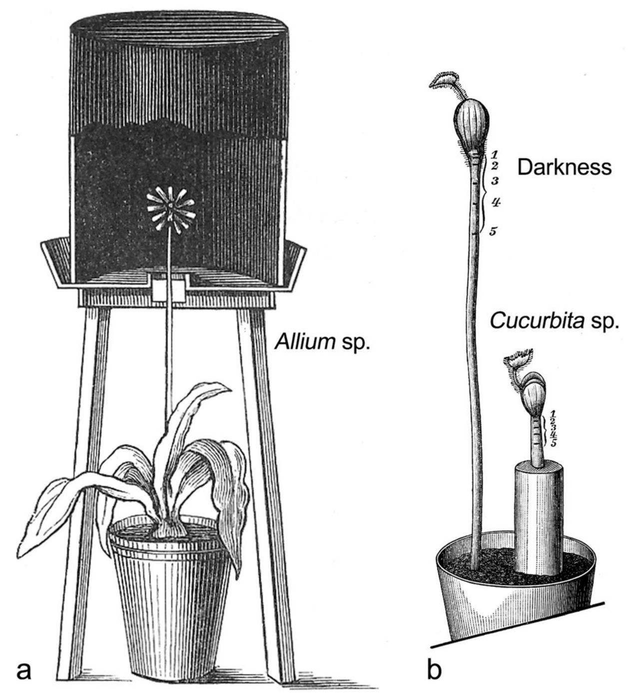


Fig. 3 Pfeffer's experiments to study the effect of light vs. darkness on plant development. The first experimental set-up was designed to analyze the effect or lack of light on flowering in an *Allium*-plant (a). In the second experiment, he placed a seedling into a sturdy chalk mantle to mechanically inhibit stem elongation (b) (adapted from Pfeffer, W.: Pflanzenphysiologie, Leipzig 1897/1904)

that these "correlations" are to a large extend due to the transport and action of phytohormones.

Recently, we analyzed the growth response of *Arabidopsis* seedlings in light vs. darkness (photo- vs. skotomorphogenesis). Based on empirical findings, we proposed that auxin-mediated elongation growth in the absence of light is coordinated at the whole organ level, and it is not a single cell response (Kutschera and Khanna 2020). This coordination of growth in darkness is multifaceted, requiring organ-specific differential regulation of gene expression and spatially regulated post-translational protein modifications. We proposed that a yet unknown universal signal, called Aleph (χ), acts to coordinate growth in darkness (skotomorphogenesis) in an organ-specific manner (Khanna and Kutschera (2020a, b). In this model, the informational signal aleph is perceived by a specific protein, called "etioreceptor", to modulate genetic and biochemical processes in the absence of light (Khanna and Kutschera 2020a, b). If true, this mechanism promotes species survival through physiological changes designed to reach favorable conditions, such as growth towards light (Khanna and Kutschera (2020a, b). We postulated that the aleph/etioreceptor acts in conjunction with the well-known light/photoreceptor systems under the diurnal dark/light environmental cues to regulate growth and development. For example, it influences the percentage of total tryptophan that becomes a substrate for auxin biosynthesis in the absence of the light signal (Khanna and Kutschera 2020a, b). Experimental studies are currently under way to assess this hypothesis (unpublished data).

Finally, it is important to acknowledge that Pfeffer (1897/1904) also analyzed root growth in etiolated seedlings. In contrast to Sachs (1887), who also investigated this process, he systematically created time-courses of organ expansion to document the exact kinetics of length increase along the growing region of the root. In Fig. 4, Pfeffer's classical studies on root development in a seedling of Faba bean (*Vicia faba*) are summarized. These data indicate that cells closer to the tip of the root expand more rapidly than those in the upper region of the sub-terranean organ.

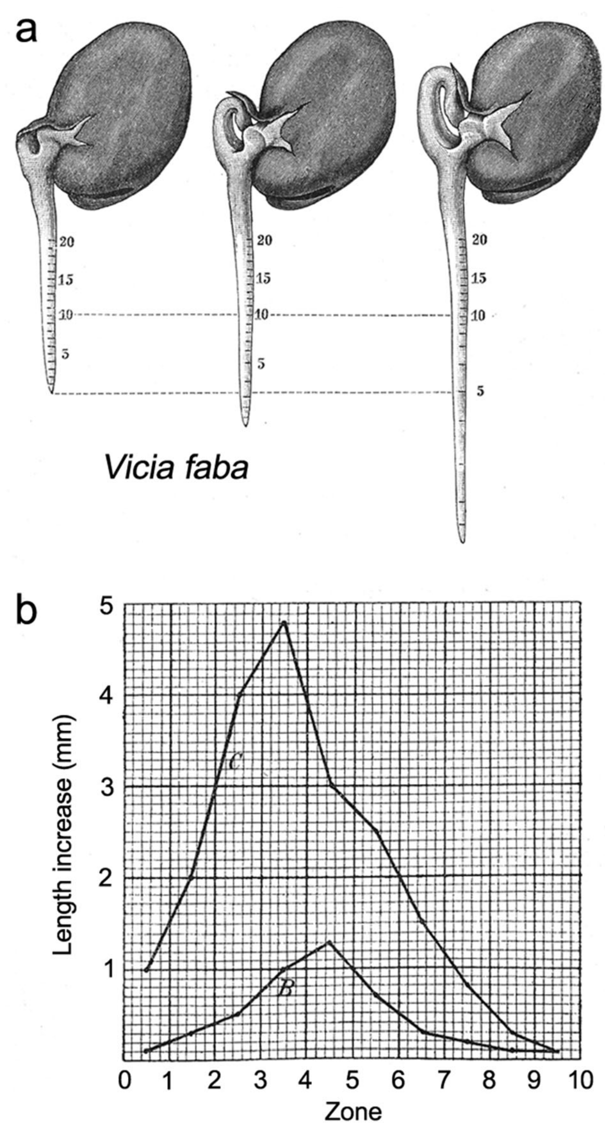


Fig. 4 Pfeffer's method for the quantitative analysis of root growth in seedlings of *Vicia faba*. By marking of a juvenile seedling (a), root elongation can be observed. The graph (b) shows that different zones along the root display a specific pattern of elongation (adapted from Pfeffer, W.: Pflanzenphysiologie, Leipzig 1897/1904)

Osmotic pressure and the Pfeffer-cell

Wilhelm Pfeffer's most famous discoveries are known under the term "Osmotische Untersuchungen" (studies on osmosis). As detailed by Bünning (1875), the botanist Pfeffer studied movements (and contractions) of plant organs, and was impressed by the physical force exerted via these turgor-driven physiological processes (Pfeffer 1873). As a result, he wanted to find out how large these "osmotic forces" really are. No quantitative data were available when Pfeffer started this research project.

In a comprehensive paper, published when he was only 32 years old, and a young professor at the University of Basel/Switzerland, he summarized his many experiments dealing with attempts to quantify osmotic pressure of diluted solutions. His device, later called "Pfeffer cell", consisted of a clay vessel that was, on its inside, coated with a membrane that displayed "semipermeable" properties. Figure 2 a shows Pfeffer's original Osmometer that he filled with corresponding solutions via the glass pipe on the top of the apparatus. After loading of this "Pfeffer cell", the cylinder was placed into pure water. As a result of osmotic water uptake, the pressure inside the vessel increased, so that the height of the solution in the glass pipe was raised (Quecksilber-Manometer). The final height of this mercury solution corresponds approximately to the initial osmotic pressure just before the system was surrounded by pure water (unit: bar; 1 MPa = 10 bar). By this means, Pfeffer (1877) was able to estimate osmotic pressures of solutions (sucrose, salty water etc.). In the summary of this 236 pages long publication, the author concluded that "Die osmotische Druckhöhe nimmt mit der Concentration der Lösung in einem für jeden gelösten Körper und jede Membran spezifischen Verhältnis zu (the osmotic pressure corresponds to the concentration of the solution, depending on the specific solute and the membrane used)" (Pfeffer 1877).

These and other measurements of osmotic pressure in diluted solutions, were analyzed by the Dutch chemist Jacobus Henricus van't Hoff (1852–1911). Based on Pfeffer's data (and those of others), van't Hoff concluded that the so-called "gas laws" also apply to diluted aqueous solutions. Accordingly, he established a famous equation ($\Pi = C \cdot R \cdot T$). Hence, osmotic pressure (Π) is proportional to the concentration of the solution (C) and the gas constant (R), times the absolute temperature (T). For the discovery of this law of nature, van't Hoff was awarded the first Nobel Prize in Chemistry (1901). The contributions of Pfeffer were acknowledged, but the fame went to van't Hoff. The gifted chemist died ten years later, at the age of 59 years, of tuberculosis (Nagendrappe 2007).

Finally, it should be mentioned that Pfeffer, using his Osmometer (Fig. 2a), was able to determine the molecular mass of the osmotic solutions placed into his custom-made device. Since it was possible to estimate relative molecular masses of different protein solutions, it is fair to say that Pfeffer was also a pioneer in the emerging fields of biochemistry and molecular biology (Bünning 1975, 1977).

Experimental work on photosynthesis

In the first Volume of Pfeffer's *Pflanzenphysiologie*, the author summarized the most important facts about plant metabolism available at that time. He discussed the processes of carbon dioxide assimilation, cell respiration and the assimilation of nitrate in many details. In these chapters, Pfeffer (1897/1904) coined the phrase "photosynthetic production of organic substance from carbonic acid and water". It should be noted, that, at that time, the word "carbonic acid" was used instead of the modern term carbon dioxide (CO_2).

Sachs (1865, 1868, 1887) described these processes under the headline "assimilation of carbonic acid by green plant organs in the light", and Pfeffer (1897/1904) adopted this classical terminology. Moreover, he developed improved devices to analyze photosynthesis. As Fig. 5a shows, Pfeffer reproduced the "aquatic plant/bubble-counting-method" of Sachs (1865), but modified this technique as follows. He placed a glass cylinder above the batch of green aquatic plants and, after irradiation in the presence of carbonic acid, collected the oxygen-rich air released by these photosynthetic organisms. Using a glowing piece of wood, the oxygen inflamed this gas mixture, so that direct proof of light-dependent O_2-production was possible (Fig. 5b). As it was common at that time, Pfeffer (1897/1904) interpreted this light-driven oxygen-production as "de-composition of carbonic acid".

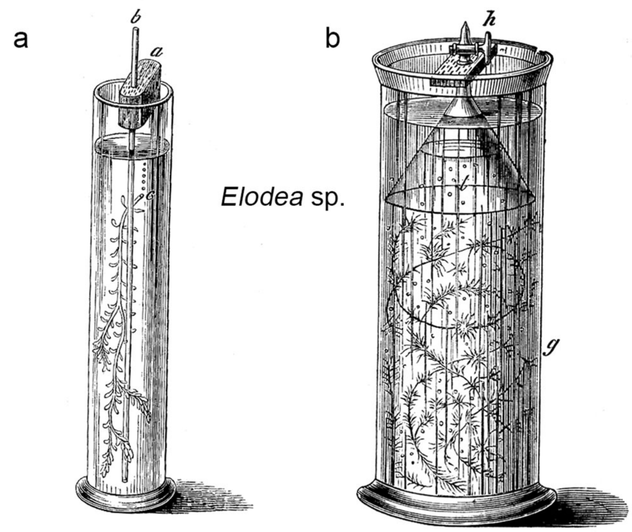


Fig. 5 Pfeffer's *Elodea*-experiments to document and analyze light-induced, CO_2-dependent photosynthetic O_2-production. The classical method of Sachs, using carbonate-enriched water, was adopted (a) and modified to collect oxygen-rich air released by the aquatic plant (b) (adapted from Pfeffer, W.: Pflanzenphysiologie, Leipzig 1897/1904)

Today we know that, during oxygenic photosynthesis, water (H_2O) is split and CO_2 assimilated via the Calvin-cycle (Kutschera and Niklas 2006). However, it was the famous biochemist Otto Warburg (1883–1970) who maintained that CO_2 may be "de-composed" in the light. This hypothesis, published almost five decades ago (Warburg 1964), was thereafter shown to be incorrect. In a detailed analysis, Clausen et al. (2005) provided experimental evidence indicating that "bicarbonate" (i.e., CO_2) is not the substrate for photosynthetic O_2-production. However, as shown by Koroidov et al. (2014), depletion of hydrogen carbonate results in a reversible reduction of oxygen production. These complex interactions are described by Junge (2019), to which we refer for further information on this topic.

The fine structure of the "chlorophyll bodies", today called chloroplasts, was largely unknown at that time, so that the original drawings of Pfeffer (1897/1904) are rather crude (Fig. 6a, b). With the development of electron

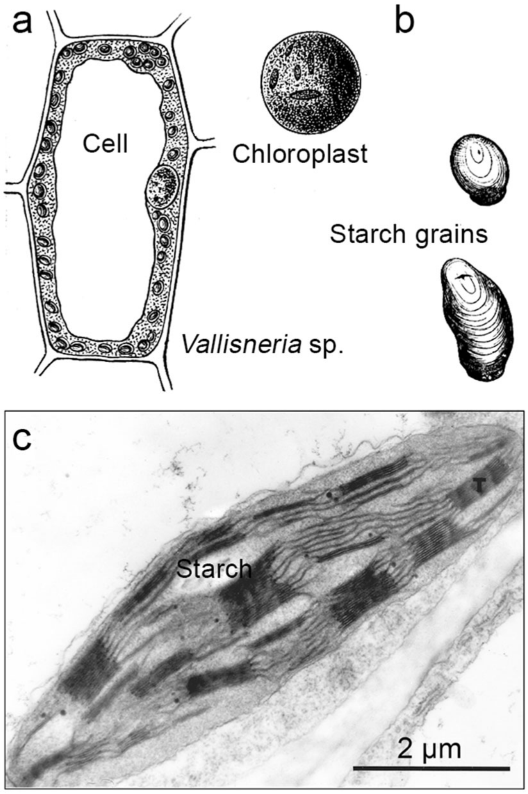
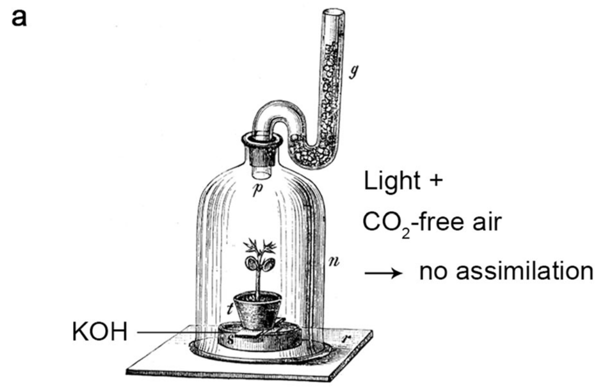


Fig. 6 Chloroplasts as "photosynthetic apparatuses" in a cell of the aquatic plant *Vallisneria* sp. (a, b) (adapted from Pfeffer, W.: Pflanzenphysiologie, Leipzig 1897/1904). These classical drawings are supplemented by a transmission electron micrograph of a chloroplast in the primary leaf of a light-grown rye seedling (c) (original micrograph of U. K.)

microscopy, the fine structure of the chloroplast has been studied in so many details (Fig. 6c) that, in the meantime, 3-d-models of these key organelles of plant life are available. Sachs (1865) had shown that chloroplasts synthesize starch in the light, and that this process is strictly dependent on CO_2. However, experimental proof that without CO_2 no starch production occurs, resulting in the death of the plant, was only briefly addressed by Sachs (1887).

Figure 7a shows a simple apparatus developed by Pfeffer, by use of which a developing seedling can be raised in air without carbon dioxide. In this environment, no CO_2-assimilation occurs. As a result, the plant ceases to grow in the light and, after several days, will die. We reproduced Pfeffer's classical "CO_2-removel-experiment" by using vegetatively grown rooted cuttings of spiderwort-plantlets (*Tradescantia* sp.). These herbaceous wildflowers (family Commelinaceae) were raised in moist garden soil. Figure 8a shows the design of this simple experiment, in which one set of plantlets was left in normal air (currently ca. 0.04 vol% CO_2), whereas the other was cultivated in the absence of this trace gas (KOH-solution removes CO_2, see Kutschera 1998). After one week of growth in a light–dark-



b **Carbon dioxide-fertilization**

CO_2-Content (Vol. %)	Air = 1	Assimilation (rel. U.)
0,03	1	100
0,06	2	127
0,11	4	185
0,56	19	209
7,26	242	230
14,52	484	266

Fig. 7 Device to raise a seedling in air without carbon dioxide (CO_2-absorption by KOH) (a). Quantitative data on the effect of rising CO_2-levels on assimilation of green leaves, compiled by Pfeffer (b) (adapted from Pfeffer, W.: Pflanzenphysiologie, Leipzig 1897/1904)

cycle, the control plants where much larger than at the start of the experiment. In contrast, the "minus-CO_2-plantlets" ceased to grow, developed brown, dry leaves and finally died. Hence, Pfeffer's original experimental design can easily be used to demonstrate that without CO_2, no plant life is possible.

Carbon dioxide and the discovery of global greening

Using the "starch-iodine-assay" to determine light-driven assimilation of carbon dioxide in green leaves (as developed by his mentor Julius Sachs), Pfeffer (1897/1904) documented that only those leaf areas that were directly exposed to solar radiation are photosynthetically active. Accordingly, he concluded that sunlight acts only in illuminated cells, and this assimilatory activity can be suppressed by the removal of CO_2 from the air.

In the context of this classical experiment (Fig. 7a; reproduced in Fig. 8a, b), the German botanist discussed the question whether or not the CO_2-content of the air (in the year 1900 ca. 0.03 vol%) is sufficient for optimizing of photosynthetic activity of the land vegetation. Based on his own experience as experimental plant biologist, Pfeffer (1897/1904) concluded that an enhancement of CO_2 should significantly promote assimilatory activity, and therefore

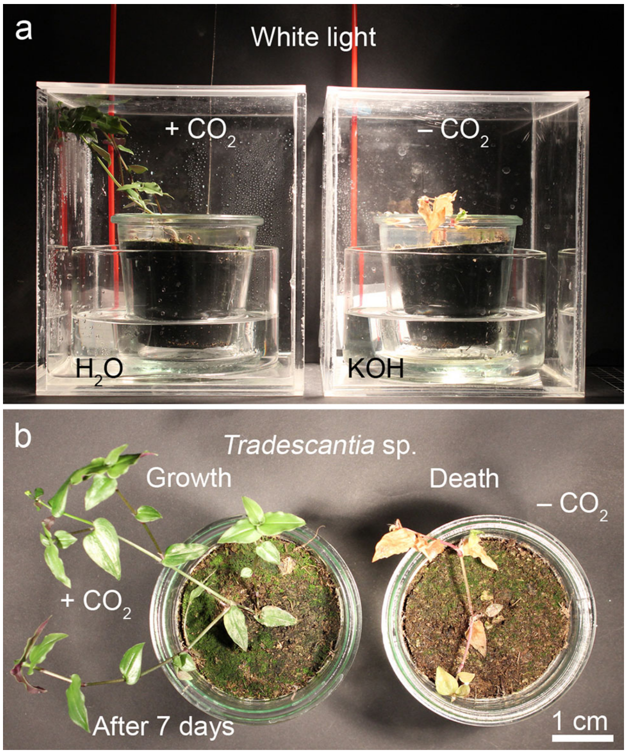


Fig. 8 Original experiment documenting the dependency of land plants (embryophytes) on the CO_2-level of the air. Cuttings of *Tradescantia virginiana*, which had developed adventitious roots, were raised in moist garden soil. The pods were either placed into Plexiglas-chambers supplemented with normal air (CO_2-level ca. 0.04 vol%) (a) or in the absence of this trace gas. CO_2 was removed by a KOH-solution on the bottom of the chamber (without contact to the soil of the plants) (b). In both chambers, relative humidity was ca. 99%, and the daytime photon fluence ca. 150 µmol m^{-2} s^{-1} (unpublished original experiment of U. K.)

plant productivity. With reference to data published in the literature, he compiled the data shown in the table reproduced in Fig. 7b. Based on a number of experiments performed on leaves of *Rubus* sp. (raspberries), *Carpinus* sp. (hornbeams), *Tropaeolum* sp. (nasurtium) etc., it was shown that experimental elevation of CO_2 in the air results in a drastic promotion of assimilatory activity of the leaves. The results of Pfeffer (1897/1904) document that a doubling of the CO_2-content from ambient (at that time ca. 0.03 vol%) to 0.06 vol% resulted in a 27% enhancement in "photosynthetic assimilation". When the CO_2-level was raised fourfold (to ca. 0.11 vol%), an almost twofold rate of assimilation was measured; values higher than ca. 7 vol% CO_2 only slightly improved carbon assimilation. Accordingly, Pfeffer (1897/1904) concluded that at ca. 4 to 10 vol% CO_2, photosynthetic activity reaches an optimum. With reference to another source, he argued that bean plants display an optimum rate of growth at ca. 4 vol% CO_2; at carbon dioxide-levels larger than ca. 10 vol%, all plants studied in these series of experiments ceased to grow and finally died. Hence, according to Pfeffer, CO_2 is an airborne nutrient for plants up to a level of 4 to max. 10 vol%; it becomes a toxic gas as soon as this concentration is exceeded. It should be stressed that these classical CO_2-fertilization experiments were performed on plants raised in well-watered garden soil, so that no limitation of the supply of mineral salts occurred.

Accordingly, Pfeffer (1897/1904) concluded that a moderate enhancement in anthropogenic CO_2-level in the air will significantly promote photosynthetic production and plant growth. He suggested that mankind, due to the burning of fossil fuels (coal etc.), will raise the level of carbon dioxide in the air. As a result, leave growth and crop productivity is promoted worldwide, so that this hypothetical "global greening" should be viewed as beneficial to humanity (Pfeffer 1897/1904).

In this context, the author also pointed out that every human being produces large amounts of carbon dioxide as a result of respiratory activity (O_2-uptake/CO_2-release). In quantitative terms, Pfeffer (1897/1904) argued that one *Homo sapiens* releases about 800 to 900 g CO_2 per 24 h. Based on the fact that the global population of human beings was ca. 1.500 million at that time, he suggested that mankind produces significant amounts of extra-CO_2 that is beneficial to plants.

Today, it is documented that the CO_2-level in outdoor environments is ca. 0.04 vol%, with a tendency to rise above this value. Since the exhaled breath from adult humans contains ca. 100-fold more CO_2 than ambient air (ca. 4 vol% vs. 0.04 vol%), it is obvious that human beings that spend long hours in closed rooms live in an environment enriched in this gaseous end-product of cell metabolism (plants also respire CO_2). Based on these insights, we have tested to what extent exhaled breath can promote plant growth, using the equipment shown in Fig. 8a. Batches of *Tradescantia*-plantlets were raised in plastic boxes in a light/dark-cycle. In one sample, the air was enriched with CO_2 by exhaled breath (application time ca. 10 min/ 24 h in a box that contained only one small hole, which was used to insert a plastic tube, see Fig. 8a). Under these artificial conditions, the CO_2-enriched plantlets grew much faster, developed adventitious roots and flowered earlier. Figure 9 shows one of four independent experiments that yielded similar results: a ca. 100% increase in photosynthetic plant mass production due to the extra CO_2 applied by the experimenter. In summary, this simple experiment (Fig. 9) corroborates Pfeffer's conclusion that CO_2 released by human beings can drastically promote plant growth and the accumulation of dry mass.

Finally, we want to point out that a number of studies have documented the negative effects of too much CO_2 on the health of humans. During the lifetime of Wilhelm Pfeffer, CO_2-levels were, as they remained over the

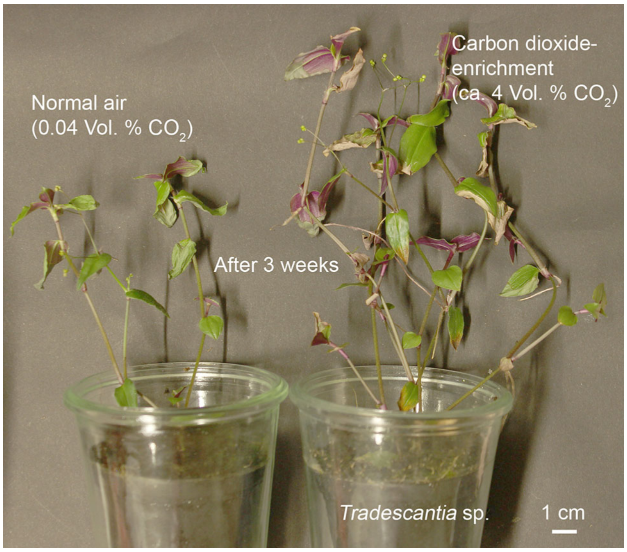


Fig. 9 Original experiment documenting the effect of a ca. 100-fold enhancement of CO_2-concentration on the growth of *Tradescantia* sp.-plantlets (see Fig. 8). The shoots were either grown in fresh air (0.04 vol% CO_2) or in air that was supplemented by exhaled breath from a human (ca. 100 times higher level of CO_2). After three weeks of growth in a natural light/dark-cycle, stem growth and flower development were drastically promoted (unpublished original experiment of U. K. and Ingo Ehnes, Kassel, Germany)

previous ca. 25 million years, in the range of ca. 0.028–0.030 vol%. With the advent of the industrial revolution, CO_2-levels steadily increased and have now reached ca. 0.041 vol% (Kutschera et al. 2020; Bierwirth 2020). As summarized in recent review article, further elevations in CO_2-levels will exert significant health risks for humanity, even at concentrations of only ca. 0.5 vol% carbon dioxide per liter of air (Jacobson et al., 2019; Bierwirth 2020).

Anthropogenic greening of the Earth

Pfeffer's hypothesis that man-made CO_2-emissions caused by the combustion of coal etc. results in an enhancement in global photosynthetic productivity was ignored over the first half of the twentieth century. However, within the context of "climate change", and the well-documented phenomenon of "global warming", the ideas of this German chemist/botanist were re-vitalized (see Idso 1991). Without reference to Pfeffer's classical book (Fig. 1), one century later, Norby et al. (1995) published a key paper entitled "Increased growth efficiency of *Quercus alba* trees in a CO_2-enriched atmosphere". The authors documented that, when oak saplings were raised at a CO_2-level of 0.065 vol%, they produced 35% more dry mass compared with the control. They concluded that this increase in growth efficiency may indicate an enhanced carbon sequestration capacity by forests. Ainsworth and Rogers (2007) summarized data from Free-air CO_2 enrichment (Face)-experiments. These studies revealed that, on average, CO_2-enrichment to a level of 0.057 vol% resulted in a 22% reduction in stomatal conduction. In C3-plants (shrubs, trees), photosynthesis was promoted by 31%. Similar results were reported by Dieleman et al. (2012), who stressed the interaction between elevated CO_2-levels and global warming.

Similar general conclusions were reached by Pfeffer (1897/1904), with reference to the data compiled in Fig. 7b. Recently, Schimel et al. (2015) concluded that increasing CO_2-concentrations in the atmosphere likely act as a significant negative feedback in the global carbon cycle by absorbing up to 30% of CO_2-emissions caused by the combustion of fossil fuels. Similar results were obtained by Campbell et al. (2017), who documented, using a variety of sophisticated methods that currently ca. 31% of anthropogenic CO_2-emissions are re-cycled by the more rapidly growing land vegetation (see Bastin et al. 2019a, b and Kutschera et al. 2020 for further discussion of this topic). Hence, as mentioned in the *Introduction*, terrestrial plants display a robust positive response to human-caused carbon dioxide-enrichment of the atmosphere, an effect that originated at the time when Pfeffer (1897/1904) published his famous book (Schönwiese 2019; see Kutschera and Farmer 2020 and the detailed analysis of Wang et al. 2020).

Based on these insights, Zhu et al. (2016) published their famous paper entitled "Greening of the Earth and its drivers". In this important contribution it is documented via NASA satellite-data that, over the past 20 years, the world became a "greener place", due to an increase in foliage of the land vegetation around the planet (Fig. 10). In quantitative terms, CO_2-fertilization (Figs. 7b, 9), resulting from anthropogenic carbon dioxide-emissions, explains ca. 70% of the documented greening (i.e., CO_2-fertilization)-effect, whereas nitrogen deposition, climate change and land cover change account for ca. 9%, 8% and 4% of this phenomenon, respectively. In a subsequent paper, Keenan et al. (2016) provided evidence indicating that man-made extra CO_2 led to increased global photosynthesis and a promotion of greening of parts of the biosphere. They also discuss these findings with respect to global warming and conclude that this CO_2-related phenomenon slowed down (ca. 1995 to 2005) as a result of the increase in the terrestrial sink-capacity of the land vegetation (see Kellogg 1987, Davis 2017, Lindzen 2018 and Nawaz et al. 2019 for a discussion of the relationship between CO_2 and global warming).

Figure 11 shows the temperature record of Germany between 1760 and 2016. These data sets, displayed as separate graphs for the summer- and winter-months, reveal the phenomenon of the so-called "Little Ice Age". This

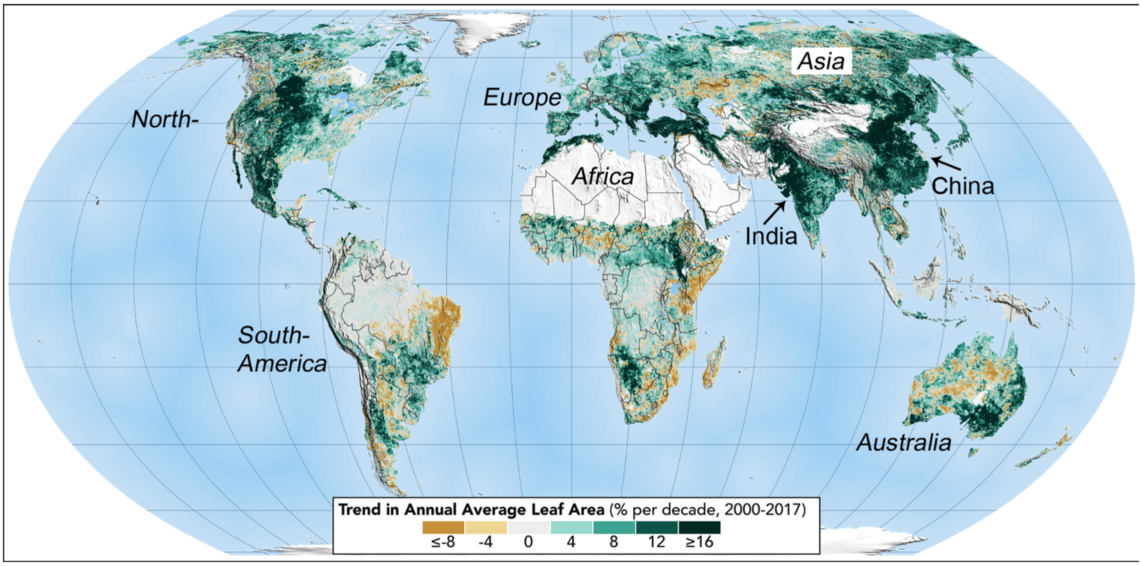


Fig. 10 Increase in foliage around planet Earth, measured in average leaf area on plants and trees per year. The data show that, over the past two decades, China and India are leading in the greening of the planet due to anthropogenic activities. The effect is to a large extent caused by man-made CO_2-emissions (adapted from Tabor, A.: nasa.gov, Febr. 11, 2019)

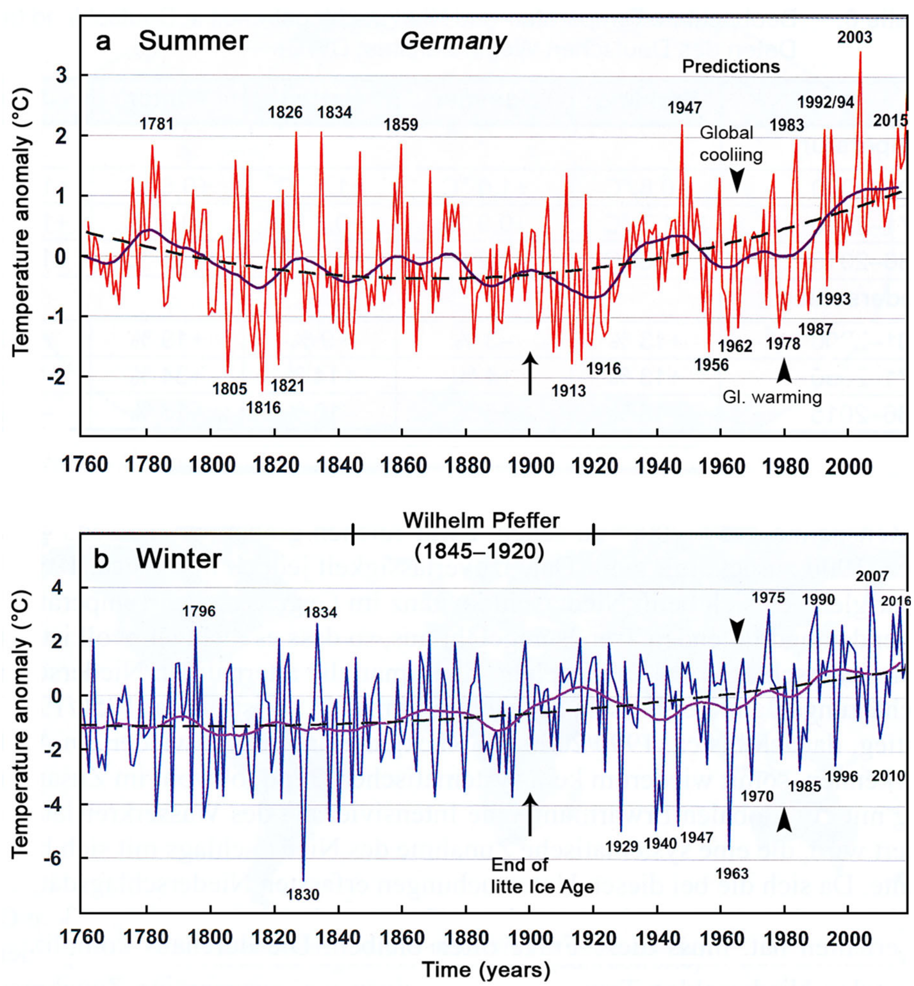


Fig. 11 Global warming, based on temperature measurements recorded from 1760 to 2016 in Germany. The results, depicted separately for summer and winter (a, b), show large variabilities in temperature anomaly. However, after the end of the little ice age (ca. 1900), a slight but significant steady increase in average air temperature occurred, inclusive of anthropogenic warming (adapted from Schönwiese 2019)

well-documented temperature anomaly can be characterized as follows. Between the 15th and mid-19th-centuries, the average temperature in the Northern Hemisphere was ca. 1 °C lower than 1960, leading to alpine glacial advances and much more ice and snow during the winter time than today. Rhodes et al. (2012) have shown that the "Little Ice Age" was not restricted to Europe, but a global phenomenon. The end of the "Little Ice Age" is not clearly defined, but most authors agree that the years between 1850 and ca. 1900 may be the time period signifying the end of this cool period (Schönwiese 2019). As shown in Fig. 11b, Wilhelm Pfeffer's life time (1845–1920) corresponds closely to the end of this cool time period that lasted at least 400 years.

The end of the "Little Ice Age" is characterized by a natural globing warming period, the exact reason of which is still a matter of debate. However, it is well documented that rising CO_2-levels, caused by human activities, may be in part responsible for this steady warming of the atmosphere (Schönwiese 2019; Wang et al. 2020). These data indicate that Pfeffer's conclusions concerning the role of CO_2 in the promotion of global plant growth were correct. A recent study has shown that China and India "lead the greening of the Earth" (Chen et al. 2019). As these authors have shown, and is apparent in Fig. 10, these two countries became greener than other parts of the planet. Notably, not only photosynthetic CO_2-assimilation and enhanced leaf growth, but also food production has increased by up to 40% in these countries (vegetables, grains, fruits etc.). Yadav et al. (2020) concluded that "The production of fruits in India has increased tremendously over the last few years and resulted in improving the economy of the country". We suggest that "global greening", also known as the CO_2-fertilization-effect, may be one reason for this positive development, but direct evidence for this hypothesis is lacking.

Pfeffer (1897/1904) must be credited with the discovery of this physiological phenomenon, and we hope that our account of his life and achievements will distribute this message throughout the plant science community.

Wilhelm Pfeffer and the discovery of the physiological clock

As mentioned above, Pfeffer (1873) studied plant movements in many details, and continued this research agenda until the final years of his life. He constructed sophisticated machines to record the movements of beans (*Phaseolus* sp.) that were raised in his constant-temperature-rooms at Leipzig University. Figure 12 shows the diurnal leaf movements, a phenomenon first described by the Swiss botanist Augustin Pyramus de Candolle (1778–1841). As detailed in a previous article, de Candolle was also the botanist who coined the name *Arabidopsis*, and provided a first outline of the systematic position of this model plant (Khanna and Kutschera 2020a, b a).

In 1907, when the Vol. II of Pfeffer's monumentous *Pflanzenphysiologie* appeared in print, the experimental botanist published a comprehensive article on the leaf movements in *Phaseolus* and other plants. Two additional articles followed, so that, on ca. 500 printed pages, Pfeffer (1907, 1911, 1915) provided a comprehensive experimental analysis of a phenomenon we today call "the physiological clock". Bünning (1975, 1977) critically read and analyzed these papers that Pfeffer published in a journal not widely distributed at that time: the *Abhandlungen der mathematisch-physikalischen Klasse der königlich sächsischen Gesellschaft der Wissenschaften Leipzig*. On these pages, the careful experimenter documented the entrainment of the rhythms by a light–dark-cycle, that deviated from the 24 h-period observed in a natural sunlight-darkness-scheme. Accordingly, Pfeffer (1907, 1911, 1915) argued that there must be a so-called "Tagesautonomie", a term that can be translated into "autonomic movements independent of the day-night-cycle" (Fig. 12). With these analyses, Pfeffer must be credited with the honor of being the discoverer of the "biological clock" in plants (Bünning 1975, 1977; Kutschera 2019).

With reference to the main topic of this article, we want to point out that in Pfeffer's experiment shown in Fig. 12, a loss of greening of the primary leaves of these bean plants occurred. When plants that developed under natural light/dark-conditions, and therefore have well-developed green leaves, are placed into continuous darkness, a rapid loss of chlorophyll occurs, and hence a de-greening of the organs can be observed. Pfeffer (1907, 1911, 1915) was well aware of this physiological response, but his focus was on leaf movements, which he characterized as driven by an internal mechanism (the biological clock). However, as Chandrashekaran (1998) has shown, chronobiology, as an independent scientific discipline, originated much earlier. Pfeffer's contributions are acknowledged in most accounts of his subject, and the reader is referred to the recent monograph of Kumar (2017) for more information on this topic.

Conclusions

In his book *History of Botanical Science*, Morton (1981) concluded that "(Julius) Sachs had, to the highest degree, the capacity of uniting the essential elements in the work of many investigators into a general theory, which, whether later proved right or wrong, was always rational in stimulating and a pointer to fresh initiatives in research. The whole course of modern plant physiology bears the

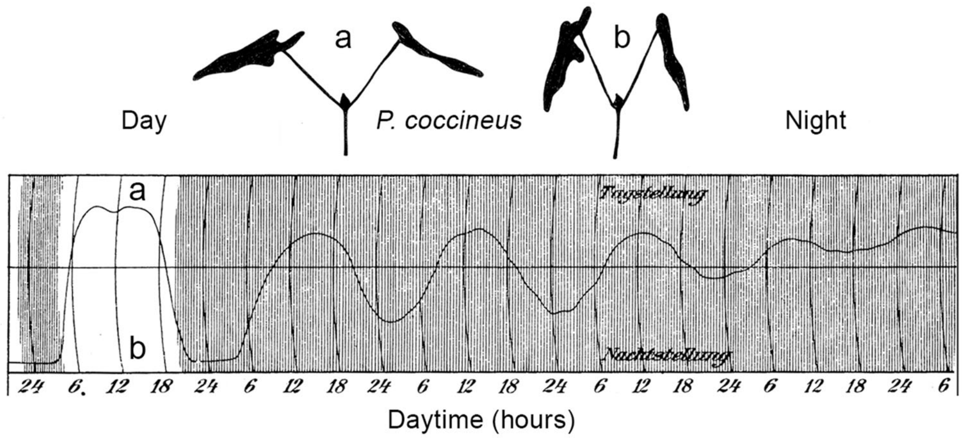


Fig. 12 Discovery of the "Tagesautonomie" (corresponding to a "physiological time-keeper") in the primary leaves of bean seedlings (*Phaseolus coccineus*). In 1907, Wilhelm Pfeffer observed that leaf movements continue in constant darkness. Six years later (1915), he concluded that these movements are driven by an internal mechanism, later labeled as "biological clock" (adapted from Pfeffer 1915)

unmistakable stamp of his incisive mind ". We agree with this general evaluation of the importance of Julius Sachs and have corroborated this interpretation in recent articles (Kutschera 2015a, b; Kutschera and Baluska 2015; Kutschera and Niklas 2018a, b). However, as the present contribution shows, Wilhelm Pfeffer further developed the concepts of Sachs to such an extent that it is fair to postulate the "Sachs-Pfeffer-principle of experimental plant science" mentioned above. This agenda, which persists to the present day, and forms the basis of all international research agendas using plants, animals and humans, rests on Pfeffer's statement that living processes are based on physics and chemistry. In addition, Pfeffer (1897/1904) was the first to include bacteria into the plant sciences (later, microbiology developed into an independent discipline), and therefore inaugurated an agenda we may label as "plant–microbe-interactions". Since Pfeffer (1895) pioneered in the construction of plant growth chambers, without which no reproducible results can be obtained in all of the physiological sciences, his contributions cannot be overstated.

We have shown here that Pfeffer (1897/1904) was the first to point out that carbon dioxide is a limiting factor for plant growth and crop productivity. Hence, the research agenda of "global greening", with the focus on CO_2-fertilization of the land vegetation, was inaugurated by this plant scientist. Piao et al. (2020) have concluded that "rising atmospheric CO_2-concentration is the main driver of global greening". This insight can be traced back to the elegant work of Wilhelm Pfeffer, who died one century ago, only one day after delivering his last lecture on the physiology of plants at the University of Leipzig, Germany.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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