Article

Worldwide Research on the Ozone Influence in Plants

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Abstract: Tropospheric ozone (O_3) is a secondary air pollutant and a greenhouse gas, whose concentration has been increasing since the industrial era and is expected to increase further in the near future. O_3 molecules can be inhaled by humans and animals, causing significant health problems; they can also diffuse through the leaf stomata of plants, triggering significant phytotoxic damage that entails a weakening of the plant, reducing its ability to cope with other abiotic and biotic stresses. This eventually leads to a reduction in the yield and quality of crops, which is a serious problem as it puts global food security at risk. Due to the importance of this issue, a bibliometric analysis on O_3 in the plant research field is carried out through the Web of Science (WoS) database. Different aspects of the publications are analysed, such as the number of documents published per year, the corresponding scientific areas, distribution of documents by countries, institutions and languages, publication type and affiliations, and, finally, special attention is paid to O_3 study in plants by means of studies about the word occurrence frequency in titles and abstracts, and the articles most frequently cited. The bibliometric study shows the great effort made by the scientific community in order to understand the damages caused by O_3 in plants, which will help reduce the big losses that O_3 causes in agriculture.

Keywords: ozone; plant; environment; health

1. Introduction

Ozone (O_3) is a gas present in the Earth's atmosphere and 90% of O_3 is found in the stratosphere. The remaining 10% is present in the troposphere, but in a lower concentration, which is known as natural background. However, since the beginning of the 21st century, the concentration of background O_3 is increasing by 0.5–2% per year in the Northern Hemisphere [1], and this upward trend is predicted to continue with mean O_3 concentrations increasing by 20–25% by 2050 and by 40–60% by 2100 [2].

The increase in tropospheric O_3 is mainly due to the rapid industrialization process of the last century and the use of fossil fuels which have caused higher emissions of O_3 precursors, such as nitrogen oxide (NOx) or volatile organic compounds (VOCs) [1,3,4]. O_3 is thus an important second air pollutant and a greenhouse gas. Its highly oxidant nature causes damage to tree species in terms of forest areas affected and their extent [5], as well as in losses in biodiversity in natural grasslands [6–8]. O_3 oxidative stress also affects most commercial crops, leading to a reduction in their yield and quality.

Wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.) are the three most important cereals in the world [9–12]. These crops stand out for their high sensitivity to O_3. O_3 damages wheat grain quality [13] and causes yield losses of approximately 7.1%, which can reach up to 15% in regions such as China and India [14]. For rice, yield losses due to O_3 are approximately 4.4%, and in regions such as China, India, Indonesia, and Bangladesh, losses can reach up to 7.5–12.5% [14]; while in maize, it is estimated that yield losses are approximately 6.1%, reaching up to 15% in countries such as China and the US [14].

The legume family is widely accepted as containing some of the most ozone-sensitive species that have been tested [15,16]; peas and beans are the most sensitive crops to O_3 [17]. Ten years ago, losses caused by O_3 were indicated to be 19.0% for beans, and 20% higher in the concentration range predicted for 2030 in Europe [17]. In addition, O_3-induced effects on fruit quality, changing the nutritional value, have been observed in beans [18], resulting in an important decrease in their market value in countries such as the UK [19]. Soybean (*Glycine max* L.) is also a species of legume that stands out for its high nutritional value. It is, together with wheat and bean, the crop that presents a greater sensitivity to O_3. Production losses due to O_3 are predicted to be approximately 12.4%, and can reach up to 20% in the US, which is the region with the greatest producer [14].

On the other hand, horticultural crop species, although covering relatively small cultivation areas, have great economic relevance. Tomato (*Solanum lycopersicum* L.) is a horticultural crop of great importance worldwide [9], whose yield and fruit composition are affected by O_3 [20]. Lettuce (*Lactuca sativa* L.), the most important green leaf vegetable at an economic and commercial level [9], was one of the first species recognized to have high sensitivity to O_3-induced oxidative stress [21], with mean reductions of approximately 15% of the market yield [22]. Potato (*Solanum tuberosum* L.) is the fourth staple food in the world [14] and an important source of energy, due to its high content of carbohydrates (mainly starch) but long-term exposure to high concentrations of O_3 can cause a drop in starch content, leading to significant yield losses [23]. The genus Brassicaceae includes numerous plants that are used as fresh food or fodder but can also be destined for industrial use or as medicinal and ornamental plants. It includes the rapeseed (*Brassica napus* L.), an extensively cultivated species [9], whose yield losses by O_3 are due to a reduction of pollination by insects [24], which consequently reduces 20% of its market value. It should be noted that this family also includes the species *Arabidopsis thaliana* (L.), which constitutes a model plant for research, as well as for the evaluation of O_3 injury.

The knowledge and understanding of changes caused by O_3 in plant species are essential to fix the damage generated, with the aim of avoiding or reducing yield losses. Therefore, it is important to know all the alterations at the genetic, cellular, and physiological levels that O_3 provokes in plants. To study the metabolic pathways and physiological effects caused by O_3 in plants, model organisms, such as *Arabidopsis thaliana* [25,26] or *Nicotiana tabacum* [27] have mainly been used.

First, O_3 enters the plant tissues through leaf stomata, which are the "first line of defence" [25]. The stomatal pore size and closure are mainly regulated by the activity of ion channels of guard cells [28,29]. One of the earliest responses to O_3 is an increase of cytosolic-free calcium (Ca^{2+}) in guard cells, as a consequence of the activation of Ca^{2+} channels [30]. This Ca^{2+} is a crucial second messenger in stress signalling and in the activation of O_3 response genes [31]. Similarly, potassium (K^+) channels of guard cells are required for fast stomatal closure induced by the reactive oxygen species (ROS) [32].

Once inside leaf tissues, O_3 triggers the formation of ROS that can lead to lipid peroxidation [33] and ROS accumulation in the apoplast. To cope with the damage of ROS, plants activate antioxidant systems, in which several enzymes play a crucial role. Among them, the activity of the enzymes ascorbate peroxidase, dehydroascorbate reductase, and glutathione reductase, all of which play a critical role in ROS-scavenging, can be highlighted [34,35]. It is also worth noting that both superoxide dismutase (SOD) and catalase play an essential role in ROS detoxification [36].

However, at a certain point, the antioxidant capacity of the apoplast is exceeded, and ROS is spread within the cell through NADPH-oxidases that generate superoxide ion (O^2) and type II cell wall peroxidases that generate H_2O_2 [37,38]. Once inside the cell, ROS triggers multiple signalling pathways that are integrated to achieve a proper response. The role of mitogen-activated protein kinases (MAPK) and calcium in the regulation of ethylene production and signalling is remarkable [39–41], causing a rapid accumulation of ethylene in leaf tissues [25]. Ethylene production is one of the earliest responses of plants to O_3 and it promotes lesion formation and cell death [40,42]. In addition to ethylene

biosynthesis, O_3 exposure also induces endogenous production of nitric oxide (NO) in guard cells of some plant species, such as *Arabidopsis*. Salicylic acid (SA) induced protein kinase (SIPK, a tobacco MPK3 orthologue) and calcium are also involved in the regulation of NO signalling pathways [25]. NO is involved together with ROS in the activation of stress responses such as hypersensitive response (HR)-like lesions [26]. This molecule is a signal inducer that enhances O_3-induced cell death, possibly by altering the ROS–NO balance. The main impact of NO is the attenuation of SA biosynthesis and other SA-related genes [26]. However, in wheat, it has been proven that NO increases the activity of both antioxidant enzymes SOD and peroxidase in leaves, allowing them to increase photosynthetic rates and to alleviate yield reduction caused by O_3 [43].

O_3 exposure also damages the electron transport chain of the thylakoid membrane of chloroplasts and alters the non-cyclical photophosphorylation process of photosynthesis, causing a decrease in photosynthetic rates and yield [44,45]. In addition, O_3 causes the inactivation of some enzymes of the Calvin Cycle, such as the small subunit of Rubisco (ribulose-1,5-bisphosphate carboxylase oxygenase), decreasing, again, photosynthesis and yields [45,46]. Rubisco plays a crucial role in carbon (C) fixation and is also the main storage protein of foliar nitrogen (N), constituting 50–70% of the total soluble protein of leaves. Therefore, the decrease of this enzyme also causes accelerated foliar senescence [45,46]. Even though the impact of O_3 on plant growth and biomass is variable, it has been proven that the damage to the photosynthetic apparatus and the low assimilation of C previously mentioned causes a lower accumulation of biomass [45] and less fertilizer efficiency [47], which results in the significant yield loss of commercial crops [47].

It has also been described that O_3 exposure can modify biogenic volatile organic compound (BVOCs) emissions from plant leaves, which could alter flower recognition [48] by insect pollinators. This could lead to a decrease in insect pollination [24], and therefore to a reduction of the market value of some crops [24,49].

All alterations caused by O_3 lead to high losses in both quality and yield of commercial crops, which already face the challenge of producing 60% more food by 2050 [50]. With the aim of understanding how the scientific community worldwide attempts to solve the losses caused by O_3 in crops, we have performed a deep bibliometric study.

2. Materials and Methods

There are diverse bibliographic databases used for bibliometric analysis such as Web of Science (WoS), Scopus, Google Scholar, and Microsoft Academic. For this project, we selected WoS since it makes downloading data easy for bibliometric purposes, fits with the scientific coverage of our research area [51], and offers robust tools for measuring science [52].

The search was conducted in January 2021 to collect academic publications from all of the databases available in the WoS from Thomson Reuters, containing the terms "Ozone" or "Ozone" and "Plant" in the title, abstract and/or keywords. The search was limited from the first publication year to 2020. The publications obtained were assessed and classified based on the following aspects: number of publications per year, subject area, countries, institutions, languages, type of document, journals, and type of publication. The results obtained were processed to allow an easier display of the results through graphs obtained with Microsoft Excel. VOSviewer was the tool used to analyse the core content and research object of the academic literature. VOSviewer is a free computer program used for the construction and presentation of bibliometric maps [53]. The frequency of word occurrence was shown by the size of the circle under the word. Additionally, the free software WordArt [54] was used to elaborate a specific word cloud using only vegetal species named in the title of the publications related to O_3 in the plant field. In these maps, the word size is directly proportional to its frequency of occurrence in the literature reports.

The number of citations allows for the assessment of the relative impact that a single publication has on the scientific community. To measure the professional quality of journals, the tool used was the 5-years Impact Factor generated by Journal Citation Reports (JCR),

based on the number of citations that their scientific articles have received [55,56]. A higher value of the H-index generally indicates greater scientific attainment. These methodologies were used successfully in other bibliometric studies [57,58].

3. Results

3.1. Evolution of Scientific Output and Distribution in Subject Categories

A total of 145,538 documents with the "ozone" term in titles, abstracts, or keywords were recovered. Remarkably, no article about O_3 appeared until the year 1855, with barely any documents until 1961. Since then, there has been a progressive increase in O_3-related publications. During this period, the year 2010 stands out due to a sudden decrease in the number of publications. Today, the number of O_3-related publications has not reached the amount of O_3 documents that were published previously to the decrease (Figure 1). The drop in the publication number could reflect the severe worldwide financial and food crises of 2008. The ways that the economic and food crises interfaced with the environment and agriculture could affect the research in said issues [59], altering the number of publications for this particular sector. A similar diminution was observed in the year 2013 in a bibliometric study about O_3 in the period 2000–2015 [60]. However, the results shown in this publication and our results are not comparable, since [60] performed a bibliometric study about O_3 using the Directory of Open Access Journals database in a limited period of fifteen years, which only retrieved 1831 articles versus 145,538 documents recovered from WoS in this project.
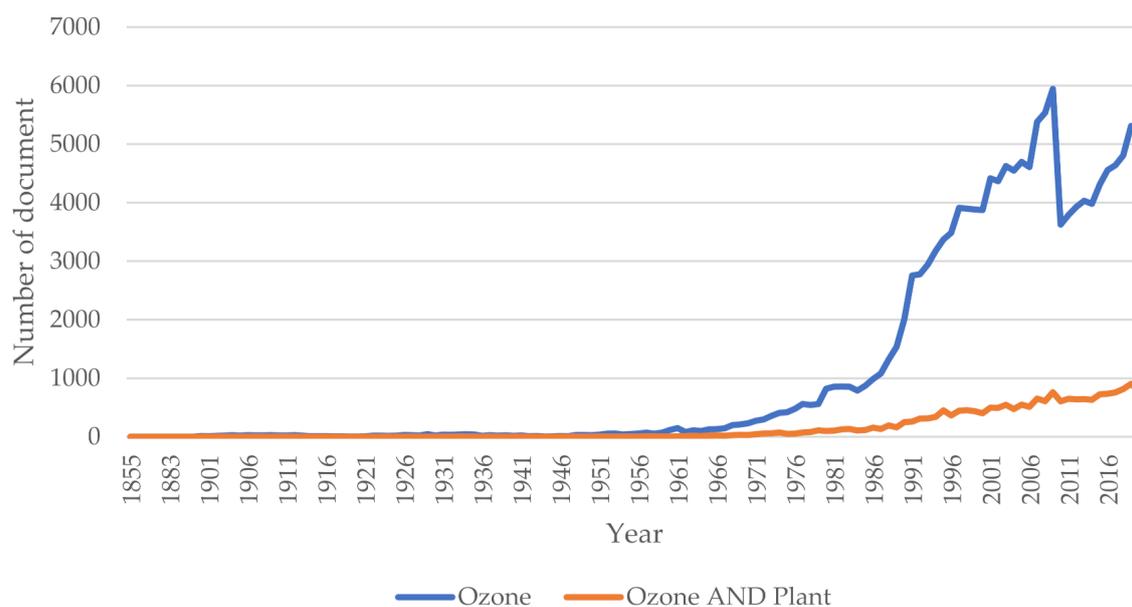
Figure 1. Trends in publications on ozone research in absolute terms (blue line) and in the plant field (orange line) in the period 1855–2020.

If the term "plant" is included in the research, only 19,202 documents are retrieved, representing 13% of O_3-related studies. This low percentage is likely due to the fact that the first report on O_3 in the plant field was published in 1902 (nearly fifty years later than the first publication of O_3), and no more than ten articles appeared until the year 1962. In this period, no manuscript was published in the years 1855–1901, 1903–1906, 1908–1909, 1912–1923, 1925–1926, 1928–1930, 1936, 1938–1939, 1941, and 1943–1946. Since 1962, the number of publications has risen slowly and continuously, until reaching a maximum of 777 documents published in 2020 (Figure 1). The results suggest that interest in O_3 should be initially focused in research areas other than the plant research field. However, an increase in publications on O_3 research in the plant studies field has been experienced

from the second half of the twentieth century to the present (Figure 1). It suggests that the interest of the scientific community on the effects of O_3 in plants is relatively recent and that a constant increment in the number of O_3 publications in the plant research field is expected in the coming years.

Based on the WoS classification, the distribution of publications in the O_3 research field covered a total of 154 subject areas. However, only 17 areas included more than 10,000 articles on O_3. The largest number of documents corresponded to Environmental Sciences Ecology (54,020 records), while the second-largest area in terms of the number of publications was Chemistry (51,924 records). The third area was Engineering (47,601 records), followed by the Meteorology Atmospheric Sciences area (40,467 records), and the fifth area was Public Environmental Occupational Health (31,982 records). The first fifteen areas were mainly related to Environmental, Technology, Engineering, Health, Physics, and Chemistry, which can be explained by the O_3 involvement in climate change and human health. No area related to Plant Science or Agriculture could be found before the sixteenth position (Figure 2). Dissimilar results were shown by [60], which showed as the first discipline Engineering and Technology followed by Chemistry, Physics, Earth and Environmental Sciences, Medicine, Biosciences, and Agriculture. Again, the different results obtained can be due to the different databases and time periods used in both projects.
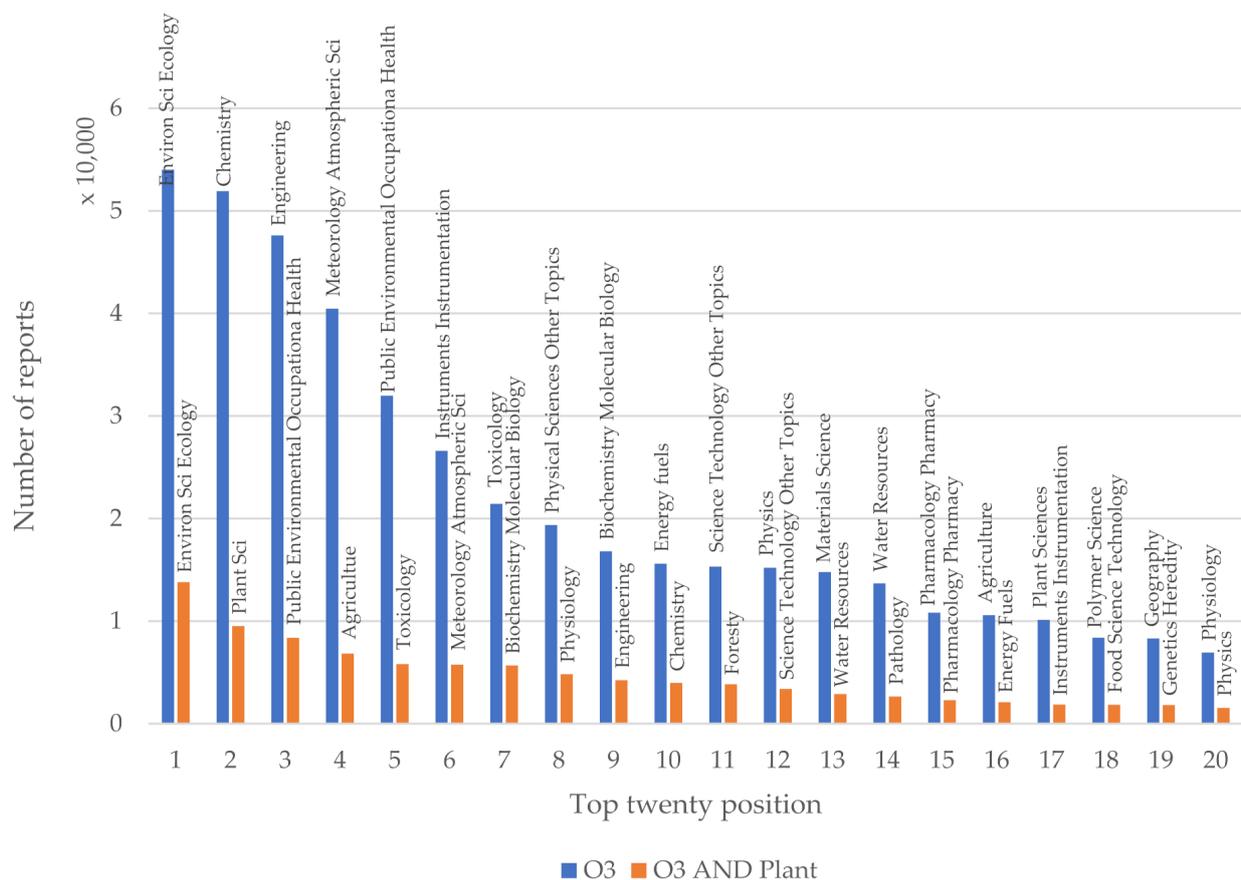
Figure 2. Distribution (number of reports) of worldwide research on Ozone (O_3) and O_3 and plant by subject area, as classified by WoS.

Distribution by areas changed when publications on O_3 were studied in the plant research field. The first area was the same as those shown by the global O_3 research field i.e., Environmental Sciences Ecology (13,796 records), while the Plant Sciences area (9523 records) and Agriculture (6832 records) moved up to second and fourth positions, respectively. In addition, the areas Public Environmental Occupational Health (8381 records)

and Toxicology (5807 records) reached third and fifth positions, respectively (Figure 2). The increase in both areas implied a reduction in areas related to Environmental, Technology, Engineering, Physics, and Chemistry. However, the search query "Ozone" and "Plant" continues considering numerous articles related to Environmental research. Therefore, the bibliometric study from this point forward will be performed considering only the areas of Plant Sciences and Agriculture. The limitation to the plant sciences and agriculture areas implies a reduction of 7658 reports, however, it should be noted that a document can be assigned to more than one area at the same time.

3.2. Publication Distribution by Countries, Institutions, and Languages

O_3 in the plant research field has been studied by 111 countries, highlighting the great impact that O_3 effects in plants have worldwide. Countries such as the United States (US), the United Kingdom (UK), China, Germany, Italy, Japan, Spain, Canada, Finland, and India (in order according to the number of O_3 publications in the plant research field) have disseminated more than 400 publications in the period analysed and together accounted for 70% of total publications about O_3 in the plant field (Figure 3). The great interest of these ten countries could be due to the high O_3 levels existent in them because of the industrialization process and the use of fossil fuel [1]. Countries in the Northern Hemisphere have experienced a continuous increase of O_3 concentration from the beginning of the 21st century (Intergovernmental Panel on Climate Change, 2013) and accumulated high O_3 concentrations, mainly during spring and summer seasons, according to the model of surface O_3 from the present-day simulation (see Figure 1 in [61]). Consequently, countries with more research about O_3 in plants are placed in the Northern Hemisphere (Figure 3). It has been described that China can reach crop yield losses due to high O_3 levels near to 15% for wheat and maize and 12.5% for rice, the US reaches losses of 15% and 20% for maize and soya, respectively [14], and the UK suffers important economic losses due to O_3 effects in crops [19]. This is consistent with the fact that the US, the UK, and China show the highest number of publications related to O_3 in the plant research field. In a similar way, high O_3 levels in the Mediterranean basin cause significant losses in the quality and yield of horticultural crops such as tomato [19], lettuce [21], potato [23], etc., which coincide with an increase in the research of O_3 in plants in the Mediterranean countries, where these kind of horticultural crops are economically and nutritionally very important.
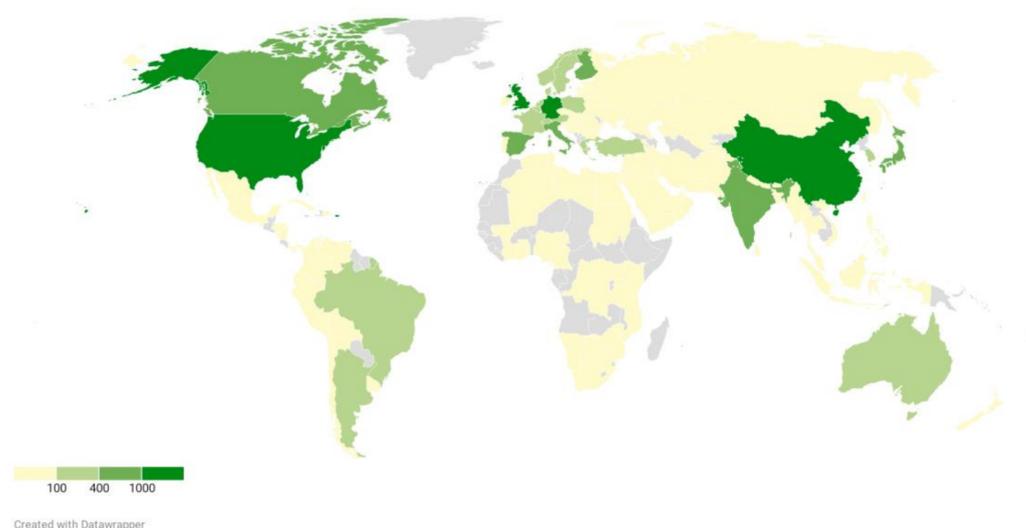
Figure 3. Contribution by country to research documents on ozone in the plant field in the period 1855–2020.

As expected, the most productive institutions were from the US and China. Table 1 shows the institutions which have more than 150 publications of O_3 in the plant field.

Eleven of these twenty institutions were from the US, accounting for 62% of total publications. It is also remarkable that all of the institutions from the top twenty belong to countries placed in the Northern Hemisphere.

Table 1. Ranking of the 20 most productive institutions in the research field of ozone in plants.

Institution	Records	Country
United States Department of Agriculture (USDA) *	866	US
University of California System	404	US
Chinese Academy of Sciences (CAS)	354	China
United States Forest Service	332	US
University of North Carolina	321	US
North Carolina State University	290	US
University of Eastern Finland	263	Finland
Consiglio Nazionale delle Ricerche (CNR)	250	Italy
Helmholtz Association	249	Germany
University of California Riverside	215	US
Pennsylvania Commonwealth System of Higher Education (PCSHE)	193	US
Pennsylvania State University	188	US
Technical University of Munich	169	Germany
l'Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE) *#	168	France
Helmholtz Center Munich German Research Center for Environmental Health #	157	Germany
Centre for Ecology Hydrology (CEH) #	157	UK
University of Pisa	154	Italy
United States Environmental Protection Agency #	152	US
United States Department of Energy (DOE) #	151	US
Cornell University	147	US

* Research Institutions focused on Agronomy. # Research Institutions related to environmental research.

Furthermore, two out of the twenty most productive centres in the O_3 research field were specific to the agricultural research field, five were related to environmental research and the rest were multidisciplinary institutes.

Research studies on O_3 in the plant field have been published in 26 different languages. The most common language on this topic is English because this is the international language for science and technology. Furthermore, if the number of publications around the world is considered, the US and the UK are the countries where more scientific documents on O_3 in plants are published, and this is in accordance with the fact that English is the most used language. Despite the low number of O_3 documents published by France, the second most used language is French. This extended use of French is related to the high number of publications on this research in Canada, where French is, together with English, an official language of the country since the "Official Languages Act of 1969". It is also remarkable that German and Chinese are the third and fourth most used languages in O_3 and plant field documents, respectively, which is also consistent with the fact that China and Germany are the countries that published the third and fourth highest number of O_3 reports, respectively, only behind two English-speaking countries (the US and the UK). The remaining documents are classified as "other". This category includes languages such as Portuguese, Spanish, Russian, Korean, Japanese, or Polish (Figure 4). Again, these results prove the high interest and importance of this trend around the world, and particularly in Northern Hemisphere countries.
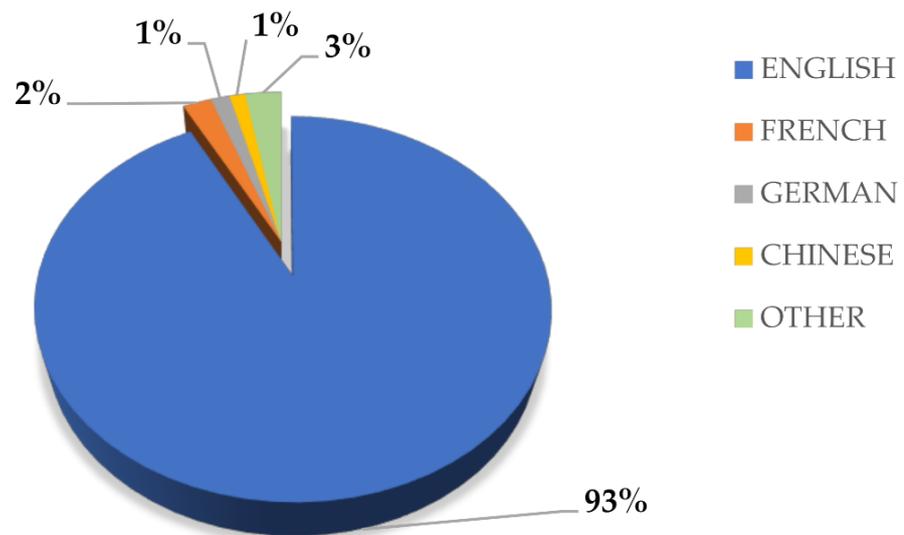
Figure 4. Languages used in the research documents on ozone in the plant field.

3.3. Distribution of Output in Journals and Types of Publications

In this research, nearly 12,000 articles were found in over 2000 different journals, although the vast majority published less than ten. This high diversity of publications, along with the heterogeneity of the journals, suggests, again, wide interest among the scientific community regarding this topic. Only 20 journals displayed more than 100 articles, comprehending 37% of the published articles overall. Of these 20 journals, 45% are exclusively related to environmental matters, another 45% on plant science, and 10% relate to plants and environment interaction. Remarkably, almost half of the journals are exclusively focused on environmental topics. This could be explained by the progressive increase in research on environmental matters in the last 30 years, due to the increasing importance of climate change and the challenges it poses to the future of agriculture and human feeding. In this sense, with regards to the publication number by source, Table 2 lists the top ten journals in which results from these topics have been published. The journal Environmental Pollution leads this list with 859 publications, comprising more than twice as many articles as the following journals. This could be due to the fact that this journal covers "all aspects of environmental pollution and its effects on ecosystems and human health", so it includes very diverse fields of study and therefore accepts a great variety of scientific topics. By the number of publications, it is followed by New Phytologist and Phytopathology, two journals about much more specific fields, however, with a deep connection to the impact of O_3 on plants. It is worth pointing out that in this top ten list of journals, none has a multidisciplinary character, possibly as the result of the particularity of the topic researched.

In reference to their scientific impact, the generic plant-related journals generally present a higher impact factor than the environmental ones, as can be seen with New Phytologist (8.795) and Plant Physiology (7.52) (Table 2). The elevated impact factor of these two journals suggests that the impact of the papers about O_3 in the plant field published in these sources is higher than those papers published in environmental-related journals with a minor impact factor such as Environmental pollution (6939) and Science of the total environment (6.419). These results point out that a journal, for instance, Environmental pollution, can publish a great number of articles about this topic (859), but have, in return, a comparatively low impact on the scientific community.

Table 2. Distribution of publications by source.

Position	Source Titles	Records	%	5 Years JCR	Country
1	*Environmental pollution*	859	7.45	6.939	UK
2	*New phytologist*	327	2.84	8.795	UK
3	*Phytopathology*	290	2.52	3.492	US
4	*Science of the total environment*	270	2.34	6.419	Netherlands
5	*Atmospheric environment*	267	2.32	4.633	UK
6	*Plant physiology*	227	1.97	7.52	US
7	*Environmental and experimental botany*	219	1.90	4.744	UK
8	*Plant cell and environment*	188	1.63	7.044	UK
9	*Water air and soil pollution*	178	1.54	2.041	Switzerland
10	*Physiologia plantarum*	172	1.50	3.947	Denmark

Documents on O_3 in the plant field recovered from the WoS database can be divided into 17 document types. The most frequently used document type was "article", which accounted for 10,848 records (65% of total publications). Another important category is "meeting", which accounted for 1373 records (8%), followed by "review", which accounted for 727 records (4%), "abstract" with 428 records (3%), "patent" with 223 records (1%), "book" with 216 records (1%), and, finally, followed by "editorial" with 113 records which accounted for less than 1% of total publications. The remaining documents are classified as "others" and correspond to minor categories such as "letter", "correction", "clinical trial", "biography", "early access", "news", and "case report". These minor categories individually contribute between 0.006–0.145%, and together accounted for 16% of total publications (Figure 5). It is important to note that a document can be included in more than one category of document type. These results indicate that most authors prefer mainly to publish their important findings in article format. Nevertheless, a lot of authors have chosen meetings and reviews as a way to disseminate the scientific research in this field, with reviews being a good way to summarize and assemble important findings and studies of specific research. It is worth pointing out the high number of patents registered in which the use of O_3 as a tool for developing equipment and procedures is included.
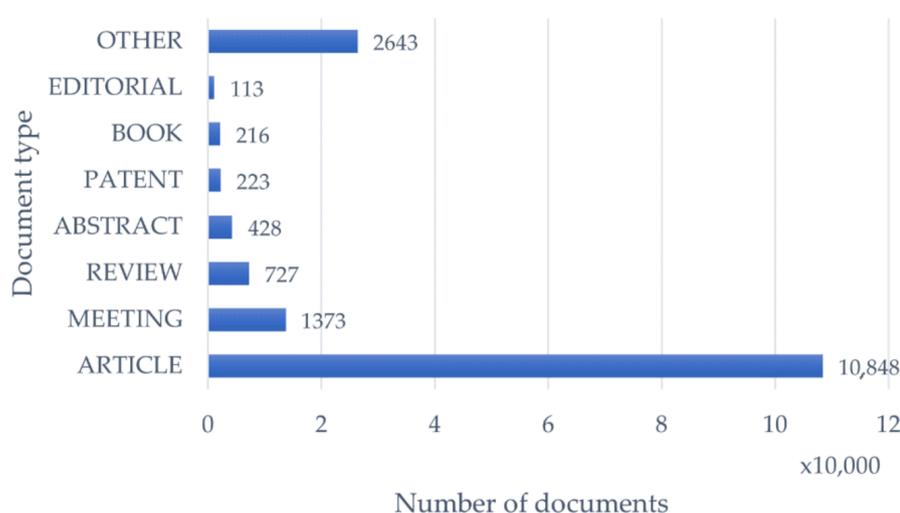
Figure 5. The most frequently used document type in the research documents on ozone in the plant field.

3.4. Analysis of Terms Used in Titles and Abstracts

To identify trends in scientific research on the topic of study, we analysed and represented the words from titles and abstracts using the VOSviewer computer program. To perform a general analysis, the research of terms mentioned more than 100 times in titles and abstracts from articles about O_3 in the plant research field was performed. The retrieved terms were divided into five clusters by the VOSviewer program, according to the relationship between the items (Figure 6).
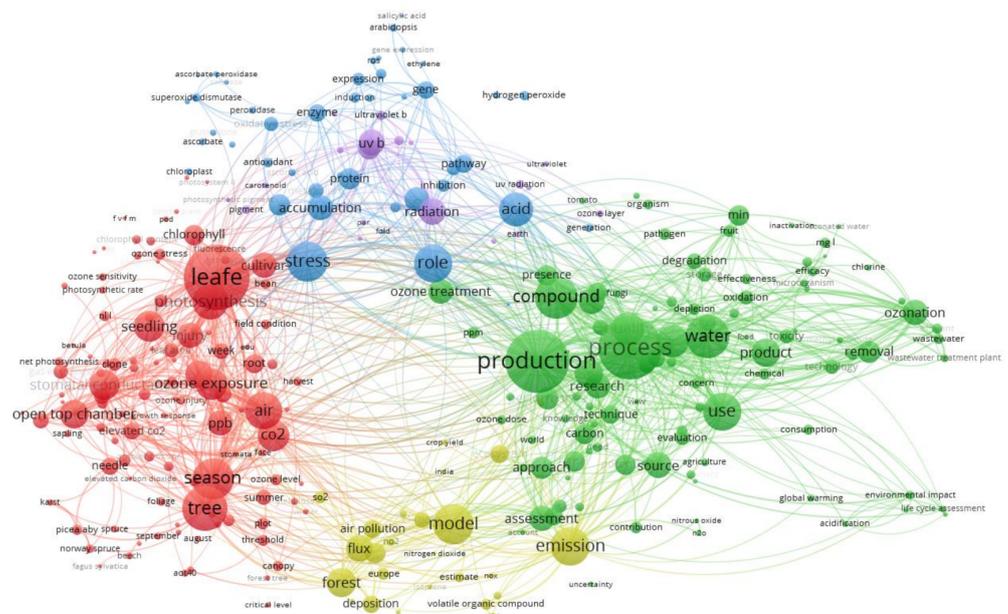
Figure 6. Word clouds based on worldwide research on ozone in the plant field. Terms named more than 100 times in titles and abstracts. The size of the circle under the word indicates the frequency of word occurrence. The five clusters, made by the VOSviewer program according to the relationship between the items, are represented in different colors.

In the first cluster, the green one, the terms "process", "production", "water", "compound" and "use" were the most frequently mentioned. These five words are habitual terms in agronomic research. However, when we analysed the term combination of the green cluster, numerous terms related to the environment and sustainability could be found: "climate change", "global warming", "degrees C", "environmental impact", "lca" (life cycle assessment), "wastewater", and so on (Figure 6). These terms are associated with O_3 research since O_3 affects climate change, because of its relationship with the ozone hole in the stratosphere and by being a greenhouse gas. In addition, terms such as "oxidation", "toxicity", "food", and "agriculture" indicate that O_3 is an important air pollutant whose oxidant capacity causes significant damage to forests and crops [5,14].

The second cluster with the most words is the red one, where the terms "leafe", "tree", "season", "air", and "seedling" are highlighted because they appear more frequently in articles about O_3 in the plant field. This group included a series of concepts related to the physiological processes affected by high O_3 levels ("photosynthesis", "rubisco", "ozone injure", "stomatal conductance", and so on), with the assays that are carried out to study such physiological processes ("open-top chamber", "otc", "aot40" (accumulated O_3 exposure over a threshold of 40 ppb), "non-filtered air", etc.), the plant tissues and organs affected ("stomata", "root", "shoot", "stem", "foliage", and so forth), as well as the altered parameters ("grow respond", "age", "dry weight", "height", "senescence", etc.) (Figure 6). As mentioned above, the first defence line of the plants against high O_3 levels is the stomata [25]. Once overcome, the photosynthesis [44] and the Calvin cycle [46] are affected, cell death [26] and the senescence are accelerated [45], and, therefore, the plants

show damage in different tissues that alter the quality and yield of the species affected [45]. The assays for analysing this kind of damage are usually carried out in open-top chambers (otc) using diverse O_3 concentrations (Aot40, non-filtered air, ambient air, etc.) [62,63]. All terms included in the red cluster speak about plant physiological processes, tissues, and parameters damaged with high O_3 levels and the assays performed to analyse them. In addition, numerous vegetal species, which will be analysed later on, are included in this cluster (Figure 6).

The third broader cluster, the blue one, highlighted words like "stress", "role", "acid", "accumulation", and "tolerance". However, this group included numerous words related to molecular and cellular alterations shown when plants are exposed to high O_3 levels such as "oxidative stress", "ascorbate peroxidase", "superoxide dismutase", "glutathione", "salicylic acid", "ethylene", "ros" (reactive oxygen species), and "H_2O_2" (hydrogen peroxide), "cell death", etc. (Figure 6). As mentioned in the introduction, the plant metabolic and molecular pathways altered by high O_3 levels are mainly the ethylene and salicylic acid biosynthesis pathways [25], and antioxidant systems [34,35], all of them represented in the terms included in this cluster. This cluster includes only two vegetal species, tobacco and *Arabidopsis* (Figure 6), which are species widely used to perform genetic and molecular studies. This is due to their exceptional characteristics, such as a small genome, short life cycle, and accessible transformation methods, all of which make them both suitable as model systems [64,65].

In the fourth cluster, the yellow one, the words "model", "emission", "forest", "flux", and "air pollution" are highlighted as frequently named in titles and abstracts of articles on O_3 in the plant research field. This cluster is characteristic since it includes words related to environmental contamination and O_3 precursors such as "NOx", "volatile organic compound", "isoprene and monoterpene", "air pollution", "nitrogen dioxide", "climate", etc. (Figure 6). As described previously, the industrialization process and the use of fossil fuels have increased emissions of O_3 precursors such as NOx or VOCs [1,3,4]. In addition, isoprene and monoterpene are the most abundant BVOCs emitted by terrestrial vegetation, particularly by forests. Similar to VOCs, BVOCs can lead to changes in the production of tropospheric O_3, depending on the NOx concentration [66]. All terms in this cluster are related to O_3 precursors that cause environmental pollution and increased O_3 levels.

The last cluster, the purple one, includes only words related to stratosphere O_3 ("ozone layer", "ultraviolet b radiation", "active radiation", "par" (photosynthetic active radiation), etc.) and with plant pigment ("flavonoid" and "carotenoid") (Figure 6). This cluster is likely generated due to the fact that O_3 in the stratosphere protects life on Earth from harmful ultraviolet radiation. The appearance of pigments in this cluster could be due to different studies about damage in the plant pigments caused by UV radiation [67].

In conclusion, this word analysis indicates that the most important issues for the scientific community on O_3 in the plant research field are the ozone hole, climate change, environmental damage in general, the stratospheric O_3 formation and its precursors, as well as the genetic, metabolic, and physiological changes suffered by plants.

These interests are easy to understand, since all damages induced by O_3 in plants cause a weakening of the plant, reducing its ability to cope with other abiotic and biotic stresses. This eventually leads to a reduction in the yield and quality of cash crops, which is a serious problem as it puts global food security at risk.

To perform an analysis about the plant species studied more by the scientific community in the O_3 research field, a word cloud using plant species named more than 25 times in titles was carried out using the program WordArt (Figure 7). First, we can appreciate that the plant species mentioned more frequently in titles is "bean" (*Phaseolus vulgaris* L."). This is because of the high importance of beans in the human diet, representing 50% of grain legumes consumed worldwide [68], and the great impact of O_3 on their growth. Almost 78% of the tested species showed detrimental effects on their total biomass relative to their growth rate due to O_3 [69]. In addition, "*Phaseolus vulgaris* L." is widely used as a bioindicator system to detect ambient O_3 effects [70,71] and is a model species for other

leguminous plants such as "soybean" [68,72], which are also represented in the cloud but with less importance (Figure 7). Other model species such as "*Arabidopsis*" and "tobacco", both widely used in molecular and metabolic research studies as mentioned above, are also included among species of interest for O_3 research in plants, mainly *Arabidopsis* (Figure 7). Similar to beans, tobacco is used as a biomarker system [71]. According to those described in the introduction, two cereals ("rice"/"*Oriza sativa* L." and "*Triticum aestivum* L."/"spring wheat"/"winter wheat") stood out among plant species mentioned more frequently in the titles of O_3 studies in plants (Figure 7). Both cereals have a high economical and commercial importance and show high sensibility to high O_3 levels, which provoke big yield losses of both cereals. Alternatively, numerous trees species are mentioned in the titles from articles on O_3 in plants (Figure 7). It is worth mentioning the presence of "betula/birch", "picea abies/Norway spruce/spruce", "Fagus sylvatica/European beech/beech", "aspen", and three species from the pinaceae family, "Scots pine", "ponderosa pine", and "eastern white pine". These findings make sense because the high oxidant capacity of O_3 damages tree species [5].
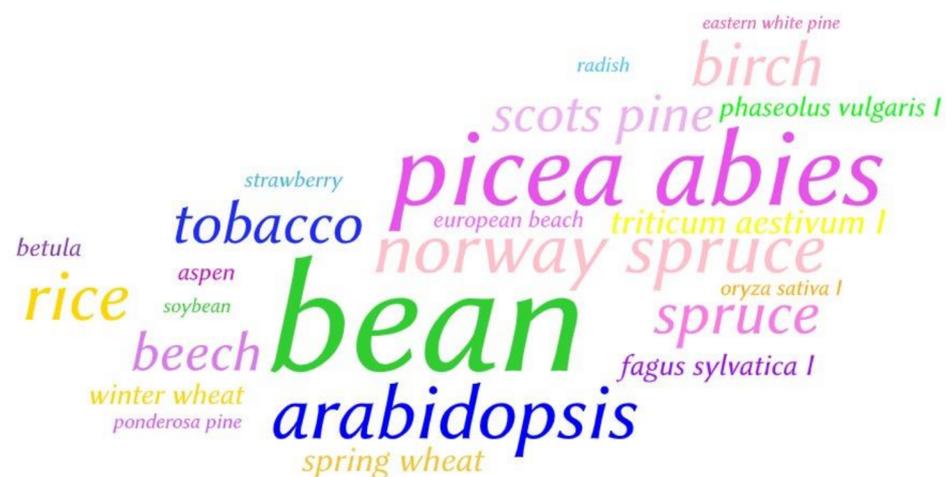
Figure 7. Word clouds based on worldwide research on ozone in the plant field. Plant species named more than 25 times in titles. The size of the word indicates the frequency of word occurrence. Leguminous plants are represented in green, the trees in pink, the cereals in yellow, and the rest, including model species, in blue.

According to this, tropospheric O_3 is a likely contributing factor to tree decline in some North American and European forests [5,73–77]. Some of the effects caused are the alterations of BVOCs mentioned in the introduction, which could alter communication among themselves or insect pollination [24,78]. However, establishing a cause and effect relationship for ambient O_3 exposure and tree growth in forests is a difficult task [79,80]. This is due to different sensitivities between species from the same natural community, which induce the selection of resistant species versus sensitive species [3,81]. Among the forest masses that suffer O_3 impact, we can underline those located in southern California, where the *Pinus ponderosa* presents high sensitivity to O_3 [82], and it is estimated that by the year 2074 it will be close to disappearing, thus changing the dominance of this ecosystem in favour of the *Quercus kelloggii* [83]. All of this reveals the considerable interest of the scientific community in the relationship between trees and O_3.

3.5. The Most Cited Articles

Another way to evaluate the main interests of the scientific community on O_3 in the plant research field is by analysing the most cited articles. Papers receiving more than 1000 citations are listed in Table 3. Two papers stand out with nearly 2000 citations. The most cited paper was published in 2015 by Lelieveld [84] in Nature (*n* = 1951). The topic

of this paper is the impact of air pollutants, including O_3, on premature mortality. The implication of the plants in this article is due to the fact that agricultural emissions make a relative contribution to outdoor air pollution in some countries. The second most cited paper (*n* = 1766) was published in Science in 1990 by Crutzen and Andreae [85]. The paper explains how biomass burning (to convert forests to agricultural and pastoral lands, control of pests and insects, prevention of brush, nutrient mobilization, etc.) in the tropics leads to high concentrations of air pollutants, including O_3, which implies damage to trees and vegetation, in addition to affecting climate, atmospheric chemistry, and ecology in the tropical regions. Two papers with nearly 1000 citations, published in Plant Cell and Environment (top three; *n* = 1182) and Plant Physiology (top five; *n* = 1011), respectively, discuss metabolic and genetic alterations generated by plant exposition to high O_3 levels. Finally, the top four articles most cited (*n* = 1063), published in Nature Protocols, describe an assay to estimate the total phenolic content and other oxidation substrates in plant tissues (Table 3). Despite the selection of Plant Sciences and Agriculture research areas to perform this project, the most cited paper discusses O_3 involvement in human health. Similarly, the second most cited paper is mainly focused on environmental changes caused by air pollutants, including O_3, although this paper also describes the damage caused by O_3 in plants. However, the other three most cited papers are focused on metabolic and genetic pathways affected in plants exposed to high O_3 levels and assays to analyse these changes. Results emphasise the high interest of the scientific community, not only in the damage caused by O_3 in plants but also to human health and to the environment.

Table 3. Ranking of the five most cited articles in the research field of ozone in plants.

Cites Number	Title	Publication Year	Journal	Reference
1951	The contribution of outdoor air pollution sources to premature mortality on a global scale	2015	Nature	[84]
1766	Biomass burning in the tropics—impact on atmospheric chemistry and biogeochemical cycles	1990	Science	[85]
1182	Oxidant and antioxidant signalling in plants: a re-evaluation of the concept of oxidative stress in a physiological context	2005	Plant cell and environment	[86]
1063	Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent	2007	Nature protocols	[87]
1011	Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*	1996	Plant physiology	[88]

3.6. Databases

This study has been carried out by assembling information from the WoS database. However, this database, besides having the WoS core collection (10,988 records), also includes other sources of information such as Business Cycle Indicators (BCI) (9547 records), BIOSIS (9546 records), Current Contents Connect (CCC) (7057 records), and Medical Literature Analysis and Retrieval System Online (MEDLINE) (4570 records) (Figure 8). Most documents can be included in more than one database, so the final number accounted for 42,069 records, despite the 11,529 documents that have been selected for the present study. BCI, with 9547 records, is a database that provides economic indicators and statistical information. A lot of publications of O_3 and plant research are included in this database, which could be due to the fact that O_3 damage causes important economic losses. BIOSIS Previews is part of the Clarivate Analytics WoS database and contains abstracts and citation indexing. CCC is a WoS platform, allowing current awareness and notifying alerts when new issues of a journal are released. Finally, MEDLINE (Medical Literature Analysis and Retrieval System Online) is a bibliographic database that includes life science and

biomedical information. Therefore, O_3 documents are registered in MEDLINE because this oxidative gas is associated with adverse health outcome damage, especially on respiratory and cardiovascular systems [89].
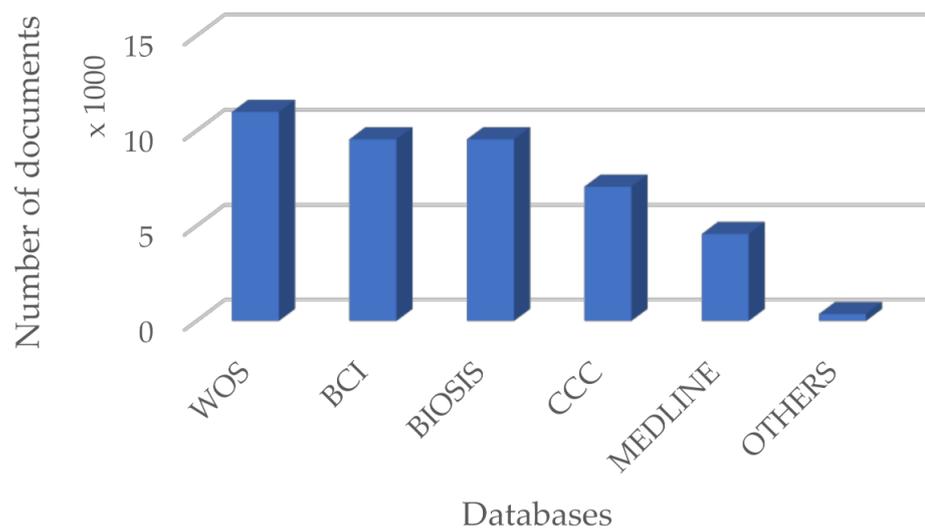
Figure 8. Number of documents on O3 in the plant research field included in the different databases of Web of Sciences.

4. Conclusions

O_3 is an important air pollutant, a greenhouse gas, and a highly reactive oxygen species that cause significant economic losses in crops, forests, and ecosystems. Even though the interest of the scientific community on O_3 in the plant research field, measured as document number per year, has been increasing since the second half of the past century, this rise is slow in relation to O_3 studies in other research areas. Research in O_3 has been studied most in the health area. Both research areas, health and environmental, are fields of high interest for humanity, and both are affected by high O_3 levels, contributing to climate change, damaging human health, and putting global food security at risk.

The countries with more studies and publications are placed in the Northern Hemisphere, where the rapid industrialization process of the last century and the use of fossil fuels have caused higher emissions of O_3 precursors such as NOx or VOCs [1,3,4]. Knowing the causes that provoke the increase of tropospheric O_3 can help avoid the continuous increase of this pollutant.

With regard to plants, alterations caused by O_3 at genetic, metabolic, and physiological levels end in important losses of quality and yield in commercial crops. Among crops studied more frequently in the O_3 research field, species with high commercial value can be underlined, such as horticultural crops (tomato, lettuce), beans, or genus *Brassicaceae* species, as well as staple food crops (soybean, maize, wheat, and rice). The latter ones are the base of the human diet, meaning that losses caused by O_3 could put at risk global food security. This important problem claims to be solved through the implementation of breeding programs, with the ultimate goal of developing commercial varieties tolerant to this contaminant. Because of this, it is paramount that the scientific community acknowledges the issue, and develops studies through which to determine the genetic and metabolic pathways that are damaged by high O_3 levels, as well as the genes that regulate tolerance to O_3. In accordance with this objective, some pre-breeding programs have been developed in rice [90,91] and soybean [92], in which some genes that confer resistance to O_3 have been identified. However, very little progress has been made with horticultural crops. Therefore, Booker et al. [93] claim the need to identify molecular markers related to O_3 tolerance in modern cultivars. With the aim of developing breeding programs, model

species have previously been used, such as *Arabidopsis* or *Nicotiana tabacum*, in which numerous metabolic and physiological pathways are known, and the effects of O_3 on them are easier to study [30,35].

In addition, numerous studies have been carried out about O_3 damage on trees, forests, and ecosystems. The effects of O_3 accumulate over time and, together with other stresses (prolonged drought, excess nitrogen deposition), may exacerbate the direct effect of O_3 on ecosystems. These alterations could influence competitive interactions among species [94], and finally, cause the disappearance of sensitive species through the selection of varieties resistant to O_3.

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