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Review

Worldwide Research on Plant Defense against Biotic Stresses as Improvement for Sustainable Agriculture

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Abstract: Agriculture is the basis for food production on a global scale. Sustainable agriculture tries to improve or maintain the quality of food without compromising the environment. As sessile organisms, plants cannot avoid adverse environmental conditions and contact with other living organisms. The damage caused to plants by other living organisms such as parasites and pathogens (virus, bacteria, fungi, nematodes or insects) brings about what is known as biotic stress. Plants are constantly exposed to biotic stress, which causes changes in plant metabolism involving physiological damages that lead to a reduction of their productivity. To fight biotic stress, plants have developed sophisticated defense mechanisms. Thus, understanding plant defense mechanisms might prevent important crop and economic losses. In this article, a bibliometric analysis of biotic stress is carried out. Different aspects of the publications are analyzed, such as publication type, research field, journal type, countries and their institutions, as well as the keyword occurrence frequency, and finally special attention is paid to the plant studied by the leading countries and institutions. As expected, journals selected by authors to publish their relevant findings are plant-specific journals. However, it should be noted that the fourth position, in terms of the number of publications per journal, is occupied by *BMC Genomics* journal. Such a journal considers mainly articles on genomics, which indicates the involvement of genetic factors in the control of biotic stress. Analysis of the keywords used in publications about biotic stress shows the great interest in the biotic–abiotic stress interaction, in the gene expression regulation in plants as well as phytohormones in the current research. In short, the great effort made by the scientific community in the biotic and abiotic stresses field with the aim to understand, regulate and control plant damages caused by biotic stress agents will help in the development of sustainable agriculture.

Keywords: sustainable agriculture; biotic stress; abiotic stress; phytohormones; plant species

1. Introduction

Agriculture faces the great challenge of providing a world population in continuous growth with food, while the natural resources remain the same [1]. This great challenge, without doubt, will be supported in the future by Agronomy and its related sciences [2], understood in a first approximation as the disciplines that combine practical knowledge and scientifically based techniques that are applied to agricultural production. Without intending to carry out an in-depth analysis of the issue, this can be supported by the sciences that study the physical, chemical and biological conditions applicable to the cultivation of plants and the general improvement of agriculture [3].

This great challenge cannot be understood without sustainability nowadays [4]. Global sustainable development "is a development that meets the needs of the present without compromising the ability of future generations to meet their own needs" [5]. There are many theories about the concept of sustainable agriculture, understood as a set of strategies, especially management, which try to improve or maintain the quality of food without compromising the environment [6–8], and without compromising the productivity of crops in the long term [9]. On the other hand, consumers are also demanding products grown in a more sustainable way [10].

Plants are faced with numerous biotic stresses and adverse environmental conditions. They respond to these stresses through several morphological, biochemical, and molecular mechanisms and evidence suggests that there are interactions among their respective signaling pathways [11]. Biotic stresses in plants are caused by pests, parasites and pathogens, which are known since ancient times. Fungi, bacteria, nematodes and viruses are the pathogens primarily responsible for plant diseases. There are two types of fungi parasites: nectrotrophs, which kill host cells using toxins, and biotrophs. Together with bacteria, they cause vascular wilts, leaf spots and cankers among other symptoms, and can infect different parts of the plant. Nematodes withdraw the contents of plant cells and can feed on all parts of the plant, but plant parasitic nematodes cause primarily soil-borne diseases and attack plants' root system. They produce symptoms related to nutrient deficiency, such as wilting or stunting. Viruses produce not only local lesions but also systemic damage that causes stunting, chlorosis and malformations affecting different parts of the plant, although they rarely kill their hosts. On the other hand, insects and mites are the pest to be highlighted. They damage plants through feeding or egg laying. Piercing–sucking insects can also act as virus vectors, transmitting them to plants through their stylets (review in [12]). In order to deal with biotic stresses, plants have developed an advanced immune system. The first line of defense in plants is passive. Plants have physical barriers such as waxes, thick cuticles and specialized trichomes that prevent insects or pathogens from settling into the plant. Plants also produce chemical compounds to protect themselves against herbivory and pathogen infection [13]. In addition, plants have two levels of pathogen recognition that trigger defense responses. The first level of recognition involves Pattern Recognition Receptors (PRRs) that recognize pathogen-associated molecular patterns (PAMPs). This type of immunity is a basal defense response called PAMP-triggered immunity (PTI) [14]. Phytophagous insects have their counterpart in the recognition of conserved Herbivore-Associated Elicitors (HAEs), Herbivore-Associated Molecular Patterns (HAMPs) or herbivore effectors by PRRs [15]. The second level of plant immune system is carried out by plant resistance (R) proteins that recognize specific effectors from pathogens or pests (Avr proteins) and activate plant defense mechanisms in a much more effective way [16,17]. This type of resistance is named effector-triggered immunity (ETI) and often activates hypersensitive responses (HR) that include programmed cell death in infected cells and the surrounding areas [18]. Most R genes encode proteins with specific domains that contain a conserved Nucleotide Binding Site, called NBS. The second most significant domain is LRR (Leucin-Rich Repeat). NB-LRR receptors can recognize pathogen effectors either directly by physical association or indirectly through an accessory protein that is part of an NB-LRR protein complex [19].

PTI and ETI trigger a first response that leads to the activation of membrane-localized ion channels and subsequently elevation of cytoplasmic calcium levels. Other important events of the early responses are the production of reactive oxygen species (ROS) and the activation of Mitogen-Activated Protein Kinases (MAPKs) [20]. Both types of resistances might activate the same responses but ETI immunity is much more robust [21].

Among the downstream signaling pathways induced by ETI and PTI, three hormones stand out: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). While the SA pathway stimulates resistance responses to biotrophic and hemi-biotrophic pathogens, the JA and ethylene pathways are generally induced against necrotrophic pathogens and chewing insects [22]. SA, in turn, activates a systemic resistance response called SAR (Systemic Acquired Resistance) that promotes the expression of PR (pathogen related) genes and provides long-term defense against a broad spectrum of pathogens [23]. Although SA, JA and ET defense signaling pathways have substantial differences in gene expression, they interact to help the plant choose the best defense strategy [24]. Recently, other hormones such as abscisic acid (ABA), auxin, brassinosteriods (BR), cytokinin (CK), gibberellic acid (GA) and peptide hormones have also been reported as important regulators of immune responses [25].

Due to the diverse feeding habits of arthropods, plant responses to insect attacks are complex and the genes involved are closely related to the herbivore feeding system. Phytophagous insects activate defense responses that are locally and systemically induced by signaling pathways which involve systemin, jasmonic acid, oligogalacturonides (OGAs) and hydrogen peroxide [26]. Yet, plants also respond to the attack of insects by producing volatile compounds that dissuade the attacking insect, induce defense responses and recruit natural enemies of their aggressors. These compounds include products from the lipoxygenase (LOX) and the terpenoid pathways [27,28]. For this reason, defense responses induced by insect attacks used to be classified either as indirect responses, characterized by the production of volatile compounds, or direct responses, designed to repair damaged tissues and activate defense mechanisms for preventing future attacks [29,30]. Although various phytohormones are involved in plant defense against herbivores, JA plays a crucial role in activating direct and indirect defense responses [31] and the JA signaling pathway works in a crosstalk network with other phytohormone signaling pathways such as ET, SA and ABA [32]. Additionally, one of the most important downstream defense responses in plants against insect herbivore attacks is the production of defensive proteins such as protein inhibitors, α-amylase inhibitors, lectins, chitinases and polyphenol oxidases. These proteins reduce herbivore insectcapacity to digest the plant [26].

Despite the numerous defense mechanisms developed by plants with the aim to overcome stress conditions and to adapt [33], important economic losses from potential crop yields due to biotic and abiotic stress conditions happen every year. Given the quick population growth worldwide and the subsequent increasing food demand, prevention of crop-yield losses as a consequence of abiotic and biotic stresses is necessary. Numerous studies about biotic and abiotic stress responses have been carried out since 1979 with the aim to understand and to be able to control plant plagues and diseases, as a deep understanding of plant defense responses to pathogens and herbivores is essential to design new methodologies and improve the current approaches to crop protection.

Scientometric [34,35], Informetric [36], and Bibliometric [37,38] sciences allow for the evaluation of scientific trends in specific research fields. The analysis of scientific production allows for the current state of research, as well as the contributions in knowledge fields from different countries, research institutions and researchers, to be valued, which will lead to upcoming research projects in specific fields [37]. Moreover, the bibliometric indicators to analyze the scientific production in a specific research field from institutions or countries can also be considered a socio-economic indicator of these countries and research centers since scientific production is closely related to investment [39]. Thus, the aim of this manuscript is to perform a bibliometric study on the worldwide scientific production in the research field of biotic stress in plants.

2. Materials and Methods

Elsevier Scopus database was used to extract the scientific publications analyzed in this study. With the aim to compile academic articles including the expression "Biotic stress" in the title, abstract and/or keywords, a search in Scopus database was conducted in November 2017. The search query (TITLE-ABS-KEY (Biotic Stress)) was used and restricted to the period between 1979 and 2016. The publications obtained from the search were evaluated and categorized according to the subsequent aspects: number of scientific documents per year, type of document, allocation by journals and by subject categories, and allocation by country and institution. Spreadsheets were used to analyze the records obtained and to make graphs to show the results more conveniently.

Given that keyword frequency in scientific documents has been commonly used to suggest the research trend of academic literature, in this project a word cloud using keywords most cited in documents about Biotic Stress was obtained from the software WordArt (https://wordart.com/). Cloud Word was used to analyze the relevance of keywords, given that the size of font in the word cloud indicates frequency in literature. With the aim to perform the word cloud adequately, keywords such as “Biotic stress”, “review” or “article” were eliminated before the analysis, since they do not contribute information to the study. Finally, duplicated keywords such as “human” and “humans” were merged as one keyword.

The number of times that a scientific publication is cited reflects the impact said research document has on the scientific community. Therefore, the number of citations was used in this work as an analytical tool to evaluate the impact of biotic stress-related reports via h-index indicator in this field. H-index, defined as h of one’s total articles has at least h citations each [40], allows for the study of a journal, an institution, a scientific or a country according to the number of publications or the number of citations [41,42]. A main research accomplishment is generally related to a high value of h-index. Other bibliometric studies have used these methodologies successfully [38,43].

The Scopus database has been selected to carry out this project since a comparative analysis between Scopus and Web of Science showed that the journal coverage is higher in Scopus than in WoS [44]. Numerous research papers show the advantages of Scopus, and use this database to perform a bibliometric analysis [39,45]. Moreover, other main databases such as PubMed are not valid to perform bibliometric reviews since massive information downloads are not allowed [46]. Furthermore, it should be noted that if the same bibliometric study is performed in a different database, e.g., PubMed, the results might be modified, but not so significantly as to change the results and conclusions obtained in this analysis.

Mean comparison (Fisher´s Least Significant Difference test, LSD) was used to determine significant differences in distribution by country of biotic stress reports and in keyword groups related to Biotic Stress worldwide research. Analyses were performed using the Statgraphics Centurion XVI.II software package.

3. Results and Discussion

3.1. Evolution of Scientific Output and Distribution in Subject Categories

A total of 5081 documents including the “Biotic Stress” term in the title, abstract or keywords were recovered from the period 1979–2016. The number of publications from the “Biotic Stress” research field has grown significantly since the first publication in 1979. Since then and until 1996, fewer than ten articles about biotic stress were published per year. However, from 1997, a constant increase in the number of documents on biotic stress has been observed until reaching 553 publications in 2016 (Figure 1). This evolution shows a slow initial growth in the number of publications while a 10% increase in reports on biotic stress is observed in the last decade. This proves that the scientific community is starting to show interest in the biotic stress field and therefore, continuous progress in the number of publications on biotic stress is predictable in the next years.

In the year 2008, one prominent rise should be noted that was not maintained during the rest of the period. To elucidate this unexpected rise, a study on the time evolution of the distribution of publications on Biotic Stress by subject area was carried out. As expected, “Agriculture and Biological Science” was the subject area where the highest number of publications related to Biotic Stress was included, while the second area in terms of the number of publications was “Biochemistry, Genetics and Molecular Biology”, followed by the “Medicine” area (Figure 2). This classification was practically maintained in the whole period analyzed. However, subject areas such as “Engineering”, “Material science”, “Physics and Astronomy” and “Computer Science”, which incorporated few articles related to biotic stress during the 1979–2016 period, experienced an unexpected increase in the number of biotic stress documents in the year 2008, which has driven a change in the areas of distribution. In the year 2008, “Engineering”, “Material science” and “Physics and Astronomy” subject areas included the highest number of reports ever on biotic stress, standing in first, second and third position respectively (Figure 2). This outcome might result from the recent interest shown by the scientific community in the new technologies, which allow for monitoring the environmental conditions in which plants grow, with the aim to optimize irrigation management. However, the largest number of documents related to biotic stress currently corresponds to the "Agricultural and Biological Sciences" area (2899 records, 33.2%), while the second area according to the number of publications is "Biochemistry, Genetics and Molecular Biology" (2396 records, 27.4%), followed by "Medicine" (637, 7.4%). These three areas account for about 70% of all publications on biotic stress (Figure 3). The fourth area is "Engineering" (597 records, 6.8%), the fifth is "Environmental Science" (337 records, 3.8%), the sixth is "Material Science" (311 records, 3.5%), followed by "Physics and Astronomy" (275, 3.1%), "Immunology and Microbiology" (263, 3.0%), "Chemistry" (206, 2.3%) and "Computer Sciences" (164 records, 1.9%) (Figure 3). These areas are the top ten in terms of the number of publications about biotic stress. However, in 2016 and based on the Scopus classification, the distribution of publications in the biotic stress research field included a total of 27 subject areas, which is a clear indication of the wide interest that the biotic stress research field has awoken in the scientific community. It should be noted that an article may be allocated in two or more areas at the same time.

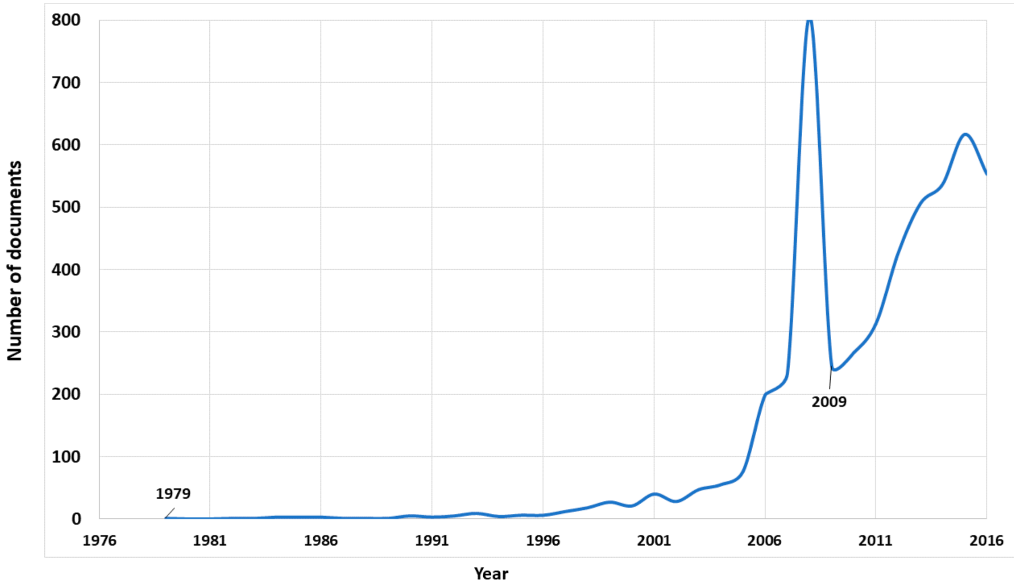

Figure 1. Trends in the publications in the Biotic Stress research field from 1979–2016.

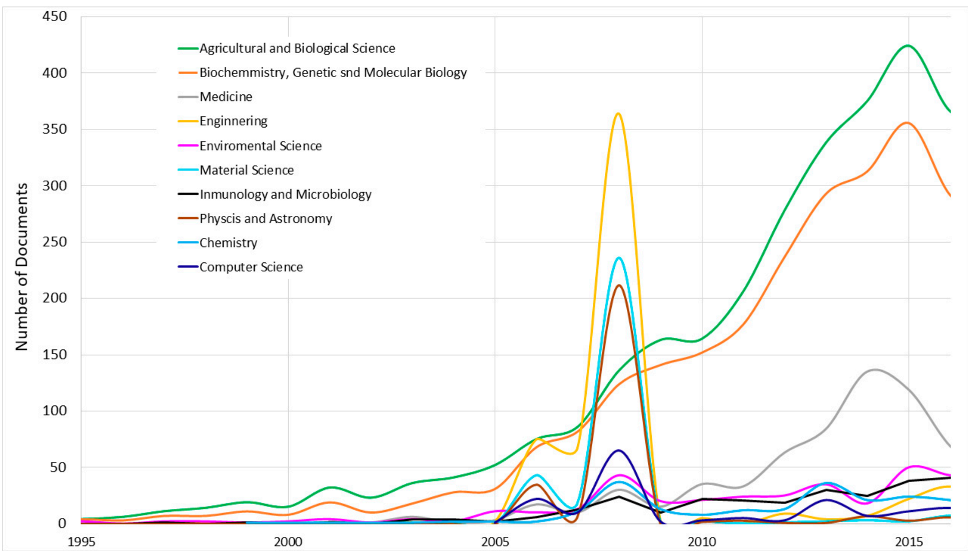

Figure 2. Time evolution of the distribution of publications on Biotic Stress by subject area.

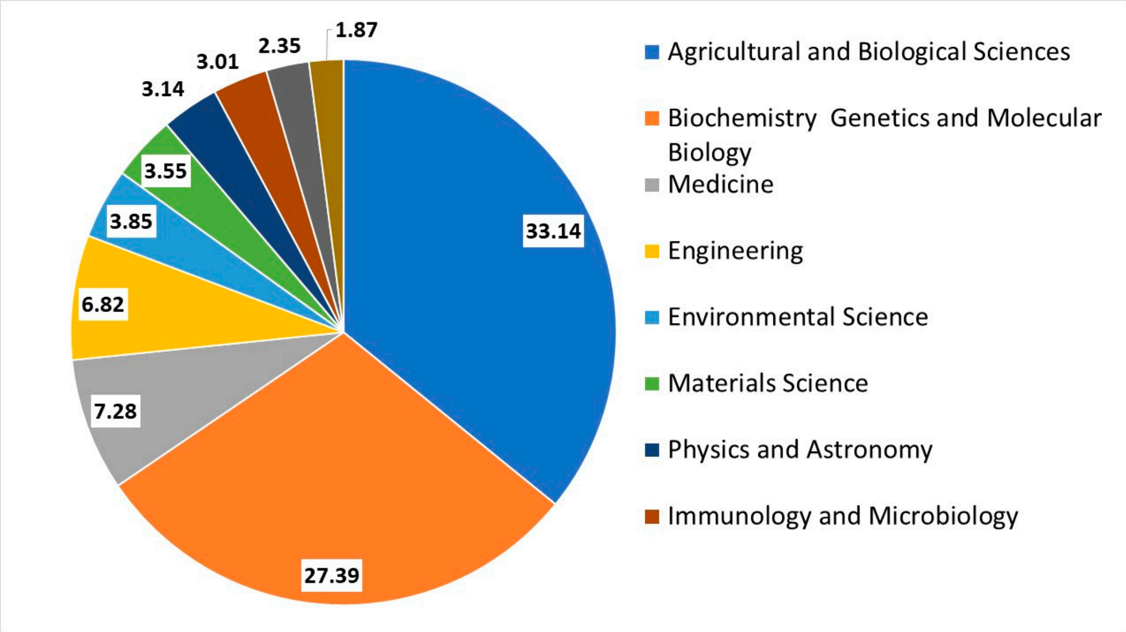

Figure 3. Distribution (%) of worldwide research on Biotic Stress by subject area, as classified by Scopus.

3.2. Types of Publications and Distribution of Output in Journals

The scientific documents about biotic stress recovered from Scopus database were classified into 11 document types. The type "articles", which accounted for 68.61% of total publications with 3486 reports, was the most common document type to publish results from the biotic stress research field. In second place, "reviews" accounted for 14.3% with 728 reports, followed by "conference papers" with 459 reports, which accounted for 9%, as well as "books chapter" with 288 documents and a percentage of 5.7%. These four document types accounted for 97.7% of the total publications. The rest of the documents, "Short Survey", "Book Editorial", "Note", "Conference Review", "Article in Press", "Letter and Erratum", only accounted for between 0.6% and 0.04% (Table 1). The results prove that articles are preferentially used by most of the authors to publish their scientific findings.

Table 1. Distribution of document types for research on Biotic Stress.

Document Type	N°	%
Article	3486	68.6
Review	728	14.3
Conference Paper	459	9.0
Book Chapter	288	5.7
Others	120	2.1

Regarding sources, results showed that 10 or more documents about biotic stress were published in 84 professional journals, of which 49 were plant specific and 8 were related to genetics. The fact that more than half of the journals were plant specific could be expected since the "biotic stress" term is mainly used for plants. However, the fact that 10% of journals in which documents related biotic stress are published should be specific to genetics suggests the great importance of genetic regulation in the biotic stress response from plants. As regards the publication number by source, Table 2 lists the top 10 journals in which results from the biotic stress research field have been published. The multidisciplinary journal *PLoS ONE*, which launched in the year 2006, led the list of the top ten journals with 213 documents. However, most of the authors chose to publish their significant findings in plant-specific journals such as *Frontiers in Plant Science*, *Acta Horticulturae*, *Journal Of*

Experimental Botany, *Plant Signaling And Behavior*, *Plant Physiology*, *Plant Physiology And Biochemistry* and *BMC Plant Biology*, which occupied the second, third, fifth, sixth, seventh, ninth and tenth positions respectively, with a number of publications about biotic stress varying between 122 and 55 documents (Table 2). Finally, a genetic-specific journal, *BMC Genomics*, was the fourth most productive journal with 122 publications, which again suggests the great impact of genetics in biotic stress research.

Table 2. Distribution of publications by source.

Rank	Source	N° Documents	H-Index	5-Year Impact Factor JCR	SJR	Country
1	*Plos One*	213	31	3.394	1.201	US
2	*Frontiers In Plant Science*	122	22	4.672	1.917	Switzerland
3	*Acta Horticulturae*	80	7	-	0.180	Belgium
4	*BMC Genomics*	75	26	4.284	2.065	UK
5	*Journal Of Experimental Botany*	72	34	6.538	2.780	UK
6	*Plant Signaling And Behavior*	70	20	-	0.641	US
7	*Plant Physiology*	68	38	7.428	3.735	US
8	*Materials Science Forum*	56	8	0.515	0.186	Switzerland
9	*Plant Physiology And Biochemistry*	56	19	3.096	1.159	Netherlands
10	*BMC Plant Biology*	55	20	4.541	1.820	UK

Regarding the h-index values of the top ten journals, *Plant Physiology* (48) and *Journal Experimental Botany* (44) had the largest h-index despite the fact that these journals did not head the top ten journals according to the number of documents published on biotic stress (Table 2). The *PLoS ONE* journal that led the top ten journals in terms of the number of publications had an h-index of 31, followed of *BMC Genomics* with an h-index of 26. Then, *Frontiers in Plant Science*, *Plant Signaling and Behavior*, *Plant Physiology and Biochemistry* as well as *BMC Plant Biology* journals had an h-index of 22, 20, 19 and 20, respectively. Finally, *Acta Horticulturae* and *Materials Science Forum* end the list with an h-index of 7 and 8 respectively. These results indicated that a journal might publish a high number of documents on biotic stress even though these publications had a low impact on the scientific community, as happened with *Acta Horticulturae*. Contrarily, *Plant Physiology* and *Journal Experimental Botany* published fewer documents on biotic stress but the h-index of these journals in this subject was very high (Table 2), suggesting that the impact of biotic stress-related publications in both journals was very high. It might be a consequence of the high impact factor of both journals in the scientific community (Table 2). Similarly, low values of h-index of *Acta Horticulturae* and *Materials Science Forum* journals were likely due to the low impact factor of both journals, which are indexed by Scopus (SJR) but not by Journal Citation Reports (JCR). It should be noted that *PLoS ONE*, *Plant Physiology* and *Plant Signaling And Behavior* journals are from US and together they have published about 40% of documents on biotic stress, while the rest of the journals are from Europe (three of them from UK) and together they have published around 60% of biotic stress reports (Table 2).

3.3. Publication Distribution by Countries and Institutions

Biotic stress has been researched by 109 countries, proving again the great interest that biotic stress in plants has awoken worldwide. Countries such as the United States (US), China, India, Germany, France, Japan, the United Kingdom (UK), South Korea, Spain and Italy have published more than 200 publications in the period analyzed, and belong (in the indicated order) to the top ten list according to the number of publications (Figure 4). These ten countries together published 63% of the total number of documents about biotic stress. However, results showed that the interest in biotic stress in each country is focused on different plant species (Figure 5). For instance, the most studied plant in the biotic stress research field was *Arabidopsis thaliana*, which is considered a model species in the plant's scientific community. Arabidopsis has a short life cycle, small genome and accessible transformation methods, all of which make Arabidopsis a suitable model species for biotic stress and other plant-specific studies [47]. Only India published more documents related with biotic stress in rice than in Arabidopsis (Figure 5). Actually, rice was the second most widely studied plant species with respect to biotic stress in oriental countries such as China, Japan and South Korea, where the national diet is based on it. On the other hand, tomato was the second most analyzed plant with respect to the biotic stress process in the US and Mediterranean countries such as Italy and Spain. Finally, wheat was the second most studied species in France and UK, while in Germany the second most studied plant with respect to biotic stress was tobacco. It is interesting to highlight that despite the fact that US headed the top ten list in terms of the number of biotic stress publications, tobacco plant was the least studied species in this country (Figure 5).

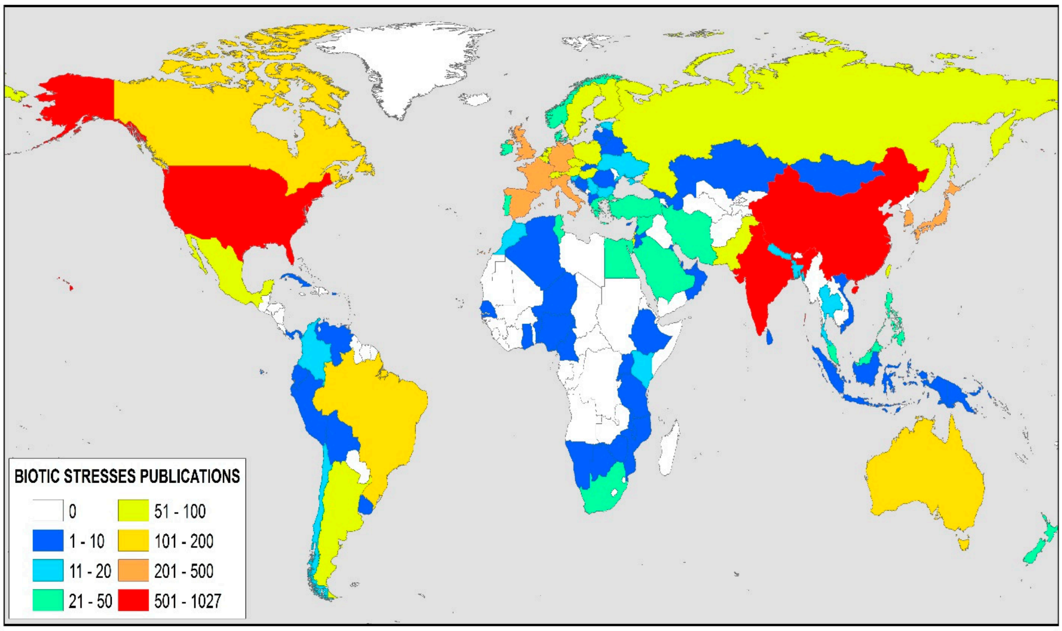

Figure 4. Worldwide research on Biotic Stresses in the period 1979–2016.

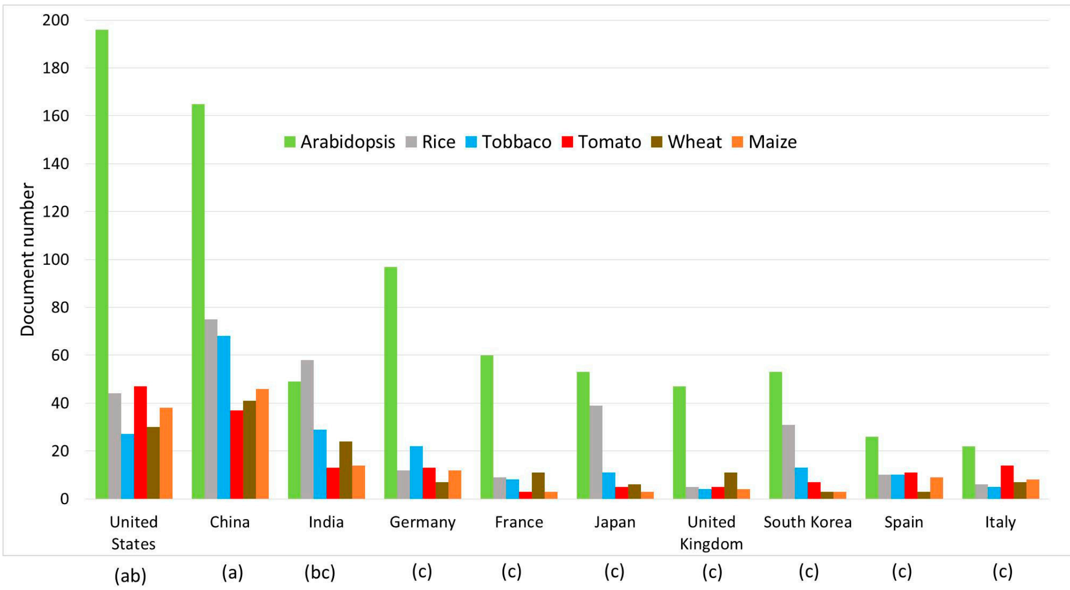

Figure 5. Distribution by country of biotic stress reports categorized by plant species. Countries followed by the same letter did not show statistically significant differences in distribution by plant species ($p < 0.05$).

If the relative importance of the species of plants is analyzed based on the percentage of publications within each country, Arabidopsis, which can be considered as a plant for basic research, is of great importance and appears in more than 60% of the publications of France, UK, and Germany, and in more than 45% in the US, South Korea and Japan. Rice accounts for more than 25% of the publications of Japan, South Korea and India. Wheat appears in more than 10% of publications in the UK, France, Italy and India. Corn study (maize) is in more than 10% of their publications in only three countries: Spain, Italy and China. The same may be said of tomato, which is studied in more than 10% of publications by only three countries: Italy, Spain and US. Tobacco appears in more than 10% of the publications of India, China, South Korea, Spain and Germany.

Results suggest that the scientific interest from each country depends on its typical habits, and thus, Asian countries show a higher scientific interest in rice, since this cereal is an essential part of the oriental diet, while countries such as Spain and Italy pay more attention to tomato research, since vegetables are essential elements of the Mediterranean diet, and this is one of the most important horticultural crops [48,49].

Despite specific differences among countries, statistic studies showed that the distribution of publication numbers by plant species in US is similar to the distribution in China and India, but showed significant differences with the other countries, among which no significant differences were shown (Figure 5).

As expected, according to the results mentioned above, the most productive institutions in terms of the number of publications related to biotic stress were from China and US. Table 3 shows the 12 most productive institutions, with more than 50 publications on biotic stress in the studied period. Eight of these twelve institutions were from China, two from US and one from France, Spain, Italy and Brazil. Also, five out of the twelve most productive centers in the biotic stress research field were specific to the agricultural research field, which was likely the cause of the high percentage of documents published in the Agricultural and Biological Science area described previously. The rest were state and multidisciplinary institutes. It should be noted that Brazil, a country that does not publish too many biotic stress documents, has a research institute in the top 12 list. This institution published almost 30% of the total number of documents from Brazil (54/180 publications), which indicates the great involvement of this institution in biotic stress research.

Table 3. Ranking of the 12 most productive institutions in the Biotic Stress research field.

Affiliation	Country	Reports	Plant Keyword
Chinese Academy of Sciences	China	110	Rice
USDA Agricultural Research Service Washington DC *	USA	82	Zea Mays
CNRS Centre National de la Recherche Scientifique	France	72	Arabidopsis
Consejo Superior de Investigaciones Cientificas	Spain	66	Arabidopsis
Nanjing Agricultural University *	China	63	Arabidopsis
Ministry of Education China	China	62	Arabidopsis
Zhejiang University	China	60	Rice
Chinese Academy of Agricultural Sciences *	China	57	Arabidopsis
Empresa Brasileira de Pesquisa Agropecuaria—Embrapa *	Brazil	54	Soybean
Consiglio Nazionale delle Ricerche	Italy	54	Tomato
Ministry of Agriculture of the People's Republic of China *	China	54	Arabidopsis
UC Davis	USA	53	Arabidopsis

* Research Institutions focused on Agronomy.

With the aim to analyze the main plant species researched in the twelve most productive institutions, the most used plant species-related keywords in the biotic stress publications from these institutions were studied. As can be observed, Arabidopsis, the model species most widely used in research worldwide, was the most used plant species to research biotic stress in seven out of the twelve most productive institutions, four located in China, one in Spain, one in France and another one in US (Table 3). However, Zhejian University and Chinese Academy of Sciences, both research centers from China, researched rice more deeply since it is a basic foodstuff in China (Table 3). Additionally, maize, a cereal widely consumed in US, was the most widely used plant species as a keyword in USDA Agricultural Research Service Washington DC, an institution whose main responsibility is "finding solutions to agricultural problems that affect Americans every day from field to table by research" (web page USDA Agricultural Research Service Washington DC). Model species tomato, a typical vegetal of the Mediterranean diet, was the most studied plant by the Consiglio Nazionale delle Ricerche, from Italy. Finally, soybean is the most used plant-species keyword in the Empresa Brasileira de Pesquisa Agropecuaria—Embrapa, which is logical, since Brazil is the second global exporter of soybean, only behind US (Table 3).

Research studies about the biotic stress field have been published in 14 different languages. As expected, English was the most used language to publish biotic stress documents (4936 reports, which account for 96.8%) since English is the international language of science and technology. English is followed by Chinese (with a mere 1.7%) which is in accordance with the fact that China was the country that published the second highest number of biotic documents. However, it should be noted that Spanish was the third most used language to publish biotic stress publications although a limited number of biotic stress-related documents were published by this country compared to others such as India, Japan, Germany or France (Table 4). In any case, Spanish is the second most spoken language in the world since Spanish is not only spoken in Spain but also in numerous countries in South America.

Table 4. Languages of scientific output in the biotic stress research field.

Languages	Reports	%
English	4936	96.7
Chinese	87	1.7
Spanish	17	0.3
Japanese	15	0.3
Russian	11	0.2
German	10	0.2
Portuguese	8	0.1
Korean	6	0.1
French	4	0.08
Czech	2	0.04
Polish	2	0.04
Croatian	1	0.02
Hungarian	1	0.02
Slovenian	1	0.02

3.4. Analysis of Keywords

The main research subject in a specific manuscript is summarized by the keywords defined in it. Thus, the assessment of the keywords of scientific documents allows for the establishment of the research trends in a specific field [50]. Figure 6 shows a word cloud made with all the keywords used in the analyzed reports. In this article, keywords of the biotic stress-related articles were analyzed. As can be seen, the word "nonhuman" (1st) is the keyword most represented in biotic stress documents, which is logical since the term biotic stress is mainly used for plants (Figure 6). For animals, the homologue concept is disease, which can also be brought about by biotic (virus, bacteria, fungus, worms . . .) and abiotic (temperature, radiations, thirst . . .) factors. In fact, the term disease is also used for plants and thus, "plant diseases" is the seventh most represented term among keywords in biotic stress publications. The second most used keyword is "physiological stress" (Figure 6), which is also logical since the term "physiological stress" can be described as an adverse effect that results from any external or internal factor that alters the homeostasis of an organism [51], in this case, the physiological stress caused by biotic stresses. Consequently, the third most frequent keyword in biotic stress-related documents is "metabolism" (Figure 6) that is defined as those chemical processes involved in maintaining the living state of an organism, which are altered in plants during biotic stress periods. Exposure of plants to biotic and abiotic stress produces changes in plant metabolism implying physiological damage [52], which makes those "physiological stress" and "metabolism" keywords highly mentioned in biotic stress publications. The fourth most used keyword in biotic stress-related reports is "abiotic stress" (Figure 6). The high number of the term "abiotic stress" as a keyword in biotic stress documents is due to the fact that a variety of biotic and abiotic factors can act in combination on plants and so, a comprehensive study of both stresses is usually carried out. Consequently, keywords related to diverse abiotic stresses, such as "salinity", "temperature", "salt stress", "drug", are included in biotic stress publications (Figure 6, in light blue). Initially, research was mainly focused on plant responses to separate abiotic or biotic stresses [53–56], although a more complex scenario is associated with the response to simultaneous stresses [57]. Interestingly, one multiple-stress exposure may enable plants to defend themselves against one stress with more resistance than against other stresses [58]. This cross-tolerance is a phenomenon observed in plants exposed to two biotic stresses (for instance, the resistance to the herbivore *Helicoverpa zea* in tomato plants is increased thanks to infection by *Pseudomonas syringae* pv. tomato (*Pst*) [59]), but biotic stress can also increase the resistance to abiotic stress (review in [60]). Finally, the fifth most used keyword is "Genetics" (Figure 6). This result again indicates the great interest that the genetic response to biotic stress has for the scientific community. It is in agreement with the appearance of the keyword "Reactive oxygen species" (Figure 6, in purple), which are metabolites produced by plants as a consequence of exposure to biotic stresses. After stress sensing, plants induce a rapid generation of "Reactive oxygen species" [61,62] whose key role is as signaling molecules in the plant cells in order to produce a genetic response in the nucleus [62–66]. The importance of genetics in biotic stress response is also suggested by the high number of genetic-related keywords, such as "gene expression regulation", "plant genome", "plant gene", "gene expression", "transgenic plants", "plant RNA", "mutation", used in biotic stress documents (Figure 6, in dark blue); however, no names of genes or genetic pathways known to regulate defense response from plants are observed in the keyword study. On the contrary, "phytohormones" names that play a key role in the stress response and that coordinate genetic response such as "Abscisic acid", "Ethylene", "Jasmonic acid" and "Salicylic acid" are included among keywords more mentioned in biotic stress publications (Figure 6, in pink). Abscisic acid is the primary hormone implicated in the sensing of numerous abiotic stresses [67], while "Salicylic acid", "Jasmonic acid" and "Ethylene" hormones mainly mediate biotic stress responses [68]. Additionally, keyword analysis indicates that plant species that awaken more interest in the biotic stress research field are "Arabidopsis", "Rice", "Tomato", "Tobacco", "Wheat" and "Maize" (Figure 6, in green), as described above. Finally, this study shows that the most researched plant organs in the biotic stress field are "leaves", "roots" and "seedlings" (Figure 6, in yellow); and that the most analyzed biotic stressors are "Bacteria", with "*Pseudomonas syringae*", "fungus" and "Hexapode" standing out (Figure 6, in orange).

This study of keywords would not be complete without analyzing the keyword groups described here during the time period analyzed. First, we evaluated the time evolution of "biotic" and "abiotic" keywords in biotic stress publications. Results showed that the evolution of both keywords did not show statistically significant differences (Figure 7a). However, despite the trend lines of biotic and abiotic evolutions showing a homogenous slope (R2 0.943 versus R2 0.947), the increase in the publication number was 20 documents for the biotic stress field and 15 reports for the abiotic field (Figure 7a). These results showed that despite the fact that there is a greater increase in the number of documents related with biotic keywords than in the number of reports related with abiotic keywords, there is a similar evolution in both biotic and abiotic reports, indicating that the study of both stresses is frequently carried out jointly and both stresses are closely related.

Second, when an evolution study was performed with plant species keywords, the results showed that "Arabidopsis" is the significantly more studied model species in the biotic stress research field during the period analyzed, followed by "rice", which began to increase with respect to "tomato", "tobacco", "wheat" and "maize" in 2006, and finally peaked in 2012, although without significant differences. So, "rice", "tomato", "tobacco", "wheat" and "maize", showed a similar evolution (Figure 7b). However, "Arabidopsis", "rice", "tobacco" and "maize" displayed a downward trend in the last year while "tomato" and "wheat" maintained an upward trend (Figure 7b), suggesting a plausible change in the interest that different plant species awoke. The fact that Arabidopsis is a model species for research and the harmful effects provoked by tobacco in humans might explain this downward trend. Similarly, changes in the commercial interest in rice and maize plants might be the reason for the alterations observed in the research trends in the last years. Vegetal starch and oil are currently used to produce biofuel. Both maize and rice are two of the main crops used for biofuel production, which has led to food crops being explicitly grown for fuel production. This action has stirred up controversy in recent years since biofuels are made from food crops grown on arable land. However, new generation biofuels try to avoid using arable land solely for the production of fuel.

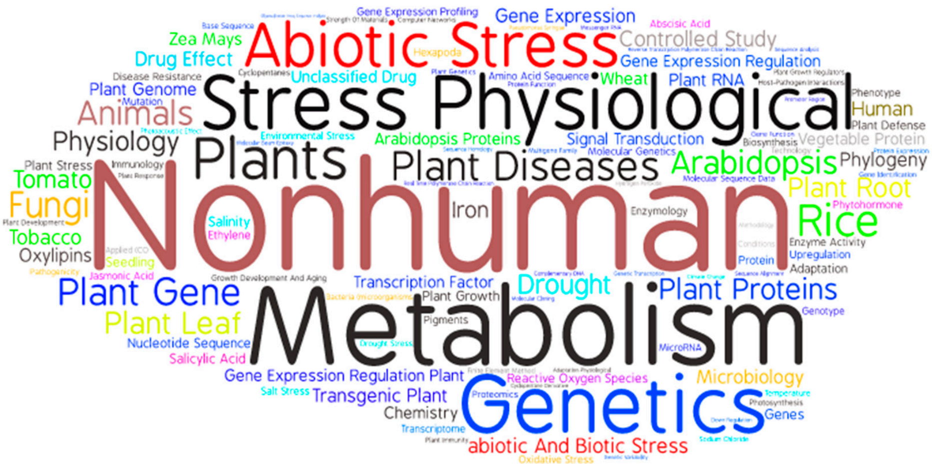

Figure 6. Cloud word based on the main keywords related to Biotic Stress worldwide research.

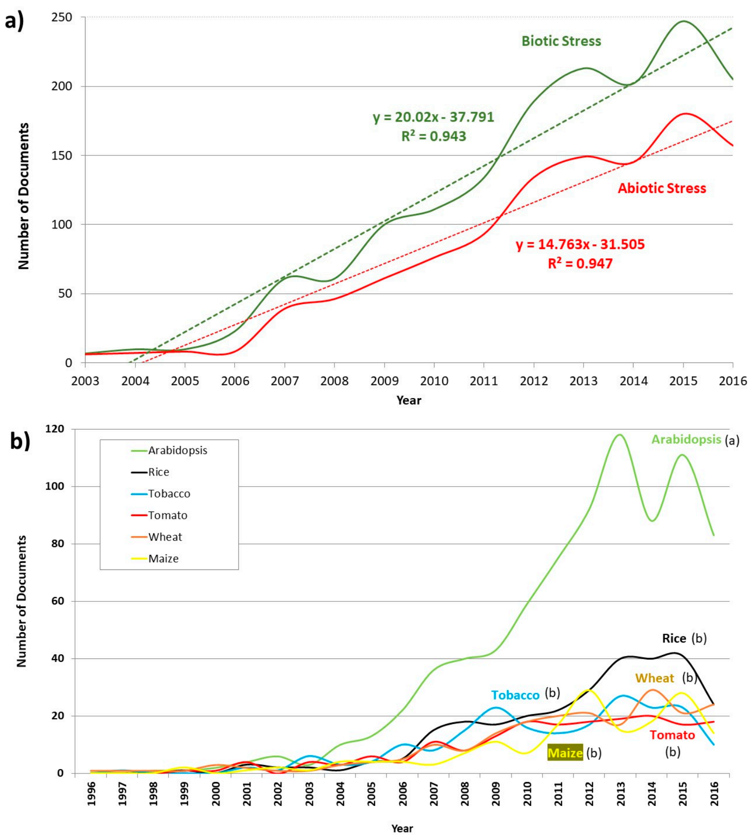

Figure 7. *Cont.*

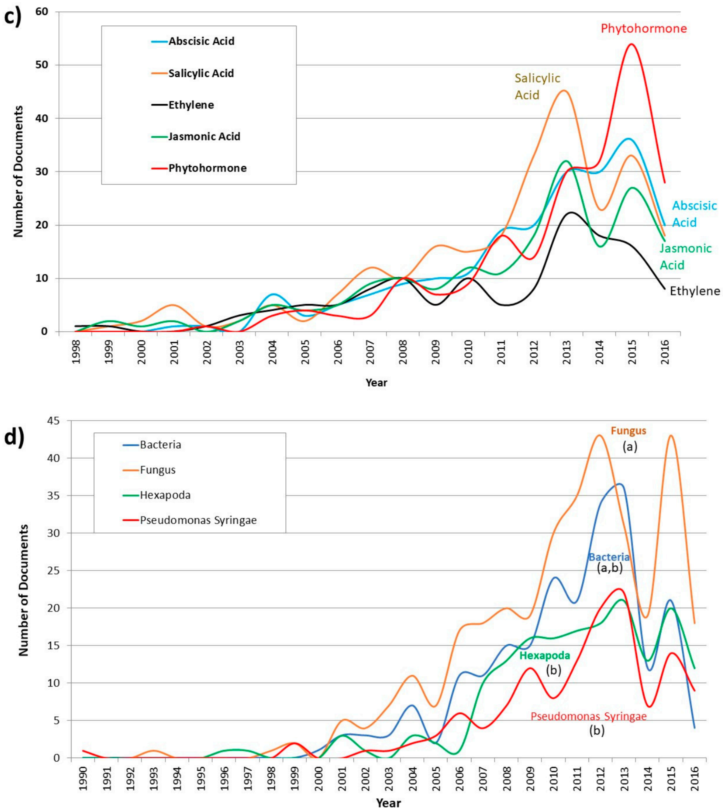

Figure 7. Time evolution of keyword groups related to Biotic Stress worldwide research. (**a**) Biotic and abiotic stresses; (**b**) plant species; (**c**) phytohormones; (**d**) stressors. Keyword-group evolution followed by the same letter did not show statistically significant differences ($p < 0.05$).

Third, the analysis of keywords related with phytohormones did not show significant differences among hormonal evolutions. However, results suggested a greater interest by "Salicylic Acid" (SA), followed by "Abscisic Acid" (ABA) and "Jasmonic Acid" (JA) (Figure 7c). However, according to results, "ABA" has had a greater impact than "SA" in recent years. ABA is a key hormone that regulates water losses via stomas. Upon abiotic stress conditions, plants induce ABA production that leads to stomatal closure and water conservation. So, ABA has an important function in the response of plants to abiotic stresses, such as drought, salinity, and extreme temperatures. In addition, the stomatal closure works as a defense mechanism in preventing pathogen attack [69,70]. Thus, recent studies have demonstrated that ABA also plays a key role in biotic stress response. In addition, this hormone plays a role in pathogen responses by interacting with other hormones related with plant defense mechanisms such as SA and JA [71,72]. However, SA and JA hormones have been typically associated with resistance to pathogens, biotrophic and necrotrophic respectively [24]. Perhaps the wide knowledge about SA and JA implication in biotic stress response and the recent findings about the role of ABA in biotic stress response might be the reason for the upward trend of the keyword ABA in the biotic stress field in recent years, with respect to typical biotic stress hormones SA and JA. Finally, the "Ethylene" has been studied less in the biotic stress field during this period. This hormone has been typically associated with developmental processes such as flowering, fruit development and ripening, root growth, etc., via the Ethylene Response Factor (ERF) superfamily [73]. Additionally, ERFs have been extensively reported to be involved in the response to pathogen attack [74,75]. However, recent research has shown that several ERFs also play a regulatory role in plant responses to abiotic stresses [76,77]. The current ensemble study of both stresses (biotic and abiotic) might have provoked the increase in the number of biotic stress documents related with ethylene in the last five years, despite ethylene, as is the case for other stressors, showing a downward trend in the last year.

Finally, the study of stressors-related keywords showed that during the period analyzed "Fungus" was significantly the most researched biotic agent, followed by "Bacteria" and "Hexapoda", suggesting that the study of diseases ("bacteria" and "fungus") has received more scientific interest than the study of plagues ("Hexapoda") in the research projects. Fungi interact with plants in various ways. While fungal pathogens have harmful effects on plant physiology, mutualistic fungi improve host defense responses to pathogens. So, fungi have potential for preventing plant diseases and for improving plant productivity [78]. Similarly, plant pathogenic bacteria provoke many plant diseases while endophytic bacteria improve plant tolerance to several abiotic stresses and can protect plants from pathogenic microbes [79,80]. On the other hand, "Hexapoda" is one of four major groups included in the Phylum Artropoda, and is formed by more than one million insect species, of which a high number are herbivores. These results evidence the great interest that "Fungus", "Bacteria" and "Hexapoda" have in the plant studies. "*Pseudomonas syringae*" has been given less importance than "Fungus", "Bacteria" and "Hexapoda" in the scientific community during this period (Figure 7d). However, it is likely because "Bacteria" represents a domain, "Fungus" a kingdom and "Hexapoda" a subphylum, all of which include numerous species, while "*Pseudomonas syringae*" characterizes a unique species. Even so, "*Pseudomonas syringae*" is a broadly-researched phytopathogenic bacterium, which has been used as a model species to study molecular bases of host–pathogen interactions. This species may be subdivided into nearly 60 pathological variants that are mainly distinguished by host range [81] and have been isolated from about 180 host plants, including main agricultural crops [81,82]. Stressor results, similar to results from phytohormone keywords, showed a downward trend in the last year (Figure 7d). However, a prominent reduction, which was posteriorly recovered, of keywords "Bacteria", "Fungus", "Hexapoda" and "*Pseudomonas Syringae*" was also observed in 2014. These results suggest that the current downward trend of stressors keywords might recover in the coming years.

It should be noted that important stressors such as virus and nematodes are not included among the main keywords from biotic stress documents. Then, the terms "Bacteria", "Fungus", "Hexapoda", "nematode" or "virus" are added to the search query (TITLE-ABS-KEY (Biotic Stress)) in order to compare time evolution among stressors, regarding the number of documents. Results show that the terms "Bacteria", "Fungus" and "Hexapoda" have a similar relation whether the number of documents (Figure 8) or the keyword occurrence frequency are analyzed (Figure 7d). In addition, the number of documents related with "Virus" in the biotic stress research field did not show significant differences with respect to the scientific production of "Fungus" and "Bacteria". Thus, "Virus" awoke a similar interest to "Bacteria" and "Fungus" in the biotic stress research field during time evolution. On the other hand, "Nematodes" made a similar impact to "Hexapoda" in the biotic stress field while it awoke significantly less interest than "Virus", "Bacteria" and "Fungus" in the biotic stress research field during the period studied. However, "Nematodes" and "Fungus" show current upward trends while "Bacteria", "Virus" and "Hexapoda" display downward trends (Figure 8). These current downward trends might suggest a loss of interest in the biotic stress field. However, it is unlikely since plant plagues and diseases alter alimentary crops which provokes significant economic losses for farmers and puts global food security at risk.

Finally, if keywords obtained with the new search queries (TITLE-ABS-KEY (Biotic Stress) AND (Virus) or (Bacteria) . . .) are analyzed, then the most studied species, genus and order of virus, bacteria, nematodes, hexapods and fungus may be elucidated. The virus, *Tobacco Mosaic Virus*, which is a member of the plant virus genus Tobamovirus, is the most studied virus in the biotic stress research field, followed by the genus Potyvirus, which includes the highly researched species *Potato Virus Y*. In addition, the genus Begomovirus, the family Geminiviridae and the species *Pepper Mild Mottle Virus* are found in the main keywords. Similarly, a keyword study with respect to bacteria has shown that the genus Pseudomonas—headed by the *species Pseudomonas Syringae*—the genus Xhantomonas—headed by the species *Xhantomonas Oryzae Pv. Oryzae*—and the order Phizobiales are the most researched bacteria in the biotic stress field. On the other hand, phylum Ascomycota and Basidiomycota, which form the subkingdom Dikarya of the kingdom Fungi, are the most researched fungi in the biotic stress field. In addition, the genus Botryotinia—headed by the species *Botrytis cinerea* or *Brotyotinia fuckeliana*—and the genus Fusarium—headed by the species *Fusarium oxysporum*—have awoken great interest in the biotic stress research field. The genus Alternaria and species *Piriformospora Indica* have also been extensively studied in biotic stress research projects in fungus. Included in the subphylum Hexapoda, the class Insecta is the most researched in the biotic stress field. Orders Lepidoptera and Hemiptera, which are included in the class Insecta, have awoken great interest in the biotic stress field. Butterflies and moths, of the order Lepidoptera, and the family Aphididae (aphid or greenfly), of the Hemiptera order, have made a great impact in the scientific community according to keyword analysis. Finally, the superfamily Tylenchoidea, the genus Meloidogyne and species *Meloidogyne javanica* and *Meloidogyne incognita* are the most studied nematodes in the biotic stress research field. These results show that parasites or pathogens have a greater impact on the crops and thus have awoken great interest in the scientific community, headed by fungus, bacteria and virus vs. hexapoda and nematode.

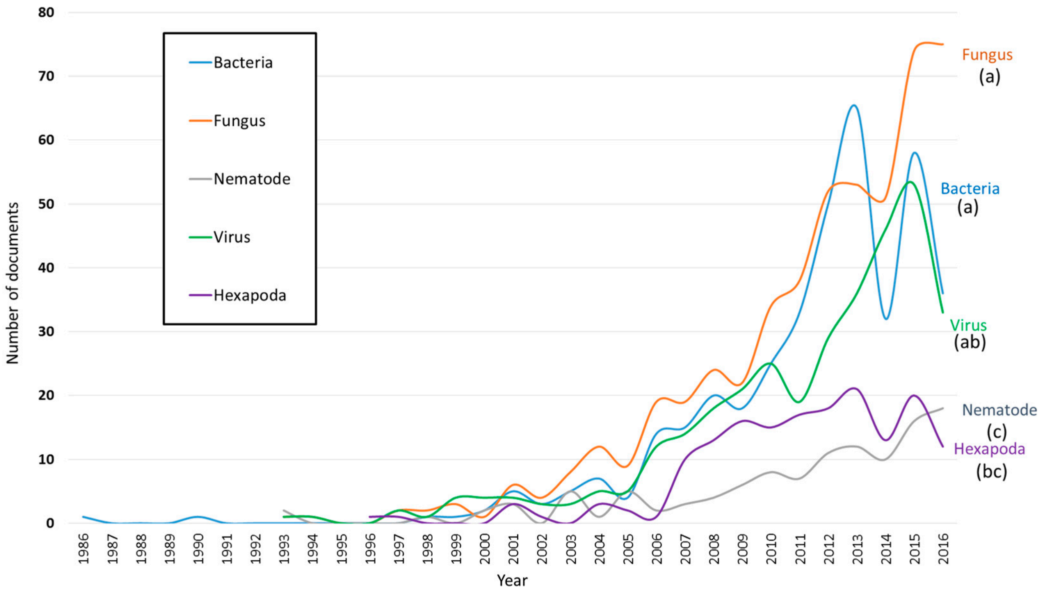

Figure 8. Time evolution of the most studied stressors in the Biotic Stress field. Stressors followed by the same letter did not show statistically significant differences ($p < 0.05$).

4. Conclusions

Grain products, fruits and vegetables, such as tomato, rice, maize and wheat form the alimentary basis on a global scale. Thus, plants are essential to food production. However, plants are sessile organisms that are very exposed to environmental damages. Therefore, both biotic (virus, bacteria, insects ...) and abiotic factors (dry, temperature, humidity ...) are enemies of global food production in a broad sense. Plagues and diseases provoke great economic losses on a global scale. So, biotic stress has been broadly studied. A review of documents related to biotic stresses in this bibliometric study has allowed us to analyze trends in the biotic stress research field. Results showed the great interest held by the scientific community in physiological stress and metabolism changes suffered by plants when they are affected by biotic and abiotic factors, as well as the genetic and hormonal defense mechanisms of plants. In addition, the fact that the term abiotic stress is closely associated to biotic stress documents proves the great importance that the interaction between biotic and abiotic stresses has for the scientific community. Most of these studies have been performed in the model species Arabidopsis, despite the fact that these species are not edible; however, its characteristics make it a good model species to be researched. Other plants also analyzed in biotic stress documents are tomato, maize, rice and wheat, all of which are of great economic importance worldwide. Finally, virus, fungus, bacteria, nematodes and insects (hexapoda) are shown as the most researched stressors in biotic stress research documents. All of the results show the great effort made by the scientific community in the biotic stress field with the aim to understand, regulate and control plant damages caused by biotic stress agents.

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