Bacillus thuringiensis pesticidal toxins: A global analysis based on scientometric study (1980 – 2021)

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Abstract

Background: Several studies have been conducted on *Bacillus thuringiensis* (*Bt*) pesticidal toxins due to their successful environmentally friendly biopesticide activity against various insect pest orders, protozoa, mites, and nematodes. However, no existing study has systematically examined the trends and evolution of research on *Bt* pesticidal toxins from a scientometric perspective. This study aimed to analyze the trends and hotspots of global research in this field.

Methodology: 5757 publications on *Bt* pesticidal toxins were extracted from the Web of Science Core Collection (WoS) from 1980 to 2021. Statistical and scientometric analyses were performed using Excel, CiteSpace and VOSviewer visualisation tools to evaluate the contributions of different countries, institutions, highly influential references, and most used keywords.

Results: Out of the 5757 publications, the USA contributed the most, with 1562 publications, 72,754 citations, and 46.58 average citations per paper (ACPP); however, Belgium had the highest (106.43) ACPP among the top 20 contributing countries. The Chinese Academy of Agricultural Sciences is the leading institution with 298 publications and 21.20 ACPP. The Pasteur Institute is ranked first (90.04) in terms of ACPP. Keywords analyses revealed that recent studies are inclined towards the evolution of insect resistance against *Bt* toxins.

Conclusion: In future, studies related to the development of resistance mechanisms by insects against *Bt* pesticidal toxins and ways to overcome them will likely receive more attention.

Background

Bacillus thuringiensis (Bt) is a Gram-positive, rod-shaped, motile, and aerobic or facultative anaerobic bacterium that produces insecticidal crystal inclusions, known as δ-endotoxins or Cry proteins, during its sporulation phase of growth. These Cry proteins have been proven to be effective against important crop pests, and also against mosquitoes that are vehicles of serious human diseases such as malaria and dengue [1].

Bt is known as the most successful environmentally friendly insecticidal microbe which acts against different orders of insect pests, such as Lepidoptera, Coleoptera, Diptera, Homoptera, Hymenoptera, Mallophaga and Orthoptera [2]. Also, certain *Bt* strains have revealed activity against protozoa, mites, and nematodes [3–5]. Unfortunately, there are still many insect pests that have shown poorly controlled or no susceptibility to the known Cry proteins [5]. Furthermore, the emergence of insect resistance to *Bt* crops in the field, which represents a major threat for using the Cry toxins, have been reported for at least five different insect species [6–10]. Therefore, discovering novel Cry toxin proteins in nature or through in vitro genetic evolution of them to enhance their toxicity against specific pests insects is of utmost importance [11–13]. During the past decade, a considerable number of studies on *B. thuringiensis* have been published so far, and pesticidal Cry proteins were the subject of intensive research [2, 14, 15]. These efforts have yielded large literature and plentiful data about the Cry proteins’ structure, mechanism of action, genetics, ecological role, performance in agricultural and other natural settings, and the evolution of resistance mechanisms in target pests [1, 2, 16, 17]. Given the importance of Cry proteins, many scholars and academic journals have focused on specific subfields of Cry proteins (which are mentioned above), with conclusions being drawn from systematic reviews or descriptive analysis. However, to our knowledge, there is no existing study that has systematically examined this field from a scientometric perspective.

Scientometric is a branch of informatics that combines information visualization technology and mathematical methods to quantitatively analyze scientific literature and understand emerging trends and the knowledge structure of a scientific research field [18, 19]. It evaluates the contributions of authors, institutions, countries, and journals in specific fields; and primarily it concentrates on the quantification properties of literature, such as publication numbers, citation frequency, and cooperative relationships [20]. Therefore, a scientometric study can help researchers to identify core entities and development trends in a research domain or a specific subject and provide new insights and directions for future research [20, 21]. Currently, an array of science mapping and visualization tools, including CiteSpace, HistCite, VOSviewer, and R-bibliometrix [22–25], are made freely available for scientometric analysis, and they have been widely applied in various scientific fields [26–29]. In this study, scientometric analysis was employed on published *Bt* pesticidal toxins' literature (from 1980 to 2021) using CiteSpace and VOSviewer servers. This study aims to summarize the knowledge structure and identify emerging trends, leading countries and organizations/institutions, the main research directions, and potential hotspots in this area in order to provide insights and guide further studies.

Materials And Methods

Source of data and search strategy

A sum of 5819 documents ranging from the period 1980 to 2021 was selected from the Web of Science Core Collection on December 21, 2021. The data acquisition strategy which reflects studies related to *Bt* biopesticide toxins was executed as follows: (TS=("Bacillus thuringiensis" OR "B. thuringiensis")) AND TS=("cry protein*" OR "cry toxin*" OR "cry gene*" OR "crystal gene*" OR "crystal protein*" OR "crystal toxin*" OR "cyt toxin*" OR "cyt protein*" OR "cyt gene*" OR "cytolytic toxin*" OR "delta endotoxin*" OR "insecticidal protein*" OR "insecticidal gene*" OR "pesticidal gene*" OR "pesticidal protein*" OR "biological insecticide*" OR "biopesticide*" OR "bioinsecticide*"). TS in WOS represents Topic Search, and it executes a search of the query provided within a bibliographic record in the fields of Title, Abstract, Author Keywords, and Keywords Plus. The wildcard asterisk (*) indicates that any string of character(s) should be considered, for example, cry protein* can find cry *proteins*, biopesticide* finds *biopesticides*, etc. Quotation marks are used to enclose terms that are phrases (e.g., "delta endotoxin*"). The document types were refined to include only articles and reviews, thus, book chapters, proceedings papers, early access, retracted publications, and data papers were exempted. Full record and cited references of the documents were imported in plain text format into CiteSpace (version 5.8.R3) and VOSviewer (version 1.6.17) for further analysis.

CiteSpace

CiteSpace is a java tool designed to analyze and visualize scholarly scientific literature of emerging trends in a knowledge domain [30]. CiteSpace performs Co-Citation Analysis (CA) which is subdivided into Author Co-Citation Analysis (ACA), Document Co-Citation Analysis (DCA), and Journal Co-Citation Analysis (JCA). In this study, CiteSpace was employed to perform documents and keywords co-citation analyses.

VOSviewer

VOSviewer is a software tool that is used to create and visualize maps of bibliographic data to build networks of scientific publications, journals, authors, research institutions, countries, keywords, and terms [31]. In this study, VOSviewer was employed to map and visualize the contributions of countries and organisations/institutions to *Bt* pesticidal toxins. The default settings of VOSviewer were used in this study. Co-authorships of countries and organizations/institutions are represented by labels and circles. The size of a circle reflects the degree of contribution of a particular country or organization. The colors in the network maps represent a network of clusters of cooperation, and the distance between items represents the strength of the connection.

Results And Discussion

Document volume and annual growth

After screening the data, 5757 qualified documents related to studies conducted on *Bacillus thuringiensis* and its biopesticide toxins were selected from the Web of Science Core Collection. The document type and the publication language are shown in Table 1 below. Out of the 5747 documents, 5375 (93.36%) were research articles whilst 382 (6.64%) were review articles. Majority of the documents were published in English (5665, 98.228%), followed by Japanese (20, 0.347%), Portuguese (20, 0.347%), Spanish (19, 0.330%), French (12, 0.208%), Russian (10, 0.174%), Chinese (8, 0,139%), Polish (8, 0.139%), and a document (1, 0.017%) each in Croatian, Czech, German, Italian and Turkish. Most of the documents were research articles published in English.

Table 1
Document type and publication language of studies related to Bt biopesticide toxins.

No.	Document type	Quantity	% of 5757	Publication language	Quantity	% of 5,757
1	Article	5375	93.36	English	5655	98.228
2	Review	382	6.64	Japanese	20	0.347
3				Portuguese	20	0.347
4				Spanish	19	0.330
5				French	12	0.208
6				Russian	10	0.174
7				Chinese	8	0.139
8				Polish	8	0.139
9				Croatian	1	0.017
10				Czech	1	0.017
11				German	1	0.017
12				Italian	1	0.017
13				Turkish	1	0.017

Evolution of Bt **pesticidal toxins literature**

Figure 2 displays the annual publications on *Bt* pesticidal toxins from 1980 to 2021. The number of publications from 1980 to 1989 witnessed a slow growth trend with less than 30 articles published per year. With increasing interest and deepening research on toxins produced by *Bacillus thuringiensis*, the number of publications has grown steadily since 1990. As studies on *Bt* biocontrol pesticidal toxins gathered pace from 1990, they are reflected in Fig. 3 with heightened annual citation frequency from1991 onwards. Another trend of increasing volatility is witnessed by annual publications exceeding 100 articles from 1994 to 2021. The peak of publications over the 42 years was recorded in 2017 when 254 articles were published. From 2017 to 2021, the average annual citation exceeded 10000, dwarfing the years from 1980 to 1992, where the average annual citation did not reach 1000. This demonstrates the continued growth of studies in this area.

Document Co-Citation analysis

The map of co-cited papers related to pesticidal toxins of *Bt* was generated in CiteSpace by selecting cited references. The reference citation analysis was employed to determine the quality of academic literature in this field by identifying documents with the most-cited references and the corresponding highly influential authors. In the analysis, time-slicing was set as 6 years per slice from 1980 to 2021 and only the top 50 references of each year slice were included, pathfinder was selected, and merged networks were pruned to reduce network density in order to improve the readability of the network [32], the rest of the parameters were set to default. Figure 4 shows a map of the co-citation network related to publication on *Bt* pesticidal toxins from 1980 to 2021. The network consisted of 242 nodes and 7883 citation links, which means that 242 authors cooperated through 7883 links. The citation threshold was set to 140 displaying only references with 140 or more citations and their respective author and publication year. The size of a node reflects the importance of a reference in the network, whereas the links connecting the nodes indicate their co-occurrence strength. The purple colors surrounding the nodes indicate node centrality which represents the prominence of a node in connecting other pairs of nodes in the network. Higher thickness indicates higher centrality.

Table 2 highlights the top 20 influential and most cited references on studies related to *Bacillus thuringiensis* biopesticide toxins, ranked based on the number of times they have been cited. It can be seen from Fig. 4 and Table 2 that the most influential reference is written by Schnepf et al. [2], having the highest citation of 1406. This review paper emphasized extensive topics covering *Bt* genome, the expression of insecticidal *cry* genes, the structure and functions of Cry toxins, the mechanism of action of Cry proteins, and insect resistance to *Bt* Cry toxins. The second most-cited document, written by Höfte & Whiteley [33], is also a review article that focuses on the nomenclature and classification scheme of crystal proteins produced by *Bacillus thuringiensis* based on the protein structure and effectiveness of their host range. Although Schnepf et al. [2], has the most citation count, the higher betweenness centrality of Höfte & Whiteley (1989) makes it more revolutionary, gives it a higher impact factor, and plays a more important role within the network. The third [4], and the fourth [34], most-cited documents talk about the nomenclature of *Bt* pesticidal crystal proteins, and the crystal structures of δ-endotoxins respectively. These and other articles in the list were crucial to the early stages of research into *Bt* pesticidal crystal toxins, and they continue to shape studies in this field today.

Table 2
Top 15 most-cited references of studies related to *Bt* pesticidal proteins

Rank	Cited reference	Count	Centrality	Year	DOI
1	Schnepf et al. (1998)	1406	0.29	1998	doi.org/10.1128/MMBR.62.3.775-806.1998
2	Höfte & Whiteley (1989)	1247	0.81	1989	doi.org/10.1128/MR.53.2.242-255.1989
3	Crickmore et al. (1998)	559	0.29	1998	doi.org/10.1128/MMBR.62.3.807-813.1998
4	J. Li et al. (1991)	434	0.27	1991	doi.org/10.1038/353815a0
5	Bravo et al. (2007)	403	0.07	2007	doi.org/10.1016/J.TOXICON.2006.11.022
6	Ferré & Van Rie (2002)	368	0.00	2002	doi.org/10.1146/annurev.ento.47.091201.145234
7	Gill et al. (1992)	347	0.02	1992	doi.org/10.1146/annurev.en.37.010192.003151
8	Bravo et al. (2011)	239	0.00	2011	doi.org/10.1016/j.ibmb.2011.02.006
9	Estruch et al. (1996)	326	0.03	1996	doi.org/10.1073/pnas.93.11.5389
10	Tabashnik BE (1994)	322	0.35	1994	doi.org/10.1146/annurev.en.39.010194.000403
11	Grochulski et al. (1995)	292	0.07	1995	doi.org/10.1006/jmbi.1995.0630
12	Gould F (1998)	291	0.31	1998	doi.org/10.1146/annurev.ento.43.1.701
13	Pigott CR (2007)	290	0.02	2007	doi.org/10.1128/MMBR.00034-06
14	Pardo-Lopez L (2013)	284	0.08	2013	doi.org/10.1111/j.1574-6976.2012.00341.x
15	Knowles BH (1987)	261	0.08	1987	doi.org/10.1016/0304-4165(87)90167-X
16	de Maagd RA (2001)	254	0.00	2001	doi.org/10.1016/S0168-9525(01)02237-5
17	Thomas WE (1983)	252	0.07	1983	doi.org/10.1242/jcs.60.1.181
18	Tabashnik BE (1990)	246	0.05	1990	doi.org/10.1093/jee/83.5.1671
19	Gahan LJ (2001)	241	0.37	2001	doi.org/10.1126/science.1060949
20	Hofmann C (1988)	223	1.00	1988	doi.org/10.1073/pnas.85.21.7844

Reference citation burst analysis

Citation burst detection in CiteSpace depends on Kleinburg's algorithm to estimate an abrupt change of frequency over a period of time [35, 36]. Burst detection is used in this study to determine cited references that received an outstanding degree of attention within a specific time range and to determine the current trend of studies by detecting references that have attracted much attention in recent years. Out of the 5,757 documents, 218 burst items were found. Table 3 lists the top 20 references with the strongest citation burst sorted by the beginning year of burst, with the right columns representing the duration of each burst – beginning of deep green lines represent publication year, and red lines segment represent burst duration. References with the same burst duration are considered to belong to the same group.

Table 3
Top 20 references with the strongest citation bursts from 1980 to 2021

References	Year	Strength	Begin	End	1980–2021
HOFMANN C, 1988, P NATL ACAD SCI USA, V85, P7844, DOI 10.1073/pnas.85.21.7844, DOI	1988	79.04	**1988**	2003	
HOFTE H, 1989, MICROBIOL REV, V53, P242, DOI.org/10.1128/mr.53.2.242-255.1989, DOI	1989	86	**1989**	2003	
VANRIE J, 1990, APPL ENVIRON MICROB, V56, P1378, DOI 10.1128/AEM.56.5.1378-1385.1990, DOI	1990	71.94	**1992**	2003	
VANRIE J, 1989, EUR J BIOCHEM, V186, P239, DOI 10.1111/j.1432 – 1033. 1989.tb15201.x, DOI	1989	68.19	**1992**	2003	
VANRIE J, 1990, SCIENCE, V247, P72, DOI 10.1126/science.2294593, DOI	1990	66.68	**1992**	2003	
FERRE J, 1991, P NATL ACAD SCI USA, V88, P5119, DOI 10.1073/pnas.88.12.5119, DOI	1991	65.13	**1992**	2003	
Schnepf E, 1998, MICROBIOL MOL BIOL R, V62, P775, DOI 10.1128/MMBR.62.3.775-806.1998, DOI	1998	89.11	**1998**	2015	
GROCHULSKI P, 1995, J MOL BIOL, V254, P447, DOI 10.1006/jmbi.1995.0630, DOI	1995	58.8	**1998**	2015	
Bravo A, 2004, BBA-BIOMEMBRANES, V1667, P38, DOI 10.1016/j.bbamem.2004.08.013, DOI	2004	64.29	**2004**	2015	
Bravo A, 2007, TOXICON, V49, P423, DOI 10.1016/j.toxicon.2006.11.022, DOI	2007	114.92	**2010**	2021	
Pardo-Lopez L, 2013, FEMS MICROBIOL REV, V37, P3, DOI 10.1111/j.1574-6976.2012. 00341.x, DOI	2013	109.76	**2013**	2021	
Bravo A, 2011, INSECT BIOCHEM MOLEC, V41, P423, DOI 10.1016/j.ibmb.2011.02.006, DOI	2011	108.93	**2011**	2021	

References	Year	Strength	Begin	End	1980–2021
Pigott CR, 2007, MICROBIOL MOL BIOL R, V71, P255, DOI 10.1128/MMBR.00034 – 06, DOI	2007	82.15	**2010**	2021	
Sanahuja G, 2011, PLANT BIOTECHNOL J, V9, P283, DOI 10.1111/j.1467-7652.2011.00595.x, DOI	2011	70.68	**2011**	2021	
Vachon V, 2012, J INVERTEBR PATHOL, V111, P1, DOI 10.1016/j.jip.2012.05.001, DOI	2012	57.61	**2012**	2021	
van Frankenhuyzen K, 2009, J INVERTEBR PATHOL, V101, P1, DOI 10.1016/j.jip.2009.02.009, DOI	2009	54.41	**2010**	2021	
Palma L, 2014, TOXINS, V6, P3296, DOI 10.3390/toxins6123296, DOI	2014	86.08	**2016**	2021	
Tabashnik BE, 2013, NAT BIOTECHNOL, V31, P510, DOI 10.1038/nbt.2597, DOI	2013	65.6	**2016**	2021	
Adang MJ, 2014, ADV INSECT PHYSIOL, V47, P39, DOI 10.1016/B978-0-12-800197-4.00002–6, DOI	2014	55.4	**2016**	2021	
Tabashnik BE, 2017, NAT BIOTECHNOL, V35, P926, DOI 10.1038/nbt.3974, DOI	2017	55.25	**2017**	2021	

Hofmann et al. (1988), is the first reference to witness citation burst, which started from 1988 to 2003. This reference is related to the specificity of *Bt* delta-endotoxins to binding sites in the brush border membrane of target insects [37]. A year later a study by Hofte & Whiteley [33], about the classification of *Bt* toxins started to witness a burst and continued until 2003. However, the top-ranked reference by burst strength is Bravo et al. [38], with a strength of 114.92, which began in 2010 and continues to experience burst. In this paper, Bravo et al.[38], discussed the mode of action of three-domain Cry toxins and cytolytic toxins in selected lepidopteran insects pests and mosquitoes. The second-ranked (Pardo-López et al., 2013) and the third (Bravo et al., 2011) references have close citation burst strengths of 109.76 and 108.93 respectively [1, 39]. These two papers stressed on the mechanism of action of *Bt* Cry and Cyt toxins but went further to highlight the resistance mechanisms certain insects have developed against these toxins and the strategies to overcome them. 11 out of the top 20 references continue to have burst and we cannot conclude when they will end. Recent papers with references citation bursts are mostly focused on the resistance of insects to *Bacillus thuringiensis* toxins.

Keywords analysis

The keywords co-occurrence analysis function in CiteSpace uses Co-occurring Author Keywords (DE) and KeyWords Plus (ID) in published documents to generate a network map of the most occurring keywords. Keyword analysis was performed to determine viral topics and trends of development in research related to toxins produced by *Bt*. Clustering analysis of the keywords was further performed to explore the potential hidden congruence between the keywords. Table 4 lists the top 20 co-occurring keywords. Figure 5 displays the map of the keyword co-occurrence network showing keywords with at least 100 appearances. The sizes of the rectangles in the figure are proportional to the frequency of occurrence of their corresponding keywords. The purple colors surrounding the nodes indicate the strength of centrality.

Table 4
Top 20 keywords with their frequency and centrality in pesticidal toxins of *Bt* research (1980–2021)

Rank	Frequency	Centrality	Year	Keyword
1	2750	0.20	1991	*Bacillus thuringiensis*
2	1544	0.07	1987	delta endotoxin
3	792	0.03	1990	resistance
4	786	0.18	1987	toxin
5	684	0.00	1991	protein
6	614	0.03	1987	gene
7	598	0.10	1987	crystal protein
8	539	0.07	1991	expression
9	537	0.00	1987	toxicity
10	472	1.00	1991	brush border membrane
11	467	0.42	1991	insect resistance
12	459	0.30	1991	strain
13	442	0.03	1991	lepidoptera
14	421	0.03	1992	*Heliothis virescens*
15	409	0.13	1991	binding
16	388	0.00	1991	identification
17	385	0.35	1991	*Manduca sexta*
18	323	0.10	2004	*Helicoverpa armigera*
19	299	0.07	1991	larvae
20	294	0.07	1991	plant

Categorization of top 20 keywords

From Fig. 5 and Table 4, the top 20 co-occurring keywords are grouped as follows:

The first group of keywords, such as "Bacillus thuringiensis (2,750 appearances in all keywords with a centrality of 0.20), "strain (459, 0.30)", and "identification (388, 0.00), is related to the identification of *Bt* strains that produce the insecticidal toxins. In 1901, a Japanese bacteriologist Shigetane Ishiwata discovered the first endospore-forming *Bacillus thuringiensis* reported it as the causal agent of sotto disease in silkworms following the ingestion of the bacterium by the silkworm larvae [40]. However, Lepidopteran insects (moths and butterflies) were popularly considered the only targets of *Bt* until the 1970s [41], when Goldberg and Margalit identified a new subspecies of *Bt* (*Bacillus thuringiensis israelensis – Bti)* that was active against mosquito and blackfly larvae (Dipteran insects) [42]. Until now, several *Bt* strains have been isolated throughout the world from various sources such as soil, diseased insects, water, grain dust, and leaf surface of many plants [43, 44]. These strains produce over 300 crystal proteins that demonstrate specific activity against several insect orders including Lepidoptera, Diptera, Coleoptera, Hymenoptera, Homoptera, Orthoptera, Mallophaga [2], and other invertebrates [41].

The keywords "delta endotoxin (1545, 0.070)", "toxin (787, 0.18)", "protein (684, 0.00)", "gene (615, 0.03)", "crystal protein (599, 0.10)", and "toxicity (539, 0.00)", form the second group of keywords and are related to the insecticidal protein toxins produced by *Bt* and the genes encoding these proteins. When there is a shortage of nutrients to *Bacillus thuringiensis*, it forms a dormant spore or large parasporal crystalline inclusions. These crystal inclusions are oftentimes referred to as δ-endotoxins (delta endotoxins), and they contain insecticidal Cry proteins that are deadly when ingested by specific susceptible insects [45]. These protein toxins are coded by a family of genes called *cry* genes [2, 4, 46].

The third group of keywords consists of, "expression (539, 0.07)", "brush border membrane (472, 1.00)", and "binding (388, 0.00). This group of keywords is related to the mechanism of action of *Bacillus thuringiensis* insecticidal toxins which involves the expression of certain *cry* genes such as *cry*1Ab, *cry*1F, *cry*9C etc. [47], by binding to the brush border membrane vesicles of specific insects. The crystal proteins of *Bt* consist of inactive protoxins. Upon ingestion, the crystals are solubilized under the alkaline conditions of the susceptible insect midgut and protoxins are processed by the proteases of the midgut to become activated [2]. The activated crystal toxin binds to a specific receptor on the brush border membrane of midgut microvillae. This causes pore formation in the insect midgut, cell lyses, and the eventual death of the insect [2, 48].

The fourth group of keywords are "resistance (792, 0.03)", and "insect resistance (467, 0.42), which are related to the evolution of insect resistance to certain *Bt* toxins. Due to the coevolution of *Bt* and insects, there were optimisms in the past that insects would not develop resistance against *Bt* toxins. However, several insect species displaying different levels of resistance to *Bt* Cry proteins by laboratory selection experiments, using insects collected from wild populations or laboratory-reared insects have been reported, starting in the mid-1980s [2, 49, 50]. Several studies have reported different levels of field-evolved resistance to *Bt* toxins by different major insect pests [51–54].

The last group of keywords comprise "lepidoptera (442, 0.03)", "*Heliothis virescens* (409, 0.13)", "*Manduca sexta* (385, 0.35)", "*Helicoverpa armigera* (323, 0.10)", and "larvae (299, 0.07)", is related to some major insect pests which *Bt* toxins have been deployed against.

Timeline clusters of keywords

Keywords are clustered and visualized in the "timeline" mode to generate a map depicting the relationship between a cluster of keywords and the lifespan of most co-occurring keywords in a cluster. Frequently co-occurring keywords are firstly clustered in CiteSpace and an appropriate cluster label is designated to each cluster. Nodes of the same cluster are aligned on the same horizontal line in accordance with the timespan, displaying the historical accomplishment of a cluster [55]. Keywords with a higher frequency of occurrence show that those keywords were *Bt* toxin research-related hotspots within that period. CiteSpace utilized the clustering modularity index (Q value) and silhouette index (S value) to compute the clustering efficacy of the map. Q value ranges from 0–1, Q > 0.3 indicates a significant network structure. A network with a mean silhouette value above 0.5 is considered rational, and if it is closer to 1, it indicates higher homogeneity of the network [35, 56]. Thus, the Q value of 0.8083, and S value of 0.9675 denote reasonable divided keywords into loose clusters and a higher degree of consistency among members in a cluster.

Figure 6 shows timeline visualization of keywords co-citation analysis, divided into 11 timelines of clusters by the Log-Likelihood Ratio (LLR) clustering method. High-frequency keywords usually appeared between 1990–1992. The largest cluster (#0 bacillus thuringiensis subsp. israelensis) has 15 members and a Mean Silhouette value of 0.936. Keywords in this cluster were hotspots in 1990, but the timeline expired around 1992. The second (#1 insecticidal toxin) and the third (#2 biological control) largest clusters have 14 members of keywords each, with a Mean Silhouette value of 1.00 each and these timelines lasted until around 2008 and 2016 respectively. This means that the keywords related to these clusters appeared early and had great influence in those periods, their popularity has declined recently.

Also, “#2 biological control”, “#3 helicoverpa armigera”, and “#4 field-evolved resistance are three highly connected clusters. The significant overlap of keywords in these clusters can be partly attributed to the detection of field-evolved resistance mechanisms in *Helicoverpa armigera* to certain *Bt* toxins (*Cry*1Ac) in *Bt* cotton fields [57]. The timelines of “#4 field-evolved resistance”, “#5 bacillus thuringiensis delta-endotoxin”, and “#6 plutella xylostela” are still active to this day. Thus, research related to keywords in these clusters has been ongoing since their emergence and they are still popular today, indicating the current research focus.

Top twenty active countries

VOSviewer was used to examine and visualize the contributions and collaborations of different countries in *Bt* pesticidal toxins related research. Only countries with a minimum of 5 documents were included. Out of the 105 countries involved, 63 met the threshold, and the visualization result is illustrated in Fig. 7. The size of a node represents the number of documents published by a particular country and the nodal linkage denote the degree of cooperation. Articles co-authored by authors from more than one country were not ignored.

As shown in Table 5, the United States of America is the leading country in terms of the number of published articles on studies related to *Bt* pesticidal toxins. Articles published by researchers from the United States have been cited 72754 times, with an average citation of 46.58 per article.

Table 5
Top 20 influential countries in *Bt* pesticidal toxin-related research from 1980–2021

Rank	Country	Documents	Citations	Mean citation/document
1	United States of America	1562	72754	46.58
2	Peoples Republic of China	933	16760	17.96
3	India	449	6583	14.66
4	Mexico	308	9986	32.42
5	Brazil	290	4740	16.34
6	England	289	17737	61.37
7	France	284	15280	53.80
8	Canada	283	10703	37.82
9	Japan	249	4945	19.86
10	Spain	245	8578	35.01
11	Germany	131	4257	32.50
12	Tunisia	123	1746	14.20
13	Switzerland	122	5320	43.61
14	Australia	113	5089	45.04
15	South Korea	111	1835	16.53
16	Pakistan	109	1243	11.40
17	Thailand	102	1463	14.34
18	Belgium	99	10537	106.43
19	Italy	98	2383	24.31
20	Turkey	79	796	10.07

The Peoples Republic of China had the second-highest published articles (933), with a mean citation of 17.96 per paper. This is followed by India (449), Mexico (308), and Brazil (290), with an average citation of 14.66, 32.42, 16.34 per paper respectively. Although Belgium is ranked eighteenth (99 documents) regarding the number of publications, it has the highest (106.43) average citation per document among the top 15 most contributing countries. This shows the quality and relative importance of articles published by researchers from Belgium in this field.

Top twenty prolific institutions

The data used contain 2899 institutions that have contributed to research publications on *Bacillus thuringiensis* pesticidal toxins from 1980 to 2021. Out of the 2899 institutions, 66 of them have published at least 25 documents. The number of published documents and the average citation number per document of an institution reflect the influence of an institution in this field. Figure 8 shows the network map of co-cited institutions with at least 25 publications.

Table 6
Top 20 institutions/organisations with the most published articles in *Bt* related research from 1980–2021

Rank	Institution	Documents	Citations	Mean Citation/document
1	Chinese Academy of Agricultural Sciences	298	6317	21.20
2	National Autonomous University of Mexico	177	8244	46.58
3	Huazhong Agricultural University	161	3141	19.51
4	University of Valencia	150	5524	36.83
5	University of California, Riverside	145	6932	47.81
6	The United States Department of Agriculture (USDA)	134	5451	40.68
7	University of Georgia	112	5578	49.80
8	University of Arizona	92	4171	45.34
9	University of Cambridge	89	5893	66.21
10	Mahidol University	85	1288	15.15
11	French National Research Institute of Agriculture (INRA)	82	4084	49.80
12	Chinese Academy of Sciences	81	1426	17.60
13	Agricultural Research Services (ARS)	79	2903	36.75
14	Cornell University	78	4411	56.55
15	Monsanto Company	76	4676	61.53
16	Ohio State University	75	5865	78.20
17	Pasteur Institute	73	6573	90.04
18	Kyushu University	70	1365	19.50
19	Iowa State University	67	2967	44.28
20	Nanjing Agricultural University	62	1739	28.04

Table 6 shows the top 20 institutions/organisations that have published the most documents on *Bt* pesticidal toxins related studies from 1980 to 2021. The Chinese Academy of Agricultural Sciences is ranked first with 298 publications and an average citation of 21.20 per document. The subsequent institutions are the National Autonomous University of Mexico, Huazhong Agricultural University, and University of Valencia, with 177, 161, and 150 publications, and average citations of 46.58, 19.51, and 36.83 per document respectively. Among the top 20 institutions, the Pasteur Institute in France, Ohio State University in the United States, and the University of Cambridge in England had the highest average citation per document of 90.04, 78.20, and 66.21 respectively. This signifies the contribution of these institutions to quality published documents on *Bt* pesticidal toxins related studies.

Conclusions

In this study, data of global scientific research publications on *Bt* pesticidal toxins from 1980 to 2021 were extracted from the Web of Science Core Collection and analyzed using CiteSpace and VOSviewer scientometric visualization tools. After refining the data, 5757 documents were extracted. The results showed that researchers from 2,899 institutions in 105 countries contributed to studies on *Bt* biopesticide toxins. The United States of America was the leading country in this field, followed by the People's Republic of China, and India. The most influential institutions were the Chinese Academy of Agricultural Sciences, the National Autonomous University of Mexico, and the Huazhong Agricultural University.

Also, Schepf et al. (1998), Höfte & Whiteley (1989), and Crickmore et al. (1998) were the most influential references based on the number of times they have been cited. Documents related to these references have contributed immensely to shaping research trends in this field. These references focused on topics such as the structure and functions of Cry proteins, nomenclature and classification of Cry toxins, expression and mode of action of Cry toxins in insects, and the evolution of resistance to Cry toxins in insects. Reference burst citation analysis revealed the most influential references and current references that are receiving the most attention [2, 4, 33]. Bravo et al. [38], had the highest citation burst of 115.06, followed by Pardo-López et al. [39] and Bravo et al. [1] with citation burst strengths of 109.83 and 109.03 respectively. Out of the top 20 references with the strongest citation burst, 11 of them are still receiving attention to this day. Most of these references are related to research on the evolution of insect resistance to *Bt* pesticidal toxins.

Keyword co-occurrence analysis revealed the most frequently occurring keywords. After the analysis, some of the most highly occurring keywords were *Bacillus thuringiensis*, delta endotoxin, resistance, toxin, protein, gene, crystal protein etc. In addition, cluster and timeline visualization of the keywords showed that keywords related to cluster labels such as field-evolved resistance, *Bacillus thuringiensis* delta endotoxin, and *Plutella xylostella* are the current active hotspots. In general, the current trend of research in this field is more focused on the evolution of resistance among pest insects against *Bt* toxins.

Research hotspots in recent years have mainly focused on the development of resistance among certain insects against *Bacillus thuringiensis* pesticidal toxins. With the latest development of research on *Bt* toxins, researchers should concentrate more on understanding the mechanisms of resistance among certain insects and put in an effort to discover novel pesticidal toxins that are effective against these pest insects.

Limitations

This scientometric study utilized CiteSpace and VOSviewer to analyze data on *Bacillus thuringiensis* pesticidal toxins' publications from 1980–2021, based on the Web of Science Core Collection database. The data used in this study were extracted from only one database. Although Web of Science consists of many journals, it is difficult to achieve full coverage of all documents on *Bt* pesticidal toxins, especially those in other databases such as Scopus, PubMed, etc. Also, data used in this study were restricted to only research and review articles, and the query terms used to search the articles. Future studies can broaden the data collection to include publication types such as book chapters, proceedings papers, early access etc., and include search terms such as parasporins, vegetative insecticidal protein (Vip) toxins, binary (Bin) toxins etc.

Declarations

Competing interests: The authors have declared that no competing interest exists.

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Figures

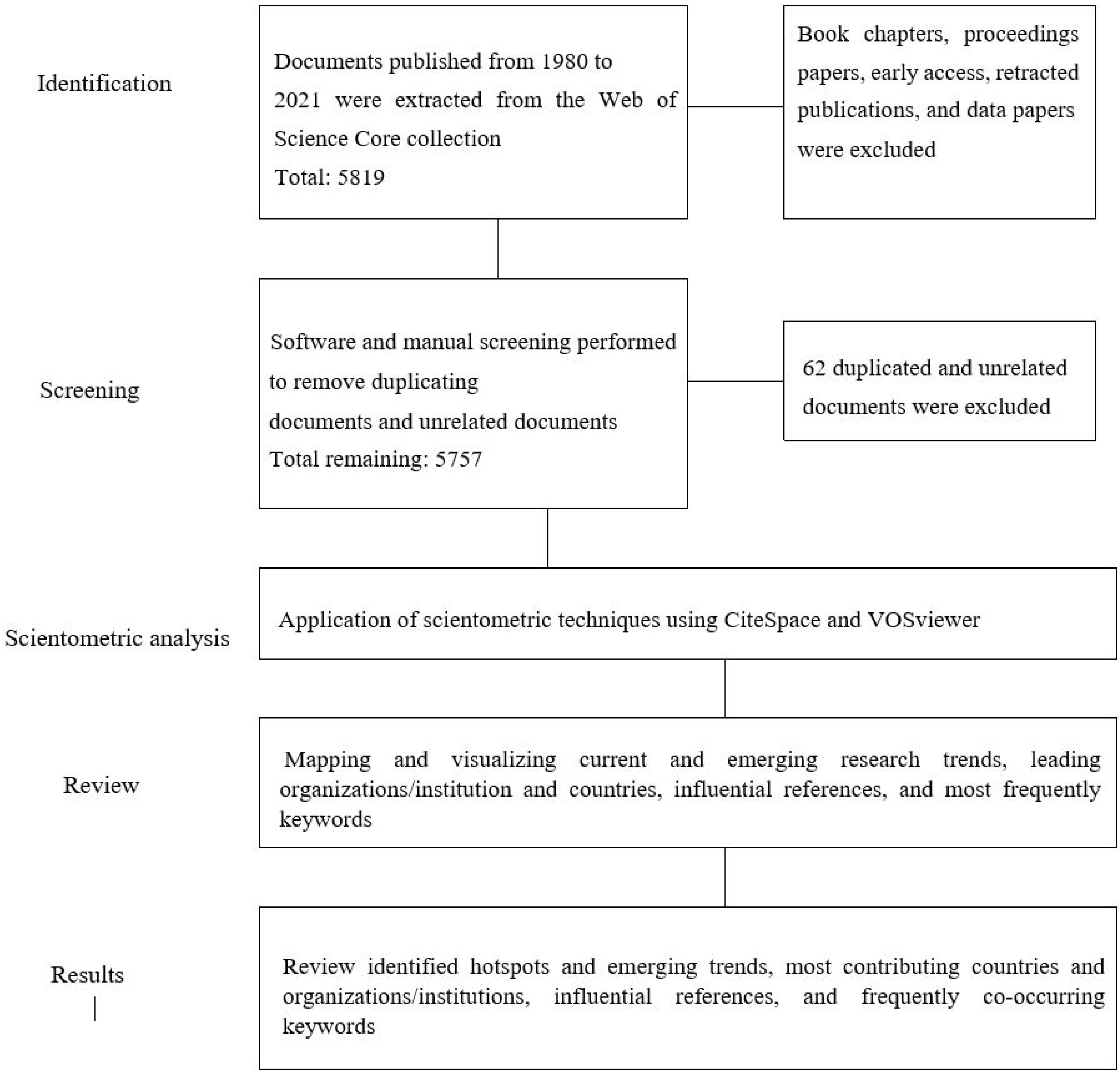

Figure 1

Flow chat of main methods

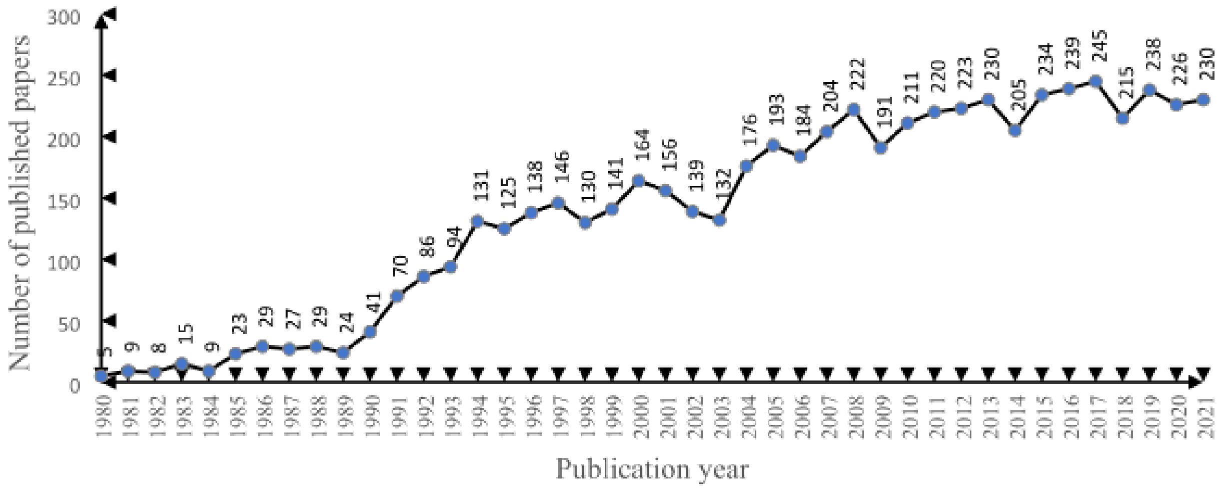

Figure 2

Number of articles published annually from 1980 to 2021

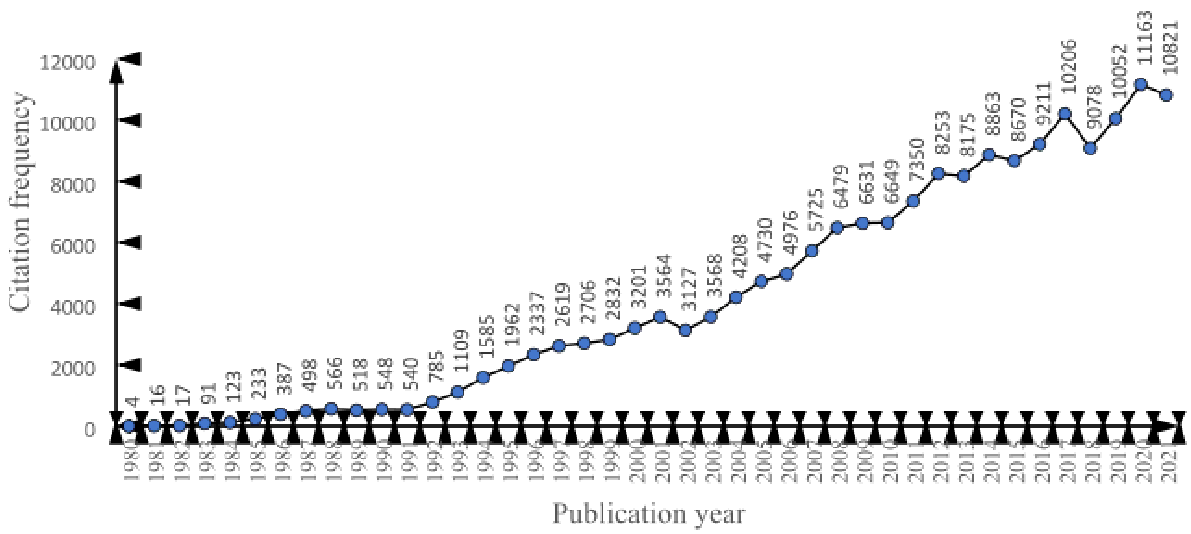

Figure 3

Frequency of literature citations on *Bt* biopesticide toxins from 1980 to 2021

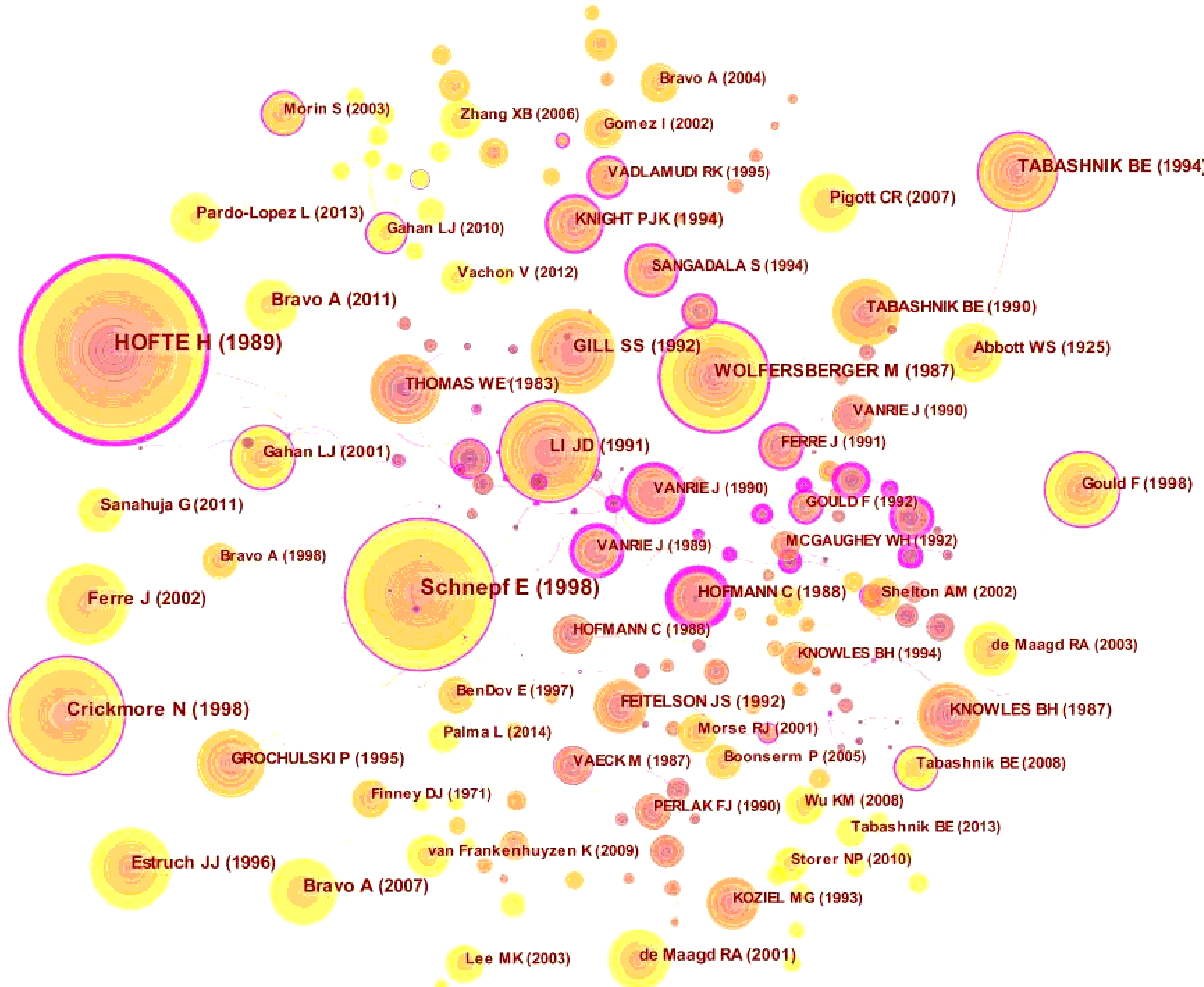

Figure 4

References co-citation map of documents on *Bt* pesticidal toxins from 1980 to 2021

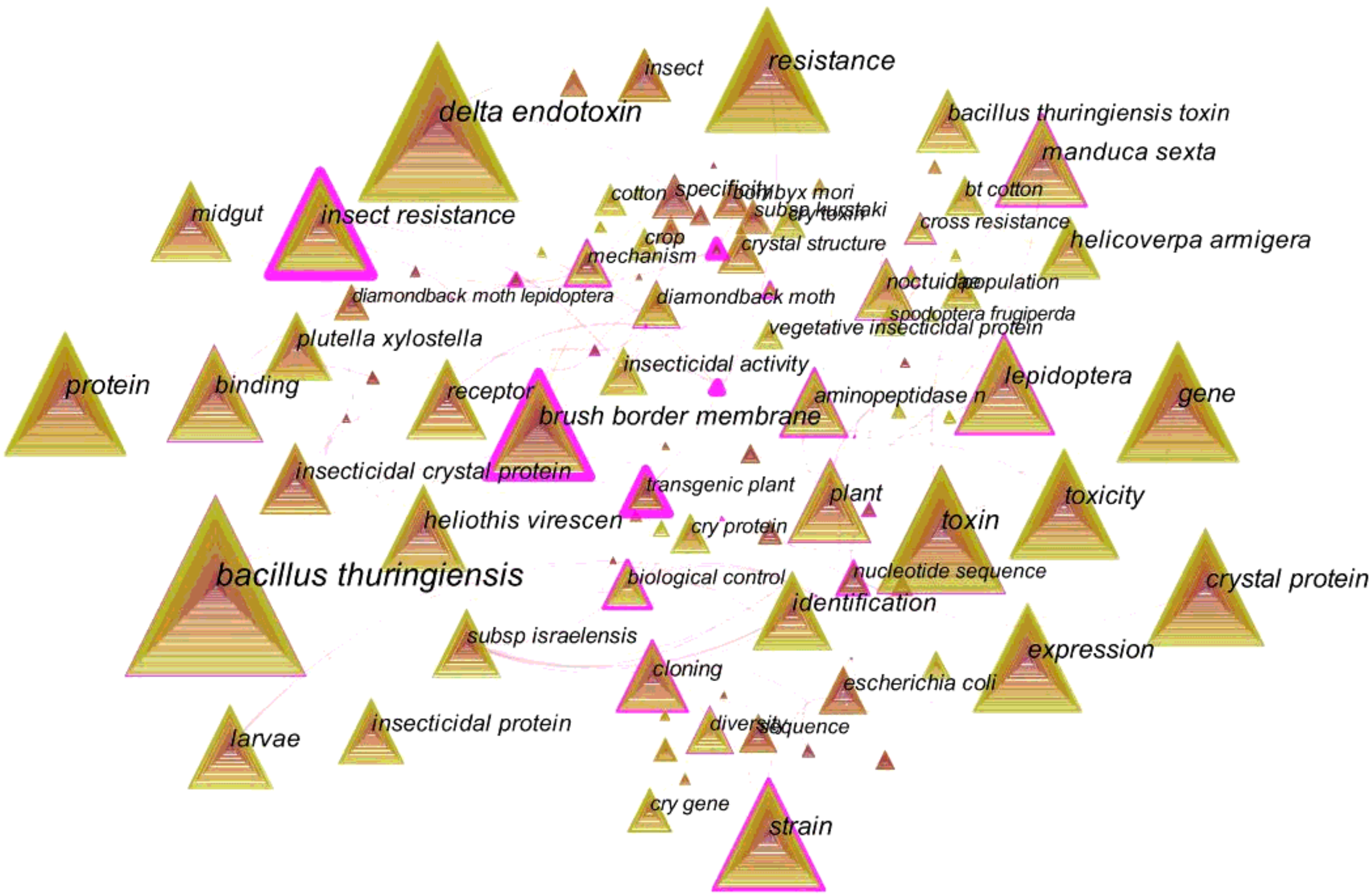

Figure 5

Keyword co-occurrence map

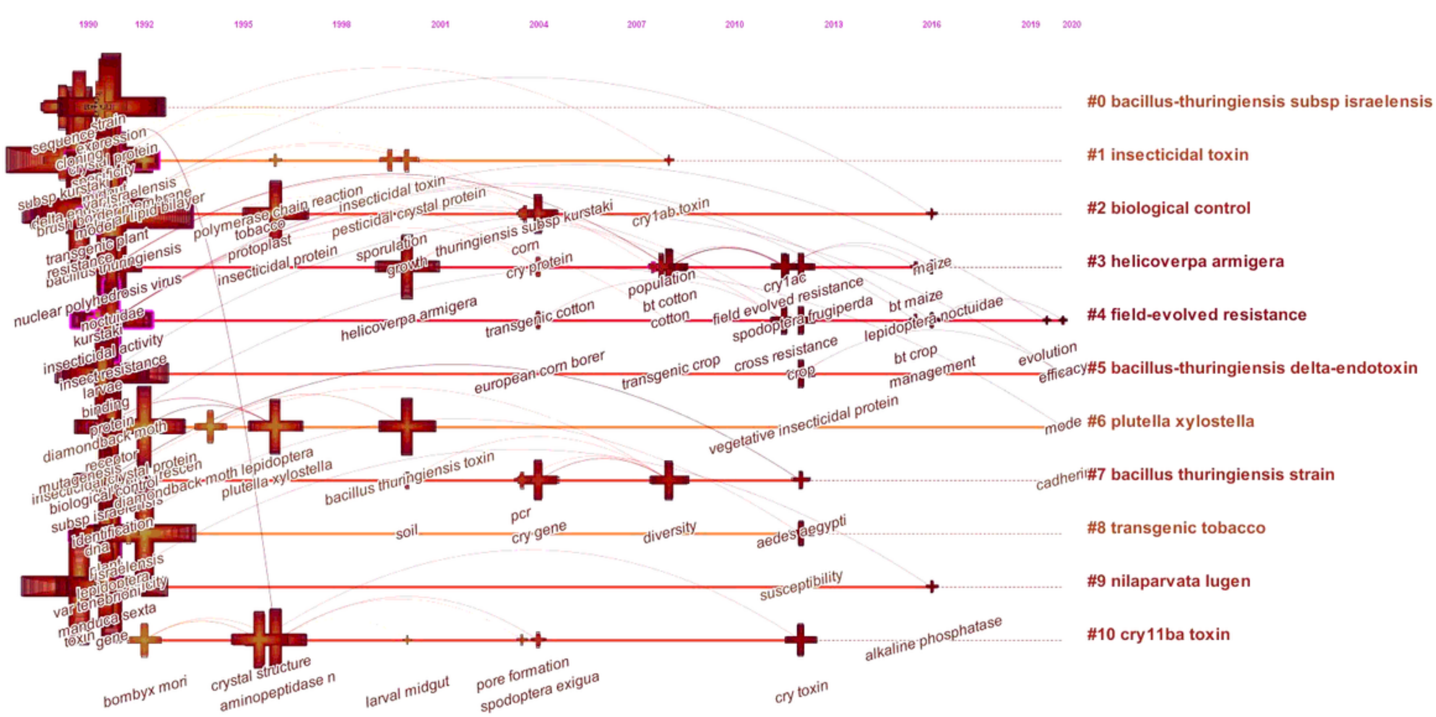

Figure 6

Timeline co-citation map of high-frequency keywords.

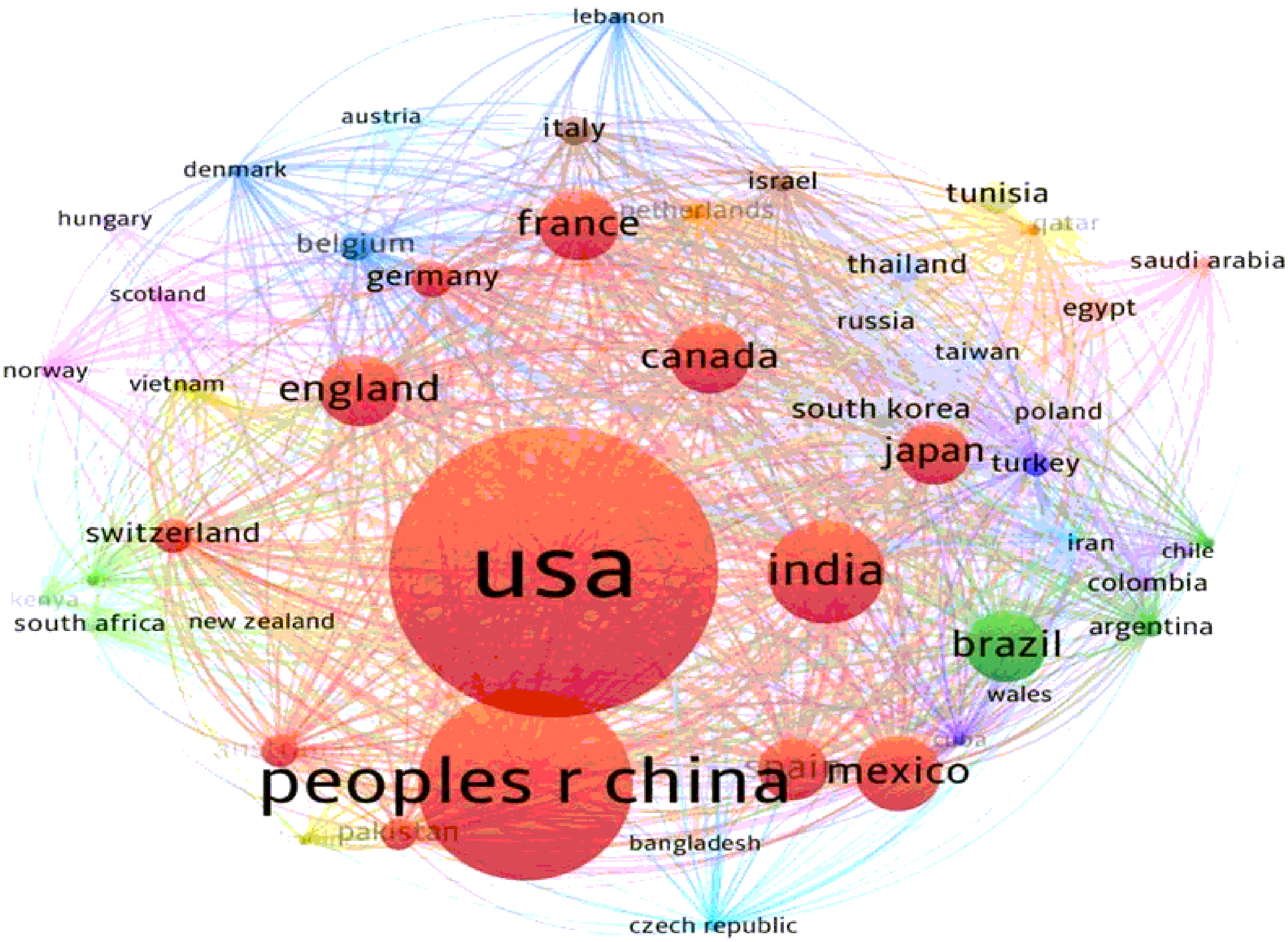

Figure 7

The co-authorship map of countries.

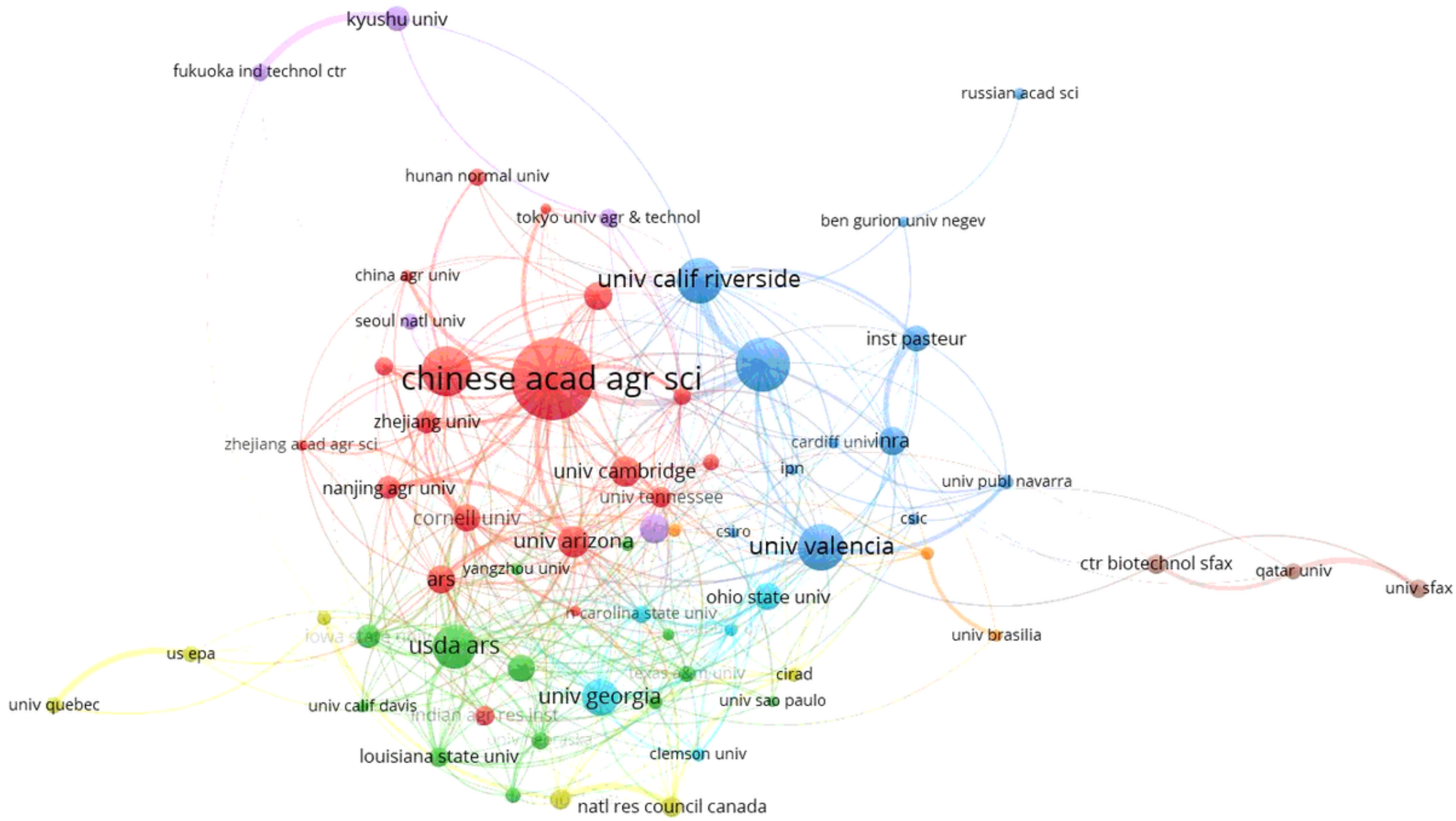

Figure 8

The co-authorship map of organisations/institutions