Bulgarian Journal of Agricultural Science, 27 (No 1) 2021, 137–146

An overview of the diversity of pathogens causing bacterial spot on tomato and pepper in Bulgaria

Nevena Bogatzevska[1*], Taca Vancheva-Ebben[2], Katya Vasileva[3], Yoana Kizheva[2] and Penka Moncheva[2*]

[1]*Agricultural Academy, Institute of Soil Science, Agrotechnologies and Plant Protection "N. Poushkarov", 1331 Sofia, Bulgaria*
[2]*Sofia University "St. Kliment Ohridski", 1164 Sofia, Bulgaria*
[3]*Agricultural Academy, Maritsa Vegetable Crops Research Institute, 4003 Plovdiv, Bulgaria*
[*]*Corresponding author:* bogatzevska@gmail.com; montcheva@abv.bg

Abstract

Bogatzevska, N., Vancheva-Ebben, T., Vasileva, K., Kizheva, Y. & Moncheva, P. (2021). An overview of the diversity of pathogens causing bacterial spot on tomato and pepper in Bulgaria. *Bulg. J. Agric. Sci., 27 (1)*, 137–146

Bacterial spot (BS) is a destructive disease affecting tomato and pepper plants. A wide diversity among the pathogens causing this disease makes them a serious threat for the tomato and pepper production worldwide, including Bulgaria, where the disease has become a major problem. To date in Bulgaria three species have been identified that infect tomato (*X. vesicatoria*, *X. euvesicatoria* and *X. gardneri*), and two that infect pepper (*X. vesicatoria* and *X. euvesicatoria*). Despite the research and published data, there is no general information about the BS agents on tomato and pepper in Bulgaria in respect to pathotypes, races, and the dynamics of pathogenic populations during the years. In this study we analyzed and summarized for the first time the data on the species, pathotype and race structure of the pathogenic population during the period 1999-2016. BS on tomato is caused by three species (*X. vesicatoria*, *X. gardneri* and *X. euvesicatoria*), and on pepper – by two species (*X. euvesicatoria* and *X. vesicatoria*). *X. vesicatoria* was detected and identified at the beginning of this period. This species is a dominant pathogen on tomato, whereas on pepper dominates the *X. euvesicatoria* species. We found a significant race diversity especially for the PT strains of *X. vesicatoria* and PT and P strains of *X. euvesicatoria*. The occurrence of new races over the past years has been registered, replacing those established at the beginning of the observed period. This indicates that the pathogenic population responsible for BS on tomato and pepper is dynamic and needs to be regularly monitored and studied, which is important to control the disease.

Keywords: bacterial spot agents; diversity; race; pathotype; pepper; tomato
Abbreviations: BS – bacterial spot; IPP – Institute of Plant Protection; IG – Institute of Genetics; MVCRI – Maritsa Vegetable Crops Research Institute; PCR – polymerase chain reaction; P – pepper pathotype; PT – pepper-tomato pathotype; NB – Northern Bulgaria; Northern-East Bulgaria; SB – Southern Bulgaria; Southern-West Bulgaria; T – tomato pathotype; WB – Western Bulgaria

Introduction

Bacterial spot is a destructive disease affecting tomato and pepper plants. A wide diversity among the pathogens causing this disease makes them a serious threat for the tomato and pepper production worldwide. Under humid conditions bacterial spot (BS) as one of the most invasive disease has the potential to cause heavy yield losses of affected pepper and tomato plants due to its seedborne nature and the low efficiency of chemical control (Quezado-Duval et al., 2004). The disease has cyclic peaks and it is observed when frequent wind-driven rainfalls and high temperature

are present (Sahin & Miller, 1998; Mitrev, 2001; Obradovic et al., 2004; Bogatzevska et al., 2007). To date, four genetically and phenotypically distinct pathogens have been defined as causative agents of bacterial spot: *Xanthomonas euvesicatoria, Xanthomonas vesicatoria, Xanthomonas gardneri,* and *Xanthomonas perforans* (Jones et al., 2004). Among the BS causative agents of tomato and pepper, three pathotypes of strains are distinguished – those that only infected tomato (T pathotype), only pepper (P type), and both tomato and pepper (PT type) (O'Garro et al., 1999). In the years, *X. euvesicatoria* and *X. gardneri* have been isolated from both symptomatic tomato and pepper, *X. vesicatoria* primarily from tomato and *X. perforans,* until recently, only from tomato (Potnis et al., 2015; Schwartz et al., 2015). The *X. euvesicatoria* strains were reported as more aggressive on pepper plants and in some countries as the prevalent pathogen (Potnis et al., 2015; Schwartz et al., 2015; Kyeon et al., 2016; Areas et al., 2015; Vancheva et al., 2014). Several races have been distinguished among the above mentioned pathotypes (Minsavage et al., 1990; Ward & O'Garro, 1992; O'Garro & Tudor, 1994; Bouzar et al., 1994a). Bacterial spot in Bulgaria was first described in 1936 and has become a major problem in the tomato and pepper-production fields (Kovachevski, 1936). For the first time on Bulgarian pepper, BS was recorded by Karov (Karov, 1965), after which BS agents have been found and species identified in both tomato and pepper. To date, three species have been described that infect tomato (*X. vesicatoria*, *X. euvesicatoria* and *X. gardneri*), and two pepper (*X. vesicatoria* and *X. euvesicatoria*) in Bulgaria (Bogatzevska et al., 2007; Kizheva et al., 2011, Kizheva et al., 2013; Vancheva et al., 2014).

The aim of the study is to review the species diversity of bacterial spot causative agents on tomato and pepper in Bulgaria, as well as their pathotype and race structure during the period 1999-2016.

Materials and Methods

A collection of 314 bacterial strains isolated from various tomato and pepper production fields in Bulgaria in the period 1999-2016 was analyzed in this study. Most of the strains were previously identified by species-specific PCR amplification and by the miniaturized identification system BiologTM (Biolog™, USA) with GN2 MicroPlate™ test plates. A large number of strains have been characterized by different molecular methods (Kizheva et al., 2011; Kizheva et al., 2013; Stoyanova et al., 2014; Vancheva et al., 2014; Kizheva et al., 2016; Kizheva et al., 2017; Vancheva et al., 2018).

The pathogenicity and the pathotype of all the BS strains were defined using tomato cv. 'Ideal' and pepper cv. 'Kalifornyisko chudo' as test plants (Bogatzevska & Sotirova, 1992). Strains were tested for race determination on the basis of the presence of a hypersensitive (HR), resistant or compatible response on different tomato and pepper genotypes. Tomato (T) races were identified using the genotypes *Hawaii* 7981 and *Hawaii* 7998, whereas pepper (P) races on the genotypes ECW10R, ECW20R, and ECW30R (Bouzar et al., 1994b). The reaction was recorded after the leaves of the corresponding genotypes were infiltrated with a bacterial suspension (10^8 cfu/ml). The tests were performed twice.

Results

Three hundred-fourteen strains (130 strains isolated from tomato and 184 isolated from pepper) were the object of this study. The strains were previously identified by species-specific PCR amplification and BIOLOG system (Kizheva et al., 2011; Kizheva et al., 2013; Stoyanova et al., 2014; Vancheva et al., 2014) as follows: 114 – *X. vesicatoria*, 19 – *X. gardneri,* and 181 – *X. euvesicatoria* (Table 1 and Table 2). The bacterial strains were isolated from different parts of tomato and pepper plants from five regions in Bulgaria – Western Bulgaria (WB), Northern Bulgaria (NB), Southern Bulgaria (SB), Southewestern Bulgaria (SW), and Northeastern Bulgaria (NE) (Table 1 and Table 2).

The species *X. vesicatoria* was identified as the main causative agent of BS on tomato in Bulgaria. Even though identified as the main agent causing BS on tomato, the

Table 1. Strain designation, year of isolation and location for *Xanthomonas* isolates from tomato in Bulgaria during the period 1999–2016

Strain designation	Year of isolation	Location
X. vesicatoria		
32t, 34t, 35t, 36t, 37t	1999	IG, Sofia* (WB)
38t, 40t	2000	Stara Zagora (SB)
39t	2000	Radnevo (SB)
41t, 42t, 43t, 56t, 57t, 58t	2006	IPP, Kostinbrod ** (WB)

Table 1. Continued

Strain designation	Year of isolation	Location
44t, 45t, 47t, 48t, 49t, 50t, 51t, 52t, 53t, 54t, 55t	2006	IG, Sofia* (WB)
59t	2007	Yarlovo (WB)
60t	2007	IG, Sofia, Bulgaria* (WB)
63t	2009	IG, Sofia* (WB)
68t	2010	MVCRI, Plovdiv *** (SB)
69t, 73t	2012	Topolovgrad (SB)
83t, 88t, 89t	2013	MVCRI, Plovdiv *** (SB)
84t	2013	Haskovo (SB)
17x, 87t	2013	Plovdiv (SB)
18x, 19x	2013	Kostinbrod (WB)
20x	2013	Bozhurishte WB)
21x, 22x, 23x, 25x, 26x, 27x, 28x	2014	MVCRI, Plovdiv *** (SB)
24x, 30x,	2014	Sadovo (SB)
29x	2014	Trudovets(SB)
33x, 56x	2014	Pazardzhik (SB)
44x	2014	Pavlikeni (NB)
46x, 47x	2014	Byala Cherkva (NB)
48x, 49x	2014	IG, Sofia* (WB)
50x	2014	Yarlovo (WB)
51x, 52x, 55x	2014	Plovdiv (SB)
97t, 98t, 99t, 100t, 101t, 102t, 103t, 104t	2015	MVCRI, Plovdiv *** (SB)
4(1)t	2015	Plovdiv (SB)
122t	2016	Sadovo (SB)
123t, 124t, 125t, 126t	2016	MVCRI, Plovdiv *** (SB)
127t, 128t	2016	Pazardzhik (SB)
129t, 130t, 131t	2016	Blagoevgrad (SWB)
132t, 133t	2016	Tyulenovo (NEB)
134t, 135t	2016	Schabla (NEB)
X. euvesicatoria		
90t, 91t, 92t, 93t, 94t, 95t, 96t	2015	Bozhurishte (WB)
105t, 106t	2015	Haskovo (SB)
107t, 108t, 109t	2015	Svilengrad (SB)
110t, 111t, 112t	2015	Sadovo (SB)
113t, 114t	2015	Byala Cherkva (NB)
115t, 116t, 139t	2016	Kostinbrod (WB)
117t, 118t, 119t	2016	MVCRI, Plovdiv *** (SB)
120t, 121t	2016	Sadovo (SB)
136t	2016	Pazardzhik (SB)
137t	2016	Pavlikeni (NB)
X. gardneri		
62t, 64t, 65t	2009	IG, Sofia* (WB)
66t, 67t	2010	MVCRI, Plovdiv *** (SB)
70t, 71t, 72t, 74t, 75t, 76t, 77t, 78t, 79t, 80t	2012	Topolovgrad (SB)
81t, 82t	2013	MVCRI, Plovdiv *** (SB)
85t, 86t	2013	Haskovo (SB)

*Institute of Genetics (part of Institute of Plant Physiology and Genetics, since 2010)
**Institute of Plant Protection (part of Institute of Soil Science, Agrotechnologies and Plant Protection 'Nikola Poushkarov', Sofia, Bulgaria, since 2012)
***Maritsa Vegetable Crops Research Institute
WB – Western Bulgaria; NB – Northern Bulgaria; NEB – North-Eastern Bulgaria; SB – Southern Bulgaria

Table 2. Strain designation, year of isolation and location for *Xanthomonas* isolates from pepper in Bulgaria during the period 1999–2016

Strain designation	Year of isolation	Location
X.vesicatoria		
1b, 2b, 3b	1999	Lovech (NB)
4b	2000	IG, Sofia* (WB)
6b, 7b	2001	IG, Sofia* (WB)
8b	2002	Lovech (NB)
9b	2002	Trebich (WB)
14b, 15b	2005	IG, Sofia* (WB)
16b	2006	Kavarna (NEB)
17b, 18b, 19b	2006	IPP, Kostinbrod ** (WB)
22b, 26b	2006	IG, Sofia* (WB)
32b, 33b	2007	Haskovo (SB)
34b, 35b	2007	Stara Zagora (SB)
36b, 37b	2008	IPP, Kostinbrod ** (WB)
53b	2011	Novi Iskar (WB)
57b, 58b, 59b	2011	MVCRI, Plovdiv *** (SB)
60b	2011	IG, Sofia* (WB)
304	2016	Trebich (WB)
305	2016	Lovech (NB)
309	2016	IG, Sofia* (WB)
X. euvesicatoria		
5b	2000	IG, Sofia* (WB)
10b	2002	Pazardzhik (SB)
11b	2003	IPP, Kostinbrod **
12b	2003	Petrich (WB)
13b	2004	IPP, Kostinbrod ** (WB)
23b, 24b, 25b, 27b, 28b, 29b, 30b, 31b	2006	IG, Sofia* (WB)
38b	2008	IG, Sofia* (WB)
39b, 40b	2009	Kostinbrod (WB)
41b, 42b, 43b	2009	Veliko Tarnovo (NB)
44b, 45b, 47b, 48b, 49b, 50b, 51b, 52b	2010	MVCRI, Plovdiv *** (SB)
54b	2011	IPP, Kostinbrod ** (WB)
55b, 56b	2011	Kostinbrod (WB)
61b, 62b, 63b, 64b, 65b	2012	MVCRI, Plovdiv *** (SB)
66b, 67b, 68b, 69b, 70b, 71b, 72b, 73b, 74b, 75b, 76b	2012	Pavlikeni (NB)
77b, 78b, 79b	2012	Durankulak (NEB)
80b, 81b, 82b	2012	Shabla (NEB)
83b, 84b, 85b	2012	Tyulenowo (NEB)
86b, 87b ,88b	2012	Kavarna (NEB)
89b, 90b, 91b, 92b	2013	Kostinbrod (WB)
93b, 94b, 95b	2013	Sadovo (SB)
96b, 97b, 98b, 99b, 100b, 101b, 102b, 103b, 105b	2013	Byala Cherkva (NB)
106b	2013	Shabla (NEB)
107b, 108b, 109b, 110b, 111b, 112b, 113b, 114b, 115b	2013	Kavarna (NEB)
116b, 117b, 120b, 122b	2014	Pavlikeni (NB)
118b, 119b	2014	Byala Cherkva (NB)
122b, 125b, 132b	2014	Durankulak (NEB)

Table 2. Continued

123b, 124b	2014	Shabla (NEB)
126b, 127b	2014	Krapets (NEB)
128b, 131b	2014	Kavarna (NEB)
129b, 130b	2014	Tyulenovo (NEB)
131b	2014	Kostinbrod (WB)
134b, 135b	2014	Svilengrad (SB)
136b, 137b, 138b	2014	Sadovo (SB)
214, 216	2015	IPP, Kostinbrod ** (WB)
260	2015	Shabla (NEB)
264, 265	2015	Kavarna (NEB)
266, 268	2015	Tyulenovo (NEB)
269, 271	2015	Durankulak (NEB)
273, 274, 276	2015	Shabla (NEB)
279, 281, 283, 284, 285, 286	2015	Krapets (NEB)
288, 289, 291, 293, 294	2015	Tyulenovo (NEB)
295, 303	2015	Svilengrad (SB)
306, 308	2016	IPP, Kostinbrod ** (WB)
307	2016	Petarch(WB)
310	2016	MVCRI, Plovdiv *** (SB)
311	2016	Kostinbrod
312, 313	2016	MVCRI, Plovdiv *** (SB)
314, 315, 316	2016	Pavlikeni (NB)
317	2016	Durnakulak (NEB)
318, 319, 326, 327	2016	Shabla (NEB)
320, 328	2016	Tyulenovo (NEB)
321	2016	Kavarna (NEB)
322, 323	2016	Sadovo (SB)
324, 325	2016	Byala Cherkva

*Institute of Genetics (part of Institute of Plant Physiology and Genetics, since 2010)
**Institute of Plant Protection (part of Institute of Soil Science, Agrotechnologies and Plant Protection 'Nikola Poushkarov', Sofia, Bulgaria, since 2012)
***Maritsa Vegetable Crops Research Institute
WB – Western Bulgaria; NB – Northern Bulgaria; NEB – North-Eastern Bulgaria; SB – Southern Bulgaria

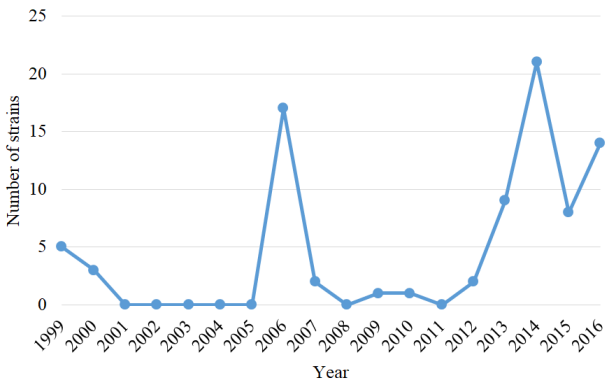

Fig. 1. Spread of the species *X. vesicatoria* on tomato in Bulgaria during the period 1999–2016

species was not isolated repeatedly every year during this study (Figure 1). *X. vesicatoria* was mostly present in 2006, 2011, 2014, and in 2016. *X. euvesicatoria* was firstly identified as a BS pathogen on tomato in Bulgaria in 2015 and found again later on in 2016, while *X. gardneri* was observed in the period 2009-2014 (Table 1).

The tomato BS pathogens were represented by two pathotypes – pepper-tomato (PT) and tomato (T) with prevalence of the PT pathotype, more pronounced among the strains of the three species isolated in the SB region (Figure 2 and Table 3).

Among the T strains of the species *X. vesicatoria* and *X. euvesicatoria* three races were identified – T1, T2 and T3, while *X. gadneri* was represented only by two races – T1 and T3 (Figure 3). Race T3 was predominant among the T strains of *X. vesicatoria*.

The races of twenty six PT strains of the three species were determined. Eight races were identified among the analyzed strains (Table 4).

During the analyzed period BS was also observed on pepper plants. One hundred eighty four strains were isolated from the five regions in Bulgaria (Table 1). The disease was shown to be caused by two pathogens: *X. euvesicatoria* (154 strains) and *X. vesicatoria* (30 strains) with *X. euvesicatoria* being the dominant one, very clearly expressed in the last years of the surveyed period (Figure 4).

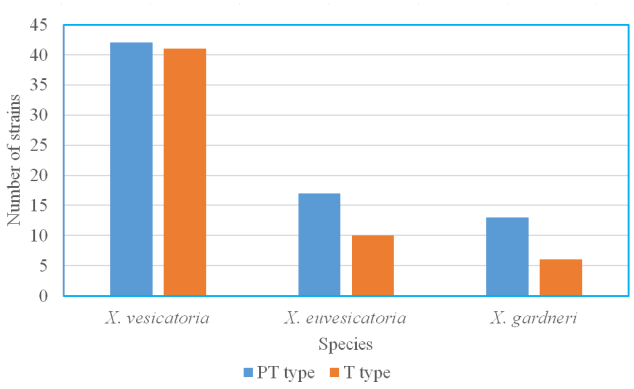

Fig. 2. Pathotype structure of the species causing BS on tomato in Bulgaria during the period 1999–2016

Table 4. Races identified among the selected PT strains causing BS on tomato

Races	Strains, %		
	X.vesicatoria	*X. euvesicatoria*	*X. gardneri*
P0T1	12.5	6.0	no
P0T2	12.5	35.0	no
P1T2	12.5	6.0	no
P3T2	12.5	no	no
P4T1	25.0	12.0	no
P4T2	12.5	41.0	no
P5T3	12.5	no	no
P10T2	no	no	100

no – no strain possesses this race

Table 3. Distribution by regions of the pathotypes of the species causing BS on tomato during the period 1999-2016

Region of isolation	*X. vesicatoria* strains, %		*X. euvesicatoria* strains, %		*X. gardneri* strains,%	
	PT	T	PT	T	PT	T
WB	36	39	18	70	23	0
NB	2	5	18	0	23	NI
SB	55	46	64	30	84	100
SWB	0	7	NI	NI	NI	NI
NEB	7	3	NI	NI	NI	NI

WB – Western Bulgaria; NB – Northern Bulgaria; SB – Southern Bulgaria; SWB – South-Western Bulgaria; North-Eastern Bulgaria
PT – pepper-tomato pathotype; T – tomato pathotype
NI – no strains isolated

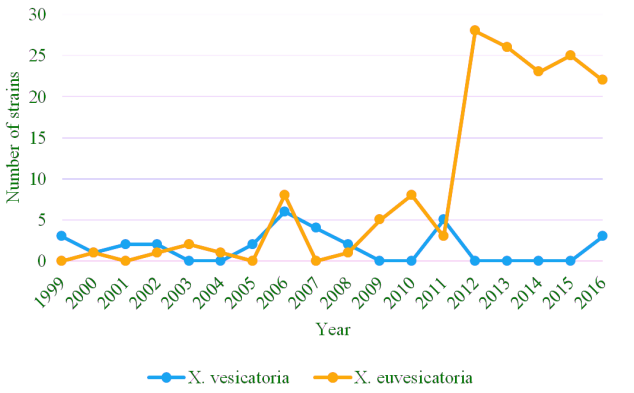

Fig. 3. Races of T strains of the species causing BS on tomato in Bulgaria during the period 1999–2016

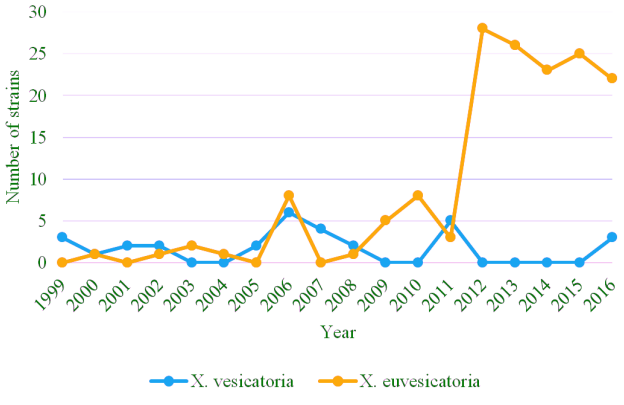

Fig. 4. Spread of the species *X. vesicatoria* and *X. euvesicatoria* on pepper in Bulgaria during the period 1999–2016

For the *X. vesicatoria* species the PT pathotype was significantly more abundant, while among the *X. euvesicatoria* the PT and P pathotypes were approximately equally presented with a slight prevalence of the P pathotype (Figure 5). The P pathotype of *X. vesicatoria* was represented by single strains isolated only in 2005. Until 2008, the *X. euvesicatoria* populations were relatively homogenous and combined only strains from PT pathotype. The distribution of the pathotypes by region of isolation is presented in Table 5.

The race structure of 93 (50% of all pepper strains) selected strains was determined. Among the PT strains of *X. vesicatoria* nine races were identified (P0T2, P1T1, P1T2, P2T3, P3T1, P3T2, P4T2, P4T3, and P6T2). The only P strain of this species belonged to race P2. PT *X. euvesicatoria* (36 strains) strains were distributed into 16 races (P0T1, P0T2, P0T3, P1T1, P1T3, P2T2, P3T2, P4T1, P4T2, P473, P5T1, P5T2, P5T3, P6T1, P6T2, and P9T1), while among the P strains (44 strains) six races were identified (P0, P1, P2, P3, P4, P5, P6, and P10).

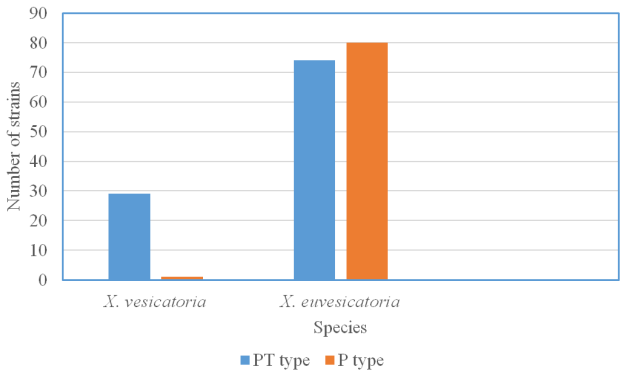

Fig. 5. Pathotypes of *X. vesicatoria* and *X. euvesicatoria* on pepper in Bulgaria during the period 1999–2016

Table 5. Distribution by regions of the pathotypes of the species causing BS on pepper during the period 1999–2016

Region of isolation	*X. vesicatoria* strains, %		*X. euvesicatoria* strains, %	
	PT	P	PT	P
WB	55	100	28	10
NB	17	no	34	11
SB	24	NI	19	18
SWB	NI	NI	1	NI
NEB	4	no	18	61

WB – Western Bulgaria; NB – Northern Bulgaria; SB – Southern Bulgaria; SWB – South-Western Bulgaria; North-Eastern Bulgaria
PT – pepper-tomato pathotype; T – tomato pathotype
NI – no strains isolated
no – no strain is of this type

Discussion

The host range of BS xanthomonads includes a wide range of plants, but tomato and pepper are the main hosts (Potnis et al., 2015). Our study reports the species, pathotype, and race diversity of BS causative agents on tomato and pepper plants in Bulgaria during the period 1999-2016. These pathogens are widely distributed in different regions of the world. Four species are the causative agents of BS on tomato and pepper – *X. vesicatoria*, *X. euvesicatoria*, *X. performance,* and *X. gardneri* (Jones et al., 2004a). Using MLSA and whole-genome sequence analysis, some authors reported that *X. euvesicatoria* and *X. perforans* belong to one species (Timilsina, 2015; Barak et al., 2016). Furthermore, a study based on the whole genome sequence comparison of the existing species *X. cynarae* and *X. gardneri* showed that they are the same species for which the name *X. cynarae* pv. *gardneri* was proposed (Thimilsina et al., 2018). In our study we found that the pathogenic populations of the hosts were heterogenic by species, type, and race structure. We identified three species: *X. vesicatoria, X. euvesicatoria,* and *X. gardneri,* of which only the first two species infect pepper. *X. vesicatoria* was a dominant species which caused BS on tomato, with 84 isolated strains. Approximately 64% of all strains belonged to this species. The species was mainly isolated from SB and WB, where most tomato plants are grown, but it was not isolated repeatedly every year during the studied period. The appearance, development and spread of BS disease on tomato and pepper depends on several factors such as the climatic characteristics of the growing area, the soil type, cultivated plant varieties, and farming technology. *X. vesicatoria* species was presented by two pathotypes – T and PT, the latter being able to infect both, tomato and pepper. Among the T strains three races were identified – T1, T2, and T3 with prevalence of the T3 race (39% of all the T strains). The three races were almost equally represented in WB, while in the SB dominated the race T3. These races were found almost every year during the survey period, except in the years when single strains were isolated. The race variety of PT strains was significant, despite the limited number of strains analyzed. Seven races were identified, six of which were equally represented. The race P4T1 predominated. Currently, four tomato races and eleven pepper races are known. Among the causative agents of BS on tomato and pepper various pepper and tomato races have been reported (Jones et al., 1995; Jones et al., 2004b; Stall et al., 2009; Kebede et al., 2014).

X. euvesicatoria was found for the first time as pathogen on tomato in Bulgaria in 2015. Similar to *X. vesicatoria*, this pathogen was represented by two pathotypes – T and PT. The latter one was dominant and more often registered in SB region (64% of all the PT strains). It is interesting to note that *X. euvesicatoria* was not found in SWB and NEB regions. Among the T strains we found three races (T1, T2, and T3), as established for *X. vesicatoria*. The races T1 and T2 were found more often than race T3. The races of selected PT strains were determined and five races were identified. The races P4T2 and P0T2 were characteristic for the largest number of strains – 41 and 35% of the tested strains and were found in all the regions, where the pathogen was isolated.

X. gardneri was established as BS agent on tomato in Bulgaria in 2009. It is interesting to note that *X. gardneri* was first reported in the United States – Ohio and Michigan also in 2009 as a causative agent of BS on tomato (Ma et al., 2011). Similar to *X. vesicatoria,* the pathogen has not been detected every year since its discovery in Bulgaria. It was established in 2009, 2010, 2012, 2014, and its maximum spread was in 2012 in SB region. *X. gardneri* was not found in NB, SWB, and NEB regions. Most of the strains were of PT type, and T strains belonged to races T1 and T2, which were equally represented. In a recent study Timilsina et al. (2015) found that this species was becoming more widely distributed in different geographic regions, as also concluded in our research.

Prior to 2009 *X. vesicatoria* was single and dominant species, causing BS on tomato in Bulgaria. Similar changes in pathogenic population were observed by other authors (Tudor-Nelson et al., 2003; Hert et al., 2005).

During the studied period we found that *X. euvesicatoria* and *X. vesicatoria* were the main pathogens in the pepper producing regions in Bulgaria. Until 2006, the BS was less manifested, followed by peaks in its spread. *X. euvesicatoria* was the dominant species, which was more strongly pronounced from 2012. Fluctuations in the presence and the distribution of the two pathogens were observed. Interestingly, the maximal abundance of one species leaded to minimal abundance of the other one, except in 2006. It is possible the climatic condition during the different years of the analyzed period to influence the emergence and development of each of the two pathogens. It was shown by other authors that the BS has cyclic peaks and it is observed when frequent wind-driven rainfalls and high temperature are present (Sahin and Miller, 1998; Mitrev, 2001; Obradovic et al., 2004; Bogatzevska et al., 2007). The two pathogens belonged to the pathotypes – P and PT. The latter was significantly more abundant for *X. vesicatoria*, while among *X. euvesicatoria* strains slight prevalence of P type was recorded. Races of 43% of *X. vesicatoria* and 52% of *X. euvesicatoria* strains, belonging to the two types were determined. In both species there was a significant diversity of races, especially in PT strains, among which 16 races were identified for the *X. euvesicatoria* species and 9 for the *X. vesicatoria* species. Largest number of *X. euvesicatoria* strains (25% of all the PT strains) belonged to the race P4T2. The largest variety of races during the analyzed period was found in WB (nine races), followed by SB regions (seven races). The greatest race diversity was found in 2016, when eight races were identified. Six of these races (P0T1, P0T3, P1T1, P1T3, P3T2, and P5T2) have not been detected in previous years. On the other hand, it is interesting to note that the races P2T2 and P5T3, which were established for strains isolated in 2000 and 2006, respectively, were not found afterwards. The majority of the analyzed P strains of *X. euvesicatoria* were isolated after 2011, and distributed into eight races. The P6 race, which was found in all the studied regions in Bulgaria, was dominant. Approximately 50% of the studied P strains related to it. The diversity of the races was greater among the strains isolated from NEB region, but it should be kept in mind that their number is higher than those isolated from the other regions. It should be noted that the four analyzed strains from SB belonged to three races. This region, as well as the NEB, is among the largest production areas of pepper in Bulgaria. Similar diversity of *X. euvesicatoria* races was found in Serbia in 2008, which is a neighboring country (Ignjatov, 2010). The authors have identified four races, two of which we also found in Bulgaria. Unlike Bulgaria, the race P8 was dominant among the Serbian strains of *X. euveicatsoria*.

The relatively small number of analyzed *X. vesicatoria* pepper strains does not allow comments to be made, but there was an obvious diversity of races, particularly in the PT strains.

Conclusion

The analysis of the results from the research on the causative agents of BS on tomato and pepper during the period 1999-2016 allows us to make some conclusions. BS on tomato was caused by three species (*X. vesicatoria*, *X. gardneri,* and *X. euvesicatoria*), and on pepper – by two species (*X. euvesicatoria* and *X. vesicatoria*). With long history as pathogen on both hosts in Bulgaria is the species *X. vesicatoria*, which was identified at the beginning of the analyzed period. This species was a dominant pathogen on tomato, whereas on pepper dominated the *X. euvesicatoria* species. In the occurrence and spread of the disease, cyclic peaks were observed. From 2001 to 2005, as well as in 2008 and 2011, BS was not found in tomato plants. It is possible that

climatic conditions during those years to be one of the reasons for its absence. Two new for Bulgaria BS pathogens on tomato were identified – *X. gardneri* and *X. euvesicatoria*. A main agent causing BS in pepper was the species *X. euvesicatoria*. Among the three species strains that can infect both hosts were identified, which is very important from epidemiological and agrotechnical point of view. The presence of significant diversity in terms of races was characteristic especially for the species *X. vesicatoria* (PT type) and *X. euvesicatoria* (PT and P types). The occurrence of new races over the past year was registered, replacing those established at the beginning of the observed period. This indicates that the pathogenic population responsible for BS on tomato and pepper is dynamic and needs to be regularly monitored and studied, which is important to control the disease.

References

Areas, S. M., Gonçalves, R. M., Soman, J. M., Sakate, K. R., Gioria, R., da Silva Jr., T. A. F. & Maringoni, A. C. (2015). Prevalence of *Xanthomonas euvesicatoria* on pepper in Brazil. *Journal of Phytopathology*, *163 (11-12),* 1050–1054.

Barak, J. D., Vancheva, T., Lefeuvre, P., Jones, J. B., Timilsina, S., Minsavage, G. V., Vallad, G. E. & Koebnik, R. (2016). Whole-genome sequences of *Xanthomonas euvesicatoria* strains clarify taxonomy and reveal a stepwise erosion of Type 3 Effectors. *Frontiers in Plant Science*, *7,*1805. doi: 10.3389/fpls.2016.01805

Bogatzevska, N. & Sotirova, V. (1992). Occurrence of two pathotypes of *Xanthomonas campestris* pv. *vesicatoria* on tomato in Bulgaria. *Report of the Tomato Genetics and Cooperative*, *42,* 11-12.

Bogatzevska, N. & Sotirova, V. (2001-2002). Bacterial spot of tomato in Bulgaria: pathotypes and races. *Genetics and Breeding*, *31 (1-2),* 59-66.

Bogatzevska, N., Stoimenova, E. & Mitrev, S. (2007). Bacterial and virus diseases spread in Bulgaria and Macedonia on field and greenhouse pepper. *Plant Protection Skopje*, *18,* 17-21.

Bouzar, H., Jones, J. B., Minsavage, G. V., Stall, R. E. & Scott, J. W. (1994a). Proteins unique to phenotypically distinct groups of *Xanthomonas campestris* pv. *vesicatoria* revealed by silver staining. *Phytopathology*, *84 (1),* 39–44.

Bouzar, H., Jones, J. B., Stall, R. E., Hodge, N. C., Minsavage, G. V., Benedict, A. A. & Alvarez, A. M. (1994b). Physiological, chemical, serological and pathogenic analysis of a worldwide collection of *Xanthomonas campestris* pv. *vesicatoria* strains. *Phytopathology*, *84 (7),* 663-671.

Hert, A. P., Roberts, P. D., Momol, M T., Minsavage, G. V., Tudor-Nelson, S. M. & Jones, J. B. (2005). Relative importance of bacteriocin-like genes in antagonism of *Xanthomonas perforans* tomato race 3 to *Xanthomonas euvesicatoria* tomato race 1 strains. *Applied and Environmental Microbiology, 71 (7),* 3581–3588.

Ignjatov, M., Gašic, K., Ivanovic, M., Ševic, M., Obradovic, A & Miloševic, M. (2010). Characterisation of *Xanthomonas euvesicatoria* strains pathogens of pepper in Serbia. *Pesticidi i Fitomedicina, 25 (2),* 139–149 (Sb).

Jones, J. B., Lacy, G. H., Bouzar, H., Minsavage, G. V., Stall, R. E. & Schaad, N. W. (2004a). Reclassification of the *Xanthomonas* associated with bacterial spot disease of tomato and pepper. *Systematic and Applied Microbiology*, *27 (6),* 755–762.

Jones, J. B., Lacy, G. H., Bouzar, H., Stall, R. E. & Schaad, N. W. (2004b). Bacterial spot – Worldwide distribution, importance and review. In: M. T. Mommol, P. Ji and J. B. Jones (eds.), Proceeding 1st International Symposium on Tomato Disease, 21-24 June, 2004, Orlando, Florida, USA. *Acta Horticulturae, 695,* 27-34.

Jones, J., Stall, R., Scott, J., Somodi, G., Bouzar, H. & Hodge, N. (1995). A third tomato race of *Xanthomonas campestris* pv. *vesicatoria*. *Plant Disease*, *79 (4),* 395– 398.

Karov, S. (1965). *Xanthomonas vesicatoria* (Doidge) Dowson of pepper. Scientific Publishing, High Agricultural Institute, Plovdiv, Bulgaria, *14,* 245-250.

Kebede, M., Timilsina, S., Ayalew, A., Admassu, B., Potnis, N., Minsavage, G. V., Goss, E. M., Hong, J. C., Strayer, A., Paret, M., Jones, J. B. & Vallad, G. (2014). Molecular characterization of *Xanthomonas* strains responsible for bacterial spot of tomato in Ethiopia. *European Journal of Plant Pathology*, *140 (4),* 677–688.

Kizheva, Y., Vancheva, T., Hristova, P., Stoyanova, M., Bogatzevska, N. & Moncheva, P. (2011). Diversity of *Xanthomonas* Spp. causal agents of bacterial spot on pepper and tomato plants in Bulgaria. *Biotechnology and Biotechnological Equipment, 25 (Suppl. 4),* 98-104.

Kizheva, Y., Vancheva, T., Hristova, P., Stoyanova, M., Stojanovska, M., Moncheva, P. & Bogatzevska, N. (2013). Identification of *Xanthomonas* strains from tomato and pepper and their sensitivity to antibiotics and copper. *Bulgarian Journal of Agricultural Science*, *19 (2),* 80–82.

Kizheva, Y., Vancheva, T., Stoyanova, M., Bogatzevska, N., Moncheva, P. & Hristova, P. (2016). 16S-23S ITS rDNA PCR-RFLP Approach as a tool for identification and differentiation of bacterial spot-causing *Xanthomonas*. *Journal of Plant Pathology*, *98 (3),* 645-649.

Kizheva, Y., Urshev, Z., Rasheva, I., Vancheva, T., Hristova, P., Bogatzevska, N. & Moncheva, P. (2017). PFGE: a tool for examination of heterogeneity between the bacterial spot-causing xanthomonads of tomato plant in Bulgaria. *Zeitshrift für Naturforshung C, 73 (7-8),* 257-264.

Kovachevski, I. (1936). New parasitic fungi in Bulgaria. *Trudove na Balgarskoto Prirodoizpitvatelno Druzhestvo*, *7,* 13–24 (Bg).

Kyeon, M. S., Son, S. H., Noh, Y. H., Kim, Y. E., Lee, H. I. & Cha, J. S. (2016). *Xanthomonas euvesicatoria* causes bacterial spot disease on pepper plant in Korea. *Plant Pathology Journal*, *32 (5),* 431–440.

Ma, X., Lewis Ivey, L. & Miller, S. (2011). First report of *Xanthomonas gardneri* causing bacterial spot of tomato in Ohio and Michigan. *Plant Disease*, *95 (12),* 1584.

Minsavage, G. V., Dahlbeck, D., Whalen, M. C., Kearney, B., Bonas, U., Staskawicz, B. J. & Stall, R. E. (1990). Gene-for-gene relationships specifying disease resistance in *Xanthomo-*

nas campestris pv. *vesicatoria*-pepper interactions. *Molecular Plant-Microbe Interactions, 3 (1),* 41–47.

Mitrev, S. (2001). Phytopathogenic bacteria on pepper in Macedonia, Working paper, PSI Institute of Southern Crops, Strumica, 90 pp. (Mc).

Obradovic, A., Mavridis, A., Rudolph, K., Janse, J. D., Arsemijevic, M., Jones, J. B., Minsavage, G. V. & Wang, **J. F.** (2004). Characterization of PCR-based typing of *Xanthomonas campestris* pv. *vesicatoria* from peppers and tomatoes in Serbia. *European Journal of Plant Pathology*, *110 (3),* 285–292.

O'Garro, L.W. & Tudor, S. (1994). Contribution of four races of Xanthomonas campestris pv. vesicatoria to bacterial spot in Barbados. *Plant Disease*, *78 (1),* 88–90.

O'Garro, L. W., Gore, J. P. & Ferguson, **E.** (1999). Races of *Xanthomonas campestris* pv. *vesicatoria* overcoming the gene Bs2 for bacterial spot resistance in pepper, prevalent on *Capsicum chinense* in Barbados and Grenada and weakly pathogenic on bell pepper an tomato in the field. *Plant Pathology*, *48 (5),* 588-594.

Potnis, N., Timilsina, S., Strayer, A., Shantharaj, D., Barak, J. D., Paret, M. L., Vallad, G. E. & Jones, **J. B.** (2015). Bacterial spot of tomato and pepper: diverse *Xanthomonas* species with a wide variety of virulence factors posing a worldwide challenge. *Molecular Plant Pathology*, *16 (9),* 907–920.

Quezado-Duval, A. M., Leite, R. P., Truffi, D. & Camargo, **L. E. A.** (2004). Outbreaks of bacterial spot caused by *Xanthomonas gardneri* on processing tomato in central-west Brazil. *Plant Disease*, *88 (2),* 157-161.

Sahin, F. & Miller, **S. A. (**1998). Resistance in Capsicum pubescens to *Xanthomonas campestris* pv. *vesicatoria* pepper race 6. *Plant Disease*, *82 (7)*, 794–799.

Schwartz, A. R., Potnis, N., Timilsina, S., Wilson, M., Patané, J., Martins, Jr. J., G. V. Minsavage, G. V., Dahlbeck, D., Akhunova, A., Almeida, N., Vallad, G. E., Barak, J. D., White, F. F., Miller, S. A., Ritchie, D., Goss, E., Bart, R. S., Setubal, J. C., Jones, J. B. & Staskawicz, **B. J.** (2015). Phylogenomics of *Xanthomonas* field strains infecting pepper and tomato reveals diversity in effector repertoires and identifies determinants of host specificity. *Frontiers in Microbiology*, *6 (Article 535),* 1-17. doi:10.3389/fmicb.2015.00535

Stall, R. E., Jones, J. B. & Minsavage, **G. V.** (2009). Durability of resistance in tomato and pepper to xanthomonads causing bacterial spot. *Annual Review of Phytopathology*, *47 (1),* 265–284.

Stoyanova, M., Vancheva, T., Moncheva, P. & Bogatzevska, **N.** (2014). Differentiation of *Xanthomonas* spp. causing bacterial spot in Bulgaria based on Biolog System. *International Journal of Microbiology*, 7-0, doi:http://dx.doi.org/10.1155/2014/495476.

Timilsina, S., Jibrin, M. O., Potnis, N., Minsavage, G. V., Kebede, M., Schwartz, A., Bart, R., Staskawicz, B., Boyer, C., Vallad, G. V., Pruvost, O., Jones, J. B. & Goss, **E. M.** (2015). Multilocus sequence analysis of xanthomonads causing bacterial spot of tomato and pepper plants reveals strains generated by recombination among species and recent global spread of *Xanthomonas gardneri*. *Applied and Environmental Microbiology*, *81 (4),* 1520–1529.

Timilsina, S., Kara, S., Jacques, M. A., Potnis, N., Minsavage, G. V., Vallad, G. E., Jones, J. B. & Fischer-Le Saux, M. (2018). Reclassification of *Xanthomonas gardneri* (ex Šutič 1957) Jones et al. 2006 as a later heterotypic synonym of *Xanthomonas cynarae* (Trébaol et al., 2000) and description of *X. cynarae* pv. *cynarae* and *X. cynarae* pv. *gardneri* based on whole genome analyses. *International Journal of Systematic and Evolutionary Microbiology*, DOI:10.1099/ijsem.0.003104.

Tudor-Nelson, S. M., Minsavage, G. V., Stall, R. E. & Jones, **J. B.** (2003). Bacteriocin-like substances from tomato Race 3 strains of *Xanthomonas campestris* pv. *vesicatoria*. *Phytopathology*, *93 (11),* 1415–1421.

Vancheva, T., Stoyanova, M., Tasheva-Terzieva, E., Bogatzevska, N. & Moncheva, **P.** (2018). Molecular methods for diversity assessment among xanthomonads of Bulgarian and Macedonian pepper. *Brazilian Journal of Microbiology*, *49 (Suppl.1),* 246-259.

Vancheva, T., Stoyanova, M., Tatyozova, M., Bogatsevska, N. & Moncheva, **P.** (2014). Sub-species diversity of *Xanthomonas euvesicatoria* Bulgarian and Macedonian strains from pepper. *Biotechnology and Biotechnological Equipment*, *28 (4),* 592–601.

Ward, H. P. & O'Garro, **L. W.** (1992). Bacterial spot of pepper and tomato in Barbados. *Plant Disease*, *76 (10),* 1046–1048.

Received: May, 2019; *Accepted*: August, 16, 2019; *Published:* February, 28, 2021