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MONITORING OF THE HARMFUL ORGANISM *CLAVIBACTER MICHIGANENSIS* SUBSP. *MICHIGANENSIS* IN ROMANIA, BETWEEN 2011-2018

Mariana-Simona Stancu [1*], Rodi Mitrea [1]

[1] University of Craiova, Faculty of Horticulture, A.I. Cuza street, no.13, Craiova, Dolj, Romania

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Abstract
Among the phytopathogenic bacteria in plants it is also Clavibacter michiganensis subsp. michiganensis, harmful organism present in most tomato growing regions. The establishment of the dissemination area is carried out by starting monitoring programs that extend over several years. In Romania, such a program has been carried out since 2011. In order to establish the contamination with this bacteria, there are not enough phytosanitary controls carried out during the vegetation period. It is compulsory to carry out laboratory tests to establish precisely the presence or absence of this harmful organism in tomato crops. Thus, the counties in which the tomato crops are contaminated with the bacteria of interest could be determined and it could be taken the phytosanitary measures of prevention and control.

Keywords: area, contamination, monitoring.

1. INTRODUCTION

Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) which produces "bacterial canker of tomato/vascular wilt of tomato" was a harmful quarantine organism until 2019, and was regulated at national level by "Government Decision no. 563/2007 for the approval of the methodological norms for applying the Government of the Ordinance no. 136/2000 regarding the protective measures against the introduction and spread of quarantine organisms for the supply of plants or plant products in Romania" and at European level by "Directive 2000/29/ EC on protection measures against the introduction into the community of plant or plant organisms and their liability in the community". Since the beginning of this year it is a non-quarantine regulated harmful organism, according to the "Commission implementing regulation (EU) 2019/2072 stablishing uniform conditions for the implementation of Regulation (EU) 2016/2031 of the European Parliament and the Council, as regards protective measures against pests of plants, and repealing Commission Regulation (EC) No 690/2008 and amending Commission Implementing Regulation (EU) 2018/2019".

Clavibacter michiganensis subsp. *michiganensis* attacks not only *Solanum lycopersicum* plants but also other solanaceae and non-solanaceae plants, being widespread throughout the world (figure 1). Every year appear new outbreaks of the disease which leading to substantial damage due to the decrease of the production of tomatoes and the deterioration of the quality and appearance of the fruits, thus becoming unfit for marketing (Rădulescu et al., 1970).

*Corresponding author, E-mail address: simosebabia@gmail.com

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Table 1. Distribution by continent and region of Clavibacter michiganensis subsp. michiganensis (modified by: https://gd.eppo.int/taxon/CORBMI/distribution)

Continent	Country/Region	Distribution
Africa	South Africa	Present, widespread
	Egipt, Kenya, Madagascar, Togo, Uganda, Zambia	Present, no details
	Marocco, Tanzania, Tunisia, Zimbabwe	Present, restricted distribution
	Democratic Republic of the Congo	Absent, unreliable record
	Algeria	Absent, invalid record
Asia	Israel	Present, widespread
	China, Iran, Japain, Lebanon	Present, no details
	India, Indonesia, Republic Korea, Syria	Present, restricted distribution
	Tawain, Thailand, Vietnam	Absent, unreliable record
	Uzbekistan	Absent, pest eradicated
North America	Canada, USA	Present, widespread
	Mexico	Present, restricted distribution
America Centrală	Belize, Costa Rica, Cuba, Dominica, Grenada, Guadelupe, Panama, Dominican Republic	Present, no details
	Martinique	Absent, unreliable record
South America	Uruguay	Present, widespread
	Argentina, Brazil, Columbia, Peru	Present, no details
	Chile, Ecuador	Present, restricted distribution
Europe	Greece, Russia, Switzerland, Turkey	Present, widespread
	Armenia, Azerbaijan, Belarus	Present, no detail
	Czech Republic, Italy, Poland, Portugal, Spain	Prezent, few occurences
	Bulgaria, Cyprus, France, Germany, Hungary, Latvia, **Romania**, Serbia, Ukraine	Present, restricted distribution
	Belgium, Jersey, United Kingdom	Absent, pest no longer present
	Slovakia	Absent, intercepted only
	Finland	Absent, no pest record
	Austria, Ireland, Lithuania, Norway, Sweden	Absent, pest eradicated
	Netherlands, Slovenia, Spain (Islas Canarias)	Transient, under eradication
Oceania	Australia	Present, widespread
	Fiji, Guam, New Caledonia, Tonga	Present, no details
	New Zealand	Present, restricted distribution

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According to EPPO Global databses, this bacteria is present on most continents, namely: Asia, Africa, America, Europe and Oceania. The detailed list by country distribution is shown in table 1.
Clavibacter michiganensis subsp. *michiganensis* was first reported and isolated, in the early 20th century (1909-1910), by Smith, in the greenhouses of Grand Rapids, Michigan, USA, North America. (De León et al., 2011; Hausbeck et al., 2000).

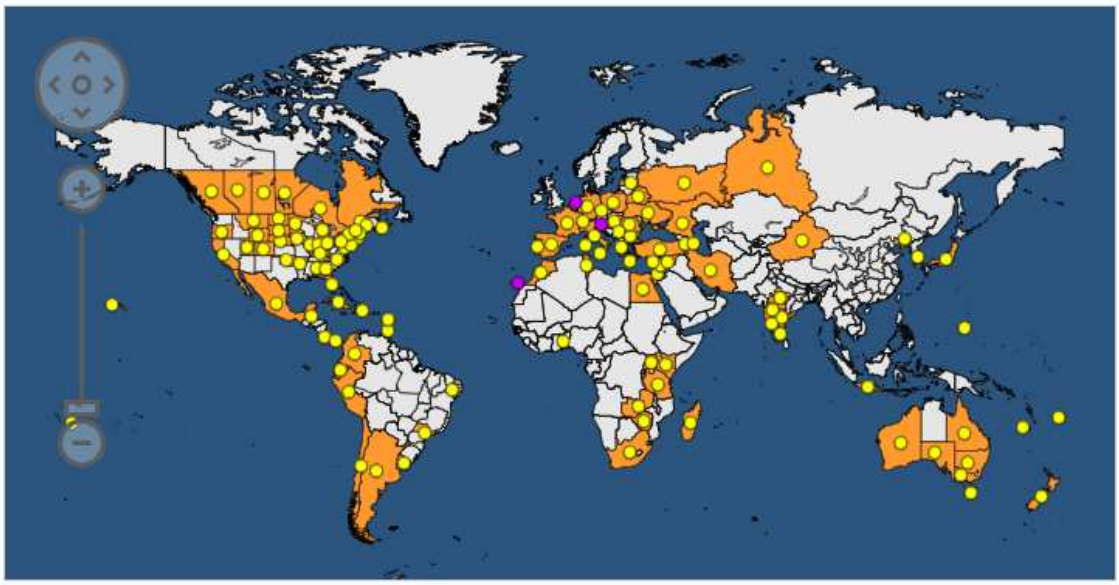

Figure 1. The geographical spread of Clavibacter michiganensis subsp. michiganensis (https://gd.eppo.int/taxon/CORBMI/distribution)
present; transient

In South America, it was first reported in 1958, in Brazil (Fatmi et al., 2017) and in 1983 in Chile. However, in the Azapa Valley area (from the Arica-Parinacota region - Chile), *Clavibacter michiganensis* subsp. *michiganensis* was not reported before 2012. It is assumed that the bacteria was introduced into this region with the infected seedlings (Chavera et al., 2013). Studies conducted in the greenhouses in the Valparaíso region showed that they were affected by 70.2% (Valenzuela et al., 2018).
In Oceania, the first report of this bacteria was in Australia, in 1925, then in New Zealand, in 1938 and in Africa, the first reporting was in 1942, in Moroc (Fatmi et al., 2017).
On the Asian continent, *Clavibacter michiganensis* subsp. *michiganensis* was first reported in Turkey, in 1950 (Fatmi et al., 2017). In 2001, this bacteria was introduced with tomato seeds or seedlings (Target cultivar) in Anatolia (Asian part of Turkey) (Şahin et al., 2002). In 2003, ”Vascular wilt of tomato” also appears in greenhouses in the western Mediterranean region of Turkey, due to non-application of bactericides and relatively high humidity (Basim et al., 2004).
In 1963, this bacteria appeared in Israel, initially sporadically, then in 2000 a severe epidemic broke out, in the south of the country, in the most important tomato growing area. Finally it spreads both in the Gaza Strip and in the north of the country (Kleitman et al. 2007).
In Iran, the first reporting took place in 1988 (https://gd.eppo.int/taxon/CORBMI/distribution), and in Cyprus, in June 1998, in the Limassol district (in the Eptagonia mountain region) (Ioannou et al., 2000).

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Then, in 2002, *Clavibacter michiganensis* subsp. *michiganensis* was detected in two batches of tomato seeds on the island of Java in Indonesia (Anwar et al., 2004), and in 2007 it first appeared in Korea, in greenhouses where cherry tomatoes were grown (Myung et al., 2008). Also, in the same year, in 2007, in Syria, the first detailed evidence of this disease appeared. In the greenhouses contaminated with *Clavibacter michiganensis* subsp. *michiganensis*, from this country, the incidence of the disease was 15% in spring of 2007, rising to 70% at the end of July (Ftayeh et al. 2008).
In the center and northwest of the Russian Federation, between 2011-2017, severe outbreaks of the disease appeared, not only in tomato culture but also in potato culture. Laboratory tests revealed that the infection was caused by the bacteria mentioned above, being isolated from both plants and potato tubers (Ignatov et al., 2018).
In northern India (Himachal Pradesh state) this pathogen was detected not only on species of *Lycopersicon* and *Capsicum*, but also on species of *Solanum douglasi*, *S. nigrum*, *S. mammosum* and *Nicotiana glutinosa* (Singh and Bharat, 2017).
In Europe, Italy, in 1914, was the first country to report the presence of *Clavibacter michiganensis* subsp. *michiganensis* (https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2014.3721). In July 2010, in the province of Viterbo, from the central of Italy, severe outbreaks of the disease occurred in sixteen fields. It has been established that the incidence of the disease was between 70 and 100% and that the hybrid Uno Rosso was the most sensitive of the studied varieties. (Lamichhane et al., 2011).
Also, there were first reports of *Clavibacter michiganensis* subsp. *michiganensis* also in Denmark (1922), Lithuania (1930), and Hungary (1959) (Fatmi et al., 2017; https://gd.eppo.int/taxon/CORBMI/distribution).
Sutic first described this bacteria in Serbia, in 1957. For more than 50 years, it did not represent a serious threat to tomato production. Between 2006-2007, there was extensive surveillance of *Clavibacter michiganensis* subsp. *michiganensis*, in greenhouse and field tomato crops, from Leskovac, Lebane, Padinska Skela, Sabac, Trstenic, Ub, Stara Pazova and Cacak. It has been found that in the field, the incidence of the disease can reach up to 100%, and in tomato greenhouses, it varies between 2 and 50% (Milijaŝević et al., 2009).
Regarding the first report of this bacteria in Romania, it was done by Elena Bucur, in 1955, in Ilfov county (Marinescu et al., 1986; Rădulescu et al., 1970). In 1970, there was a devastating attack, with losses of over 90%, in the greenhouses of our country. Bacteriologists from the Institute of Plant Protection from Bucharest have established a set of measures for the protection of tomato plants, which have led to decreasing losses in the following years. Also, they in collaboration with the researchers from the Research Station Işalnița initiated a system of thermal disinfection of tomato seeds (Severin and Iliescu, 2006).

2. MATERIALS AND METHODS

In 2011, in Romania, the first monitoring program was started which was named “Monitoring plan for quarantine organisms *Clavibacter michiganensis* subsp. *michiganensis* and *Xanthomonas campestris* pv. *vesicatoria*”. Then, in 2016, this plan was extended to other harmful organisms in tomato and pepper crops, becoming "Program for monitoring quarantine viruses in tomatoes and quarantine bacteria in tomatoes and peppers" (https://www.madr.ro/docs/fitosanitar/programe-monitorizare/pm-virusi-carantina-tomate-ardei-update-22.11.2016.pdf).
The main objectives of this monitoring program are:

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- verification of the conformity of the plants intended for planting with the specific requirements, in order to issue the phytosanitary passport;
- updating the spread of the target organisms;
- the eradication of the harmful organisms detected or the prevention of their spread, when eradication is not possible;
- checking the health status of seeds and plants intended for planting, which come from intra-Community circulation or from import.

Under this program, the phytosanitary inspectors, from the County Phytosanitary Offices, have the obligation to inspect, not only the commercial crops and the seed crops of tomatoes and peppers, during the vegetation period, but also the planting material and the seeds, from the internal production. The planting material and seeds, coming from the intra-Community circulation and from the import, are controlled either by the phytosanitary inspectors from the Border Inspections Points or either by the inspectors from the County Phytosanitary Offices. Both services belong to the Phytosanitary Direction of the National Phytosanitary Authority.

According to this monitoring program, the inspection is performed on both diagonals of the culture. Depending on the surface of the culture, a number of control points are established, as follows: areas up to 15 hectares are controlled in 5 points, and the areas over 15 hectares are controlled in 10 points. The number of plants examined at each control point is 200. As for the control in greenhouses, this is done according to the size of the greenhouse, the percentage of plants examined being 1-4%.

If during the inspections carried out during the vegetation period, plants with characteristic symptoms of the disease are observed, they are collected and sent for laboratory analysis. Each County Phytosanitary Office determines the number of samples it will collect send for analysis. This thing depends on: the number of registered producers, the phytosanitary status of the host plants and the symptoms observed during the inspection.

If the inspection takes place at the seed material, sampling is required (10000 seeds or 10 seedlings) according to the monitoring program.

All inspections will be recorded in a form ("Inspection sheet", "Phytosanitary passport inspection sheet" or "Import phytosanitary inspection sheet"), as the case may be (https://www.madr.ro/docs/fitosanitar/programe-monitorizare/pm-virusi-carantina-tomate-ardei-update-22.11.2016.pdf).

All the samples collected by the phytosanitary inspectors are packaged appropriately, to avoid contamination between the samples and sent, as soon as possible, to avoid deterioration, to the National Phytosanitary Laboratory of the National Phytosanitary Authority. The samples sent to the laboratory are necessarily coded and accompanied by a request for analysis. Once arrived at the destination, the samples are taken by an operator from the "Reception of samples" and they receive a laboratory code. According to the requested pest organism, the samples are taken from the bacteriology and/or virology laboratory. If the samples are not analysed as soon as they arrive, they are kept at room temperature (in the case of seeds) or in the refrigerator (in the case of plant material). After performing the bacteriological and/or virological analyses and determining whether the analysed sample is infected with one or more harmful organisms concerned, a “Laboratory report” is issued, which is sent to the County Phytosanitary Offices/Border Inspections Points, from where the sample was sent. After receiving the results of analyses, the phytosanitary inspectors have the obligation to inform the beneficiary of the test about the result of the analysis.

At the end of the year, the phytosanitary inspectors have the obligation to complete " The evaluation sheet of the situation regarding the quarantine viruses in tomatoes and the quarantine bacteria in

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tomatoes and peppers" and send it to the national rapporteur (the person designated by the National Phytosanitary Authority with centralization data). The national rapporteur draws up a report which it sends to the National Phytosanitary Authority.
During this monitoring we analyzed the samples received from phytosanitary inspectors, establishing whether the samples received are contaminated or not with *Clavibacter michiganensis* subsp. *michiganensis* and also, at the end of each monitoring year, we centralized the data received from the inspectors, drafting a report about bacteria and viruses monitoring at tomatoes and peppers.

3. RESULTS AND DISCUSSIONS

If following the laboratory analyses, at the plants covered by this monitoring program, at least one sample is contaminated with one or more monitored organisms, the parcel or lot is declared contaminated and are subject to plant protection measures.
In such situations, the phytosanitary inspectors have the obligation to write "Notification of measures" and to send it to the beneficiary. They estabilish the phytosanitary measures that the producer is obliged to apply in order to eradicate and prevent the spread of the harmful organism detected. Phytosanitary inspectors also draw up an " Information regarding the investigation carried out in order to establish the path of introduction of the harmful organism". The two above mentioned documents are transmitted, within a maximum three working days, to the Phytosanitary Direction of the National Phytosanitary Authority (https://www.madr.ro/docs/fitosanitar /programe-monitorizare/pm-virusi-carantina-tomate-ardei-update-22.11.2016.pdf).
According to the "Guide for the recognition and control of diseases and pests in tomatoes grown in protected areas (greenhouses, indoor protected area and tunnels)", drafted by the National Phytosanitary Authority, the phytosanitary measures to be applied in the situation of detection of *Clavibacter michiganensis* subsp. *michiganensis* are: preventive measures (use of certified planting material; performing thermal and chemical treatments on the seed material; appropriate seed extraction; use of resistant varieties; crop rotation; drip irrigation; weed control; removal of attacked plants and surrounding plants; destruction by burning of vegetal debris; treatment of soil with copper products; application of appropriate decontamination measures) and curative measures (chemical control with: Curenox 50, Bordeaux juice, "MIF", Super Cupertine and Alcupral 50 PU) (http://www.anfdf.ro/sanatate/ghid/ghid_tomate_2019.pdf).
Regarding the situation of the harmful organism *Clavibacter michiganensis* subsp. *michiganensis*, in Romania, following the centralization of the data received from the phytosanitary inspectors, between the years 2011-2018, has been established:

- the annual number of samples of *Solanum lycopersicum* collected and analyzed for the detection of *Clavibacter michiganensis* subsp. *michiganensis* (table 2);
- the annual number of the samples contaminated with *Clavibacter michiganensis* subsp. *michiganensis* (table 3);
- the area of *Solanum lycopersicum* monitored/inspected/contaminated with this bacteria (table 4);
- the surface of *Solanum lycopersicum* contaminated with *Clavibacter michiganensis* subsp. *michiganensis*, by county (table 5);

After centralizing the data, we were able to draw up maps with the counties in Romania where the target bacterium was detected (figure 2).

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During this period, in addition to the samples of the plants/part of plants of *Solanum lycopersicum*, contaminated with *Clavibacter michiganensis* subsp. *michiganensis*, additionally were found contaminated 3 samples of tomato seedlings: 1 sample from Bacău (domestic production, 2015) and 2 samples from Hunedoara (intra-Community circulation/import, 2016). Aso, there were found infected with *Clavibacter michiganensis* subsp. *michiganensis*, 11 samples (2 Buzau, 2 Iasi, 7 Ilfov) of tomato plants from the beneficiaries, in 2011 and 2 samples (1 Gorj, 1 Suceava) from the the territory surveillance, in 2014.

Table 2. The annual number of samples of Solanum lycopersicum analyzed for the detection of Cmm

Year of monitoring	Samples from internal production		Samples from County Phytosanitary Offices (intra-Community circulation)	Samples from Border Inspections Points (intra-Community circulation/import)	The total number of the samples
	Plants/part of plants	Seeds			
2011	107	32	28	86	253
2012	80	24	42	59	205
2013	82	26	71	59	238
2014	61	11	58	37	167
2015	66	21	45	31	163
2016	66	27	55	39	187
2017	87	21	31	25	164
2018	117	26	37	46	226

Table 3. The annual number of the samples contaminated with Cmm

Year of monitoring	The total number of the samples of plants/part of plants contaminated with *Cmm*	The total number of the samples of plants/part of plants contaminated with *Cmm / county*
2011	42	1 Alba, 1 Arad, 7 Argeş, 1 Constanța, 13 Dolj, 5 Galați, 2 Harghita, 1 Hunedoara, 2 Olt, 2 Prahova, 5 Sălaj, 2 Vâlcea
2012	21	1 Alba, 1 Arad, 3 Argeş, 7 Dolj, 1 Iași, 2 Olt, 5 Prahova, 1 Suceava
2013	6	1 Cluj, 3 Olt, 2 Prahova
2014	11	3 Argeş, 1 Harghita, 3 Olt, 1 Prahova, 2 Suceava, 1 Sălaj
2015	5	1 Gorj, 1 Harghita, 1 Ilfov, 2 Suceava
2016	7	1 Ilfov, 2 Prahova, 4 Dolj
2017	6	1 Galați, 1 Hunedoara, 1 Iași, 1 Olt, 1 Mehedinți, 1 Vâlcea
2018	8	1 Bacău, 3 Argeş, 1 Harghita, 2 Olt, 1 Vrancea

After analyzing the aforementioned data, regarding the number of samples from the units served to take samples, it was found that the largest number of samples of *Solanum lycopersicum* comes from domestic production, compared to the number of samples from import and intra-Community circulation (figure 3). Also, although the number of samples varied from year to year, the number of cases contaminated with *Clavibacter michiganensis* subsp. *michiganensis* was initially large, then decreased to eventually remain relatively constant (figure 4).

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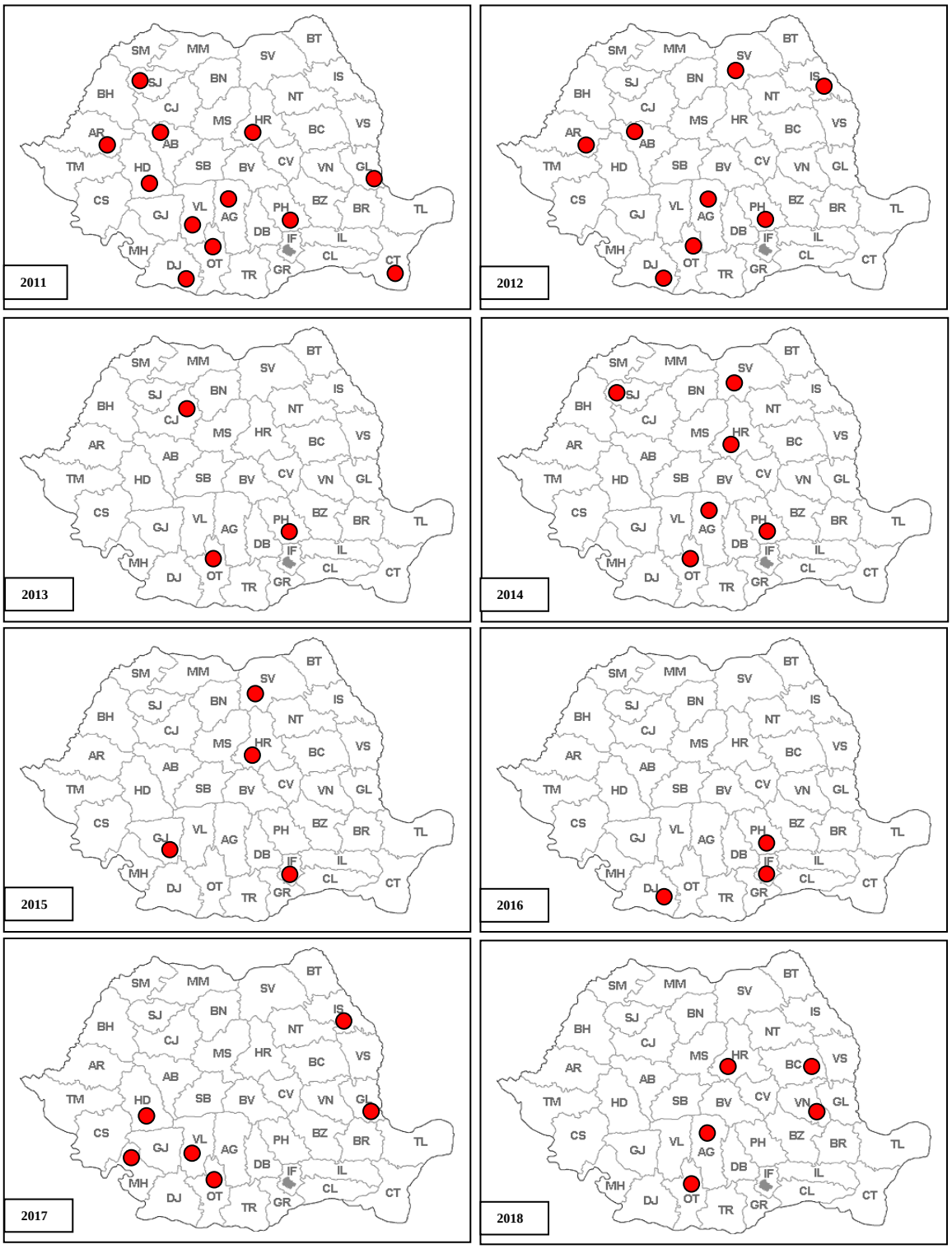

Figure 2 Counties contaminated with Cmm (2011-2018)
contaminated

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Table 4. The area of Solanum lycopersicum monitored/inspected/contaminated with Cmm

Year of monitoring	The surface of tomatoes monitored	The inspected surface / the percentage of the inspected surface of the total surface	Surface contaminated with *Cmm* / percentage of contaminated surface of the inspected surface
2011	*37034.230 ha*	*3230.511 ha / 8.72%*	*13.050 ha / 0.40%*
2012	*36344.630 ha*	*2340.380 ha / 6.43%*	*4.150 ha / 0.17%*
2013	*36254.420 ha*	*2211.460 ha / 6.09%*	*0.080 ha / 0.0036%*
2014	*35196.131 ha*	*1847.874 ha / 5.25%*	*0.808 ha / 0.04%*
2015	*34629.245 ha*	*2042.455 ha / 5.89%*	*0.440 ha / 0.02%*
2016	*36374.650 ha*	*1799.780 ha / 4.94%*	*0.860 ha / 0.04%*
2017	*38178.546 ha*	*1354.593 ha / 3.54%*	*0.880 ha / 0.06%*
2018	*39208.555 ha*	*1396.730 ha / 3.56%*	*1.1 ha / 0.07%*

Table 5. Surface of Solanum lycopersicum contaminated with Cmm by county

Year of monitoring	Surface contaminated with *Cmm* / county
2011	Alba - 0.04 ha, Arad - 0.12 ha, Argeş - 7 ha, Constanța - 2 ha, Dolj - 0.95 ha, Galați - 0.15 ha, Harghita - 0.06 ha, Hunedoara - 0.00075 ha, Olt - 0.05 ha, Prahova - 0.04 ha, Sălaj - 0.64 ha, Vâlcea - 2 ha
2012	Alba - 0.4 ha, Arad - 0.5 ha, Argeş - 0.42 ha, Dolj - 1.82 ha, Iaşi - 0.01 ha, Olt - 0.03 ha, Prahova - 0.92 ha, Suceava - 0.05 ha
2013	Cluj - 0.02 ha, Olt - 0.01 ha, Prahova - 0.05 ha
2014	Argeş - 0.6 ha, Harghita - 0.02 ha, Olt - 0.01 ha, Prahova - 0.035 ha, Sălaj - 0.11 ha, Suceava - 0.033 ha
2015	Gorj - 0.2 ha, Harghita - 0.02 ha, Ilfov - 0.2 ha, Suceava - 0.02 ha
2016	Dolj - 0.63 ha, Ilfov - 0.13 ha, Prahova - 0.1 ha
2017	Galați - 0.01 ha, Hunedoara - 0.08 ha, Iaşi - 0.46 ha, Olt - 0.03 ha, Mehedinți - 0.1 ha, Vâlcea - 0.2 ha
2018	Argeş - 0.5 ha, Bacău - 0.14 ha, Harghita - 0.05 ha, Olt - 0.2 ha, Vrancea - 0.21 ha

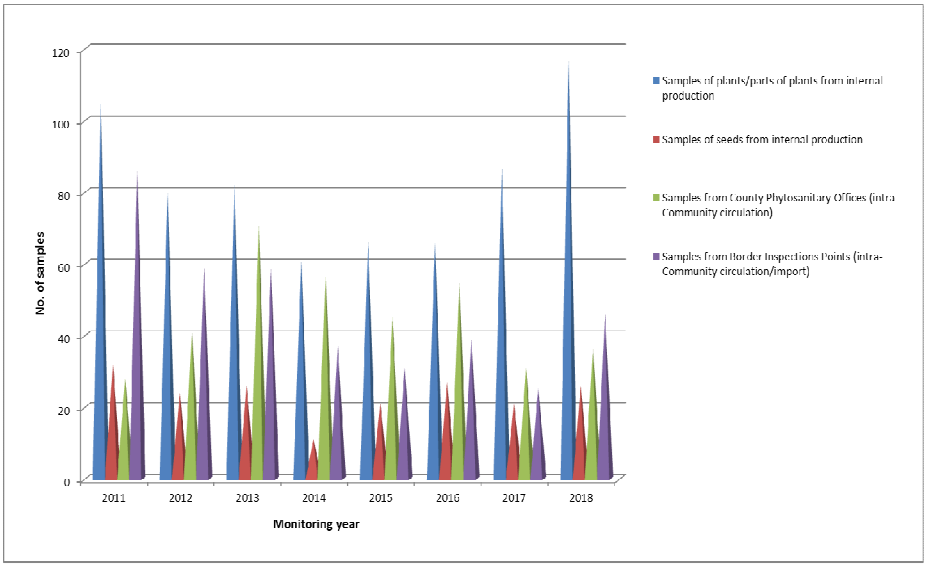

Figure 3. Evolution of the number of samples of Solanum lycopersicum, between 2011-2018

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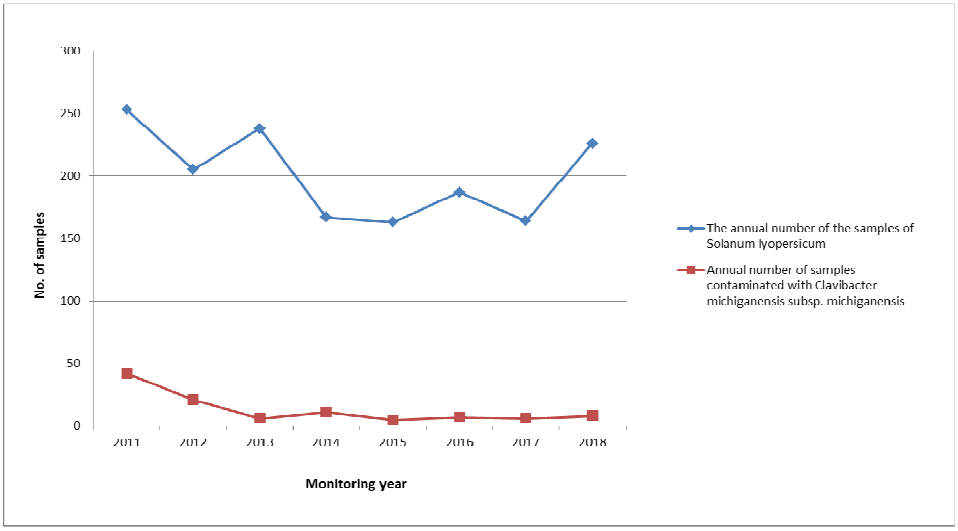

Figure 4. Comparative graph of the evolution of the total number of samples of tomatoes, with the number of samples contaminated with Cmm, between 2011-2018

4. CONCLUSIONS

Between 2011-2018, the number of the samples sent for analysis, by the County Phytosanitary Offices from the territory of Romania and analyzed by the National Phytosanitary Laboratory has varied, being the highest in the first year of monitoring. In the following years, although the surface area of tomato cultivation increased, the surface area inspected decreased, which implicitly led to a decrease in the number of samples collected and analyzed. This thing led to decrease the number of samples contaminated with *Clavibacter michiganensis* subsp. *michiganensis* and implicitly at reducing the surface of tomatoes contaminated with this harmful organism.

The number of positive cases was not constant in the counties either. The number of samples contaminated with this bacteria decreased or was equal to zero in the counties where the control and prevention measures recommended by the phytosanitary legislation were properly applied. In counties where these measures were not properly applied, the number of contaminated samples remained constant or even increased. Therefore, phytosanitary measures have a very important role in increasing or decreasing the number of positive cases.

Given the relatively small number of samples and implicitly the small number of positive samples, it is very likely that, if the number of samples collected and analyzed would increase, the number of samples contaminated with *Clavibacter michiganensis* subsp. *michiganensis* would also increase.

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*Corresponding author, E-mail address: simosebabia@gmail.com

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*Corresponding author, E-mail address: simosebabia@gmail.com