

Genetics and Breeding

Races of bacterial spot pathogen infecting genus *Capsicum* in Bulgaria

K. Vasileva^{1*}, N. Bogatzevska²

¹Maritsa Vegetable Crops Research Institute, 32 Brezovsko Shosse Str., 4003 Plovdiv, Bulgaria

²Institute of Soil Science, Agrotechnologies and Plant Protection "N. Pushkarov", 7 Shosse Bankya Str., 1331 Sofia, Bulgaria

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Abstract. The causative agents of bacterial spot disease infecting pepper are the species *X. euvesicatoria* and *X. vesicatoria* common in the typical pepper growing areas (Northern: Black Sea Coastal and Central; Southwest; Southern - Upper Thracian valley) in Bulgaria. The pathogens refer to pepper P pathotype (22 strains) and pepper-tomato PT pathotype (52 strains). The natural population of *X. euvesicatoria* is heterogeneous of pathotype and physiological races. Widespread is race P6 in the P of the pathogen, followed by race P1. Single strains are assigned to P3, P9 and P10. In PT, races P0, P1, P4, P5 are differentiated, the dominant race in PT is the P4 race in combination with the tomato race T2. For the first time in Bulgaria in the natural population of *X. vesicatoria*, are detected strains which infect only pepper, differentiated are races P0, P2 and P3. The population of *X. vesicatoria* PT prevails in private gardens and vegetable areas near tomatoes. Differentiated races are P1, P3 and P4 in combination with tomato races T1, T2 and T3. Race P3 occurs in P and PT pathotype.

Keywords: pepper races, bacterial spots, spread

Introduction

Bacterial spot of pepper (*Xanthomonas euvesicatoria* and *X. vesicatoria*) has cyclic peaks and it is observed when frequent wind-driven rainfalls and high temperature are present (Jones et al., 1998; Bogatzevska et al., 2007; Stall et al., 2009; Vancheva, 2015). *X. vesicatoria* and *X. euvesicatoria* are phenotypically and genotypically heterogeneous species of the genus *Xanthomonas* and cause spot on the foliage, stems, and fruits on various varietal types and cultivars of field grown pepper (Bogatzevska et al., 2006; Bogatzevska and Pandeva, 2009; Vancheva et al., 2014, 2018). Bacterial spot disease on pepper was recorded for the first time in Bulgaria by Karov (1965) and as a severe disease with a significant economic impact (Bogatzevska et al., 2007; Vancheva, 2015). In the populations of the causative agents of bacterial spot disease on tomato and pepper, three pathotypes are differentiated: tomato (T), pepper (P), and pepper-tomato (PT) (Minsavage et al., 1990; Bogatzevska and Sotirova, 1992; Jones et al., 1998). All tested genotypes of *Capsicum* are resistant to the T pathotype and vice versa, all genotypes of *Solanum* are resistant to the P pathotype. Bacteria of PT are pathogenic to the genus *Capsicum* and *Solanum* (Jones et al., 1998). The study of resistance in the genotypes of tomatoes and pepper to the bacterial spot disease causative agents allows the determination of their racial structure. Race is a term used to classify a subgroup of bacterial strains within a species according to the pattern of HR that they cause or the specific cultivars that a pathogen can infect within a host (Agrios, 2005). Races have no nomenclature value, they are denoted by letters or numbers (Young et al., 1991). Bacterial spot pathogens of peppers are classified into 11 races,

based on the differentiator response: *Capsicum annuum* cv. Early Calwonder (ECW), its near-isogenic lines (ECW 10R, ECW 20R, ECW 30R), and PI235047 (*Capsicum pubescens*). The hypersensitive reaction (HR) of pepper plants is known to be induced by an interaction between the Bs genes in pepper plants and the corresponding effector (avirulence, avrBs) genes in the pathogen in a gene-for-gene model (Minsavage et al., 1990; Stall et al., 2009; Potnis et al., 2015). Cook and Stall (1982) consider that the races of bacterial spot disease causative agents on tomato and pepper have specific and local geographic distribution, their structure is constantly changing in different countries and continents.

In the present work, the results show the differentiation of the races of the established and identified causative agents of bacterial spot disease on pepper (*X. euvesicatoria*, *X. vesicatoria* -P and PT pathotype) spread in the country.

Material and methods

Bacterial strains

A collection of 74 pathogenic bacterial strains, 59 of I and 15 of *X. vesicatoria*, were isolated from leaves, flowers, fruit stalks and fruits of different types of pepper from the regions of Northern Black Sea Coast (Durankulak, Kavarna, Krapets village, Tyulenovo village, Shabla), Central Northern Bulgaria (Byala Cherkva, Veliko Tarnovo, Lovech, Pavlikeni), Southwest Bulgaria (Blagoevgrad, Kostinbrod, Petrich, Petarch village, Trebich village, Sofia - IG, IFRG), South Bulgaria - Upper Thracian valley (Plovdiv - VCRI Maritsa, Sadovo, Plovdiv and Pazardzhik region, Svilengrad, Haskovo, Stara Zagora) during the period 1999-2017 (personal collection of prof. N. Bogatzevska). Pathogens are identified with the Biolog sys-

*e-mail: kkvasileva@abv.bg

tem (Bogatzevska et al., 2007; Stoyanova et al., 2014) and molecular methods (Kizheva et al., 2011, 2014; Vancheva et al., 2014, 2018; Vancheva, 2015).

Differentiation of pathotype and races

Pathotype

The pathotype of the pathogenic strains is identified by test-plants: tomatoes cv. Ideal and pepper cv. Kaliforniisko chudo (Bogatzevska and Sotirova, 2001-2002). Pepper pathotype (P) – the strains are pathogenic only to pepper. Pepper-Tomato pathotype (PT) – the strains are pathogenic to both hosts.

Pepper races of pepper and pepper-tomato pathotypes

Races (P-22; PT-52 strain) were determined based on hypersensitive (HR) and susceptible (Sus) reactions in response to infiltration of the bacterial suspension (10^8 cfu/ml) into leaf tissues, according to the race identification system reported by Kurowski et al. (2015) and Wai et al. (2015). Two leaves were used as replication for each isolate in two replications. The occurrence of HR 24-48h is registered at the injection site on the leaves of resistant differentiator. Watery, dark green to brown, necrotic areas are formed 4-5 days after leaf infiltration of sensitive isogenic lines. Resistant reaction (R) is the absence of HR and the typical signs of bacterial spot on the leaves (Figure 1).

Tomato races of pepper-tomato pathotype

The races' grouping of 52 strains is determined based on Sus and HR on the leaves of the differentiator lines: *Hawaii* 7981, *Hawaii* 7998 and cv. Ideal (Bogatzevska and Sotirova, 2001-2002). HR reaction on the 24-48h after infiltration of the leaves of the test plants is reported. The appearance of necrotic brown spots without halo on the leaves, stalks and stems are observed 4-5 days after infection.

Results and discussion

The causative agents of bacterial spot disease are the species *X. euvesicatoria* and *X. vesicatoria* common in the typical pepper growing areas (Northern: Black Sea Coastal and Central; Southwest; Southern - Upper Thracian valley) in Bulgaria. The pathogens, natural populations of *X. vesicatoria* and *X. euvesicatoria*, refer to pepper P pathotype (22 strains) and pepper-tomato PT pathotype (52 strains) in a ratio of 22 to 52. The main cause of bacterial spot disease on the territory of the country is the closely specialized on pepper species *X.euvesicatoria* (39PT:19P). From the identified 15 strains of *X. vesicatoria*, 3 strains are pathogenic only to pepper and refer to P pathotype and 12 strains infect both hosts and belong to the PT (Figure 2).

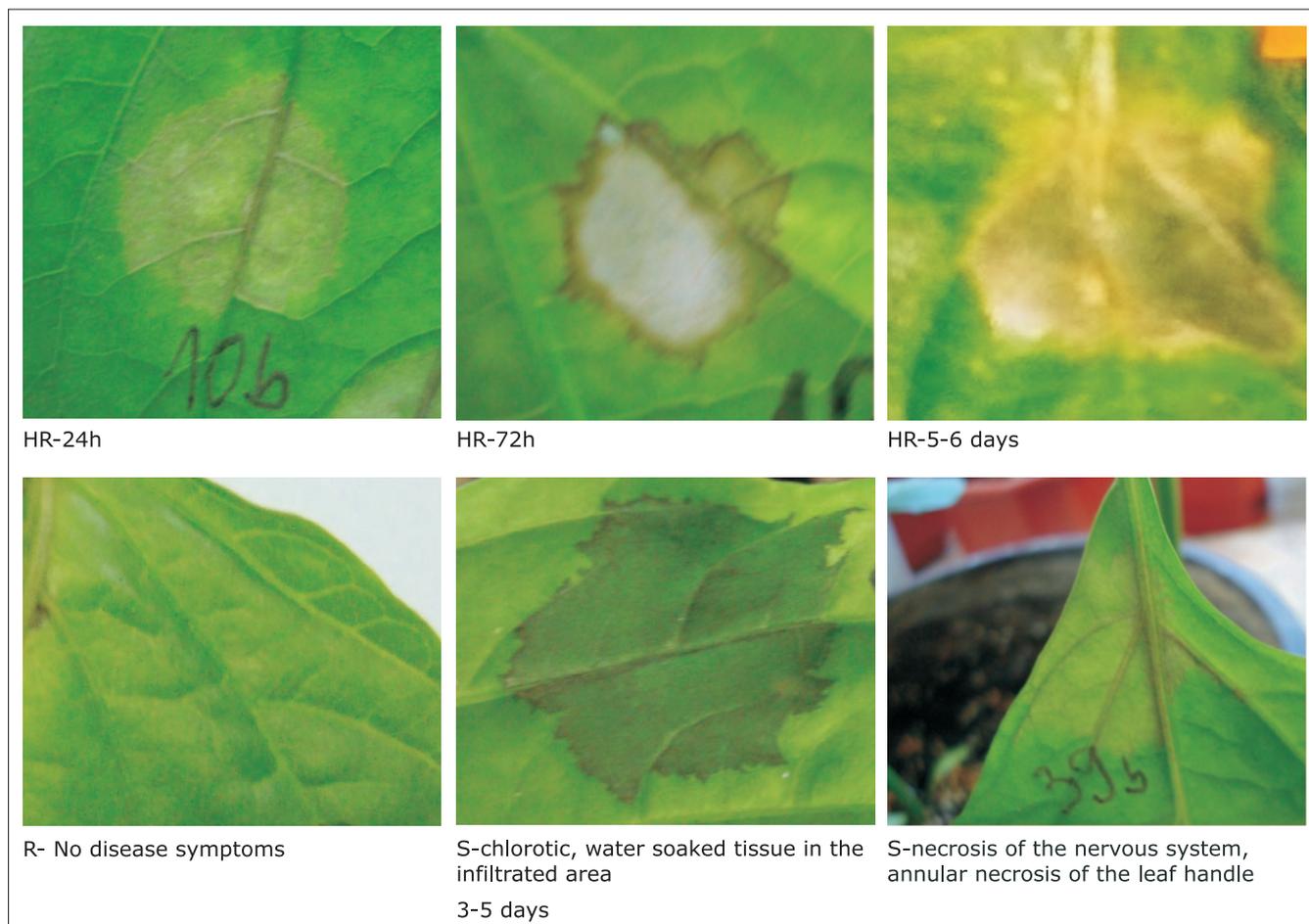


Figure 1. Hypersensitive (HR), resistance (R) and susceptible (Sus) reaction to infiltration of bacterial spot pathogen (S= Susceptible reaction; HR= Hypersensitive-resistant reaction; R= Resistant reaction non hypersensitive)

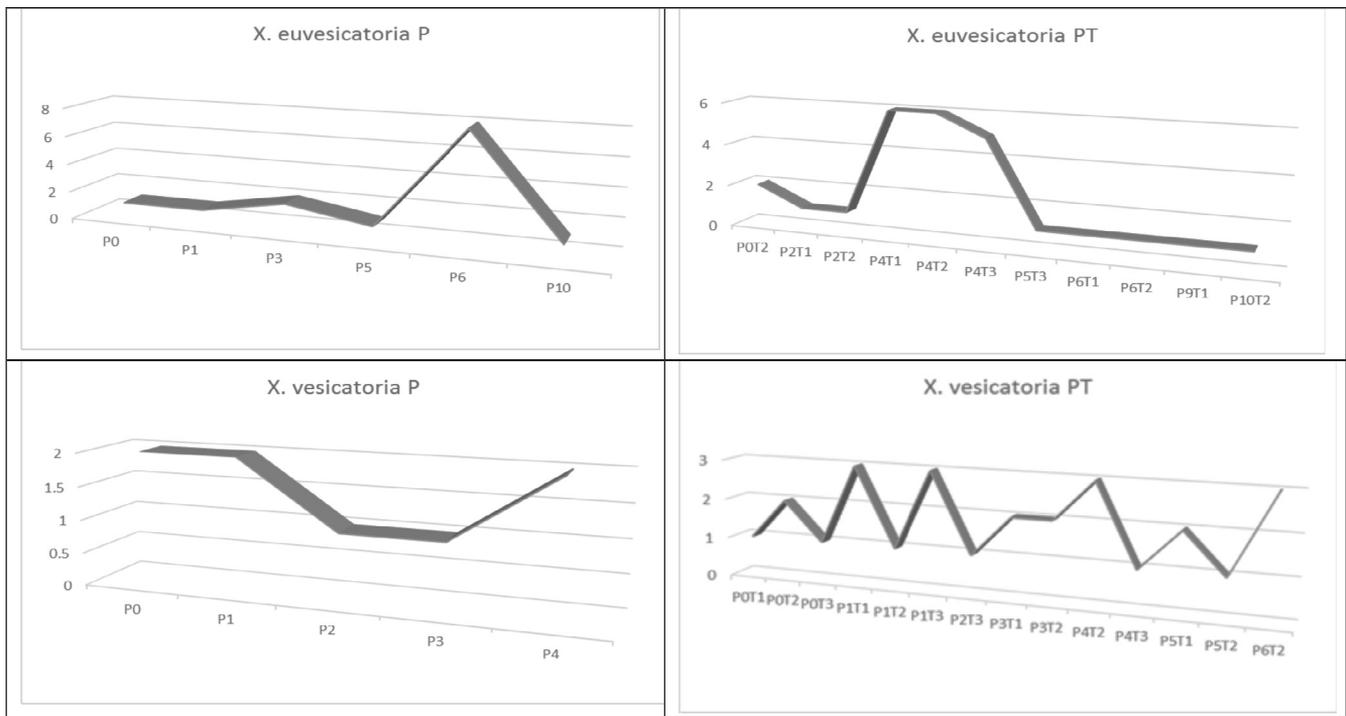


Figure 2. Ratio between the pathophytes and the races of bacterial spots of *Xanthomonas vesicatoria* and *Xanthomonas euvesicatoria* during the period 1999-2017

The emergence, development and spread of bacterial spot disease on pepper in the country depend on the climatic characteristics of the growing area, the soil type, the varietal composition, the farming technology.

The Northern Black Sea region is under the influence of continental Europe climate and the Black Sea Basin and is a maritime variation of temperate continental climate. The soil cover is various. Fluvisols and rendzinas soils are suitable for vegetable growing (Shishkov and Kolev, 2014). This district is a specific productive for the cultivation of pepper: the varietal types - kambi and kapia, the main and sole cause of bacterial spot disease is the species *X. euvesicatoria* (P, PT). Races P1, P4 and P6 are differentiated in the P pathotype. In the PT pathotype predominate the race P4 and the tomato race T2. Single strains refer to races P1 and P6 in combination with tomato T1 race. In PT pathotype predominates T1 (P1T1, P4T1, P6T1).

North Central region is characterized by moderate continental climate and various soil cover. Soil climatic conditions are extremely suitable for growing green and red pepper. A bacterial disease on the leaves of the varietal type kapia was observed in the Lovech region in 1999, 34 years after reporting the disease and the causative agent by Karov in 1965 (Karov, 1965). Isolated and identified is the phytopathogenic bacteria *X. vesicatoria*, which infects tomatoes and pepper and refers to a PT pathotype (Bogatzevska et al., 2007). In the population of the pathogen, races are differentiated: P0T2, P2T3 and P6T2. Bacterial spot disease on pepper (*X. vesicatoria* PT) is recorded near tomatoes or in crop rotation where the two crops alternate. The species *X. euvesicatoria* (P, PT) is the main cause of the disease in this region in recent years (2012-2017). In the natural population of the pathogen, the

PT pathotype prevails. In the P pathotype, races P6 and P3 are differentiated, the major race is P6. The dominant race in PT is the pepper P4 and the virulent tomato race T2. Races P0T2, P1T3 and P3T2 are only recorded in fields that are close to tomatoes. In the PT population of *X. euvesicatoria*, the three tomato races T1, T2, T3 are present with a tendency for T2 to prevail.

Southwestern Bulgaria is a region characterized by a varied climate, moderate continental in the high and northern valleys and Mediterranean influence in the Sandanski-Petrich field. The soils are chernozem-vertisols in the northern valleys, leptosols and fluvisols in the river valleys (Shishkov and Kolev, 2014). Vegetable production (tomatoes, pepper) is highly concentrated in the region due to favourable soil and climatic conditions. Different varietal types of pepper are grown – kambi, kapia, long pungent fruited, small-fruited (ribki, shipka), conical (sivria). An important disease in peppers is the bacterial spot disease with causative agents *X. euvesicatoria* and *X. vesicatoria*. Dominating is the natural population of the highly specialized, for this type of crop, species *X. euvesicatoria* pathotypes P and PT. In pathotype P most common is the P6 race, single strains are differentiated as P0 and P1. A great diversity is registered in the race composition of pathotype PT. Predominant are strains of the bacteria that refer to race P4 in combination with tomato races T1, T2 and T3, the most common is the combination P4T2. In the population of the intermingle pathotype of *X. euvesicatoria* sporadically can be found races P0, P6 and P10, always in combination with tomato race T2. In the selection fields of IG (IRFG - Sofia), PT strains are registered, they include pepper races P2, P5 and tomato races T1, T2, T3, respectively P2T1 (T2) and P5T1 (T2, T3). For the first time in Bulgaria in

the natural population of *X. vesicatoria*, are detected strains which infect only pepper, differentiated are races P0, P2 and P3. Differentiated races are P0, P2 and P3, which are distributed in the region of Strumitsa town (Mitrev et al., 2001). This species is highly specialized to the genus *Solanum*, a general cause of bacterial spot disease on tomatoes in the country. The species *X. vesicatoria* pathotype P was found in 2005 in selection test fields (IRFG) and pepper fields around Petrich. In the same year, this pathogen was isolated and identified in the region of Strumitsa and Kochani, Macedonia, which are located near the Bulgarian southwestern border (Bogatzevska et al., 2007; Vancheva, 2015). The population of *X. vesicatoria* PT prevails in private gardens and vegetable fields to tomatoes. Differentiated races are P1, P3 and P4 in combination with tomato races T1, T2 and T3 (P1T2, P3T2, P4T2).

Central South Bulgaria includes the largest and most fertile lowland on the Balkan Peninsula – Thracian valley with transcontinental and Mediterranean climate. The main soil types are vertisols, fluvisols are found along the streams and the confluents of the Maritsa and Tundzha rivers (Shishkov and Kolev, 2014). The warm climate favours the growing and production of vegetables (tomatoes, pepper, eggplant, etc.). The main cause of bacterial spot disease on the pepper is the *X. euvesicatoria* (P, PT) species. In P pathotype are differentiated races P5 and P10. The strains of the bacteria are isolated from the introduced pepper varieties in the experimental field of the Vegetable Crops Research Institute – Maritsa. Widespread is the PT population of *X. euvesicatoria* in private gardens and fields located near tomatoes. PT is characterized by a variety of pepper (P0, P4, P5, P6, P9) and tomato races (T1, T2, T3). Single strains refer to P5 and P9 in combination with tomato race T1 (P5T1, P9T1). Pepper races P0 and P6 are in combination with the most virulent tomato race T2 (P0T2, P6T2). The species *X. vesicatoria* P0T2 was isolated from single fruit of red kapia in a field with pepper around Stara Zagora.

Races are differentiated based on interactions between the Bs1, Bs2, Bs3 and Bs4 genes that confer a HR reaction in the pepper and corresponding avirulence genes (*avrBs1*, *avrBs2*, *avrBs3* and *avrBs4*) in the pathogen (Stall et al., 2009). Avirulent genes defining pathogenicity in tomatoes and pepper are *avrBs4* (HR on both hosts - tomatoes and pepper) and *avrBsT* (HR on pepper) (Minsavage et al., 1990; Ballvora et al., 2001; Potnis et al., 2015). Both avirulent genes are located in plasmids and their loss allows the respective strain to cause disease on a particular host (Canteros et al., 1991; Minsavage et al., 1999). The aggressiveness of the agents is determined by the race structure of the bacterial population and the interaction in the pathogen-host system. *X. euvesicatoria* strains have been found to be more aggressive when interacting with pepper plants than with tomatoes and are closely specialized to the genome of the genus *Capsicum* (Bogatzevska and Pandeva, 2009; Ignjatov, 2013; Vancheva et al., 2016). *X. euvesicatoria* is divided into races, which correspond to the pepper resistance genes they defeat (Alfano and Collmer, 2004). The population of *X. euvesicatoria* was heterogeneous, consisting of four physiological races: P1, P3, P7 and P8 in Serbia. The most common was pepper race P8, followed by P7, P1 and P3 (Ignjatov, 2013).

In the Bulgarian population of the species *X. euvesicatoria*, causative agent of bacterial spot disease on pepper, by PCR with primer pairs were detected two types of effector proteins *AvrBs3* and *AvrBs4*. The predominant part of the Bulgarian strains isolated until 2008 have the *AvrBs3* gene, which also correlates with their established affiliation to PT. Among the Bulgarian strains isolated after 2008, dominates P pathotype and the presence of *avrBs4* gene. The presence of species characteristic genes (*AvrBs3* and/or *AvrBs4*). The absence of these genes in 6.5% of the strains of *X. euvesicatoria* suggests the involvement of other pathogenicity factors (Vancheva, 2015). These strains of the bacteria refer to races P2, P5 and P10. Characteristic for race P2 are virulence genes *AvrBs1* and *AvrBs2*, for race 5-*AvrBs1*, for race P10 the genes have not been determined (Stall et al., 2009; Kurowski et al., 2015). Analysis of the newly sequenced *X. euvesicatoria* strains revealed interesting findings among the type 3 (T3) effectors, relatively ancient stepwise erosion of some T3 effectors, additional *X. euvesicatoria*-specific T3 effectors among the causal agents of bacterial spot of tomato and pepper (BST), orthologs of *avrBs3* and *avrBs4*, and T3 effectors shared among xanthomonads pathogenic against various hosts. The results from this study support the finding that T3 effector repertoire and host range are fundamental for the study of host-microbe interaction but of little relevance to bacterial speciation (Barak et al., 2016).

Conclusion

The natural population of *X. euvesicatoria* is heterogeneous according of pathotype and physiological races: P consists of races P0, P1, P3, P4, P6, single strains refer to races P5 and P10. Widespread is the race P6 in the P of the pathogen, followed by race P1. In PT, races P0, P1, P4, P5 are differentiated. Single strains are assigned to P3, P9 and P10. The dominant race in PT is the P4 race in combination with the tomato race T2. The only cause of bacterial spot disease in the area of the Northern Black Sea region is the species *X. euvesicatoria*. In the heterogeneous population of the closely specialized causative agent of bacterial spot disease in Bulgaria, the physiological races P4 and P6 predominate. Races P4 and P6 are more aggressive in field conditions than other pepper races. In PT of the pathogen are differentiated tomato races T1, T2 and T3. Dominant is the highly virulent, for the tomato host, race T2 followed by T1 race. Single strains of *X. euvesicatoria* refer to races P3, P9 and P10. In both pathotypes of the natural population of the pathogen that inhabit the pepper plants have been differentiated as P0, P1 and P4. Characteristic of P is race P6, and for PT - race P4. The species *X. vesicatoria* is a major cause of bacterial spot disease on tomatoes, individual strains also infect pepper. The population of *X. vesicatoria* PT prevails in private gardens and vegetable areas adjacent to tomatoes. Differentiated races are P1, P3 and P4 in combination with tomato races T1, T2 and T3. For the first time, strains that are pathogenic only for pepper are detected. P pathotype of the bacterium is found only in the experimental breeding fields of IRFG - Sofia field and in areas with pepper near the southwestern border with Macedonia.

Differentiated races are P0, P2 and P3, which are distributed in the region of Strumitsa town (Macedonia). Race P3 occurs in P and PT pathotype.

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