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Bulgarian Local Cherry Genetic Resources towards Sustainable Agriculture

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Abstract: In the conditions of global climate change and invasion of new diseases and pests, the utilisation of the local gene pool is a vital step towards sustainable agriculture. Local cultivars and forms are generally considered as better adapted to the conditions of the originating region than widely spread commercial cultivars bred elsewhere. In order to collect, study, and preserve old and local fruit and vine cultivars and forms, a series of expeditions have been conducted throughout Bulgaria under the frame of project KP-06-N46/3 funded by the National Science Fund of the Bulgarian Ministry of Education and Science. During these scientific expeditions, several local cherry forms were discovered without visual symptoms of brown rot *Monilinia laxa* (Aderh. and Ruhl.) Honey or cherry leaf spot *Blumeriella jaapii* (Rehm) Arx. To confirm the degree of resistance/low susceptibility of the specimens, laboratory tests with artificial inoculations of fruits and leaves were conducted. In the current study, two specimens found in Damyanovo region (a village in the municipality of Sevlievo, Gabrovo Province, in northern central Bulgaria) were included. They were compared to two old local cherry cultivars, 'Volsko sartse' and 'Ranna cherna edra', and two new selections of FGI-Plovdiv, 'Asparukh' (El.17-90) and 'Kossara', using 'Bigarreau Burlat' as reference cultivar for resistance to economically important diseases. The artificial inoculations confirmed the field observation of the cherry specimen with designation SM-58 with 0.00% of infected fruits. Therefore, it is recommended for use in the breeding programme of the Fruit Growing Institute, Plovdiv for the creation of new cultivars.

Keywords: cherry; local cultivars; genetic resources; *Monilinia*; *Blumeriella*

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1. Introduction

Globally, the increasing population, globalization, urbanization, and climate change impose more demands on agricultural productivity. It is recognised that in the last century the increase of trade and competition has led to the use of a limited number of cultivars, often of a similar origin. At the same time, research and practices are mainly focused on improving the productivity of a small number of existing crops that form the cornerstone of the global food economy rather than increasing crop diversity. The result is a loss of agrobiological diversity, which leads to unsustainable practices in the food industry and reinforces the pressure from the changing climate and the multiplication and transfer of pathogens from one country to another.

The use of 15 species that represent 90% of all human food makes clear the narrow basis on which we depend. A limited number of species that we use for our livelihood presents a huge concern about the genetic vulnerability of agriculture [1].

The limited gene pool used in crop breeding can lead to vulnerability (susceptibility) to diseases and pests, as well as the emergence of new resistant races and pressures on the environment [2].

The selection and breeding for the creation of new cultivars and rootstocks often uses the same parenting forms and varieties with proven qualities, but this leads to a restriction of the used gene pool [3–5]. In general, many of the main fruit cultivars and vines are originated from common ancestors. At present, it is unclear how much genetic diversity is available in the breeding programmes. The pedigree information of the cultivars does not give an accurate indication, since some of the original main clones, although having different names, may actually be the same or genetically similar [6]. The successful development of new combinations of characteristics in the future cultivars may be limited by the lack of a broad genetic base, and this could require the use of exotic sources and gene modifications.

Conservation of genetic resources is of regional and global importance [7,8]. It has a direct relationship with breeding programs and research in the field of integrated plant protection and organic farming, as it is the basis for creating genetic diversity, bearing valuable biological and agricultural qualities and resistance to pests and diseases [1,9].

A series of connected organizations working at the global level is addressing some of the major constraints in collecting, describing, and preserving plants; managing information systems; and integrating new technologies in conservation of plant genetic resources (PGR) [8,10–14]. There are a number of strategies for the collection, conservation and sustainable use of genetic resources for agricultural productivity and food safety [14–16].

In Bulgaria, the latest large-scale expedition studies on scientific programs for local resources collection were conducted in the 1960s and 1970s [17–19]. Later, on a much smaller scale, this problem was partially developed in projects of the Agricultural Academy and the Ministry of Education and Science. Through these projects, some valuable local forms and cultivars have been preserved and researched—mainly apples, pears, plums, and cherries [20–24]. The cited authors emphasize the importance and plasticity of these fruit specimens. Some of them are recommended, though for limited distribution, and others are expected to find a place in the new breeding programs. In order to enrich the variety of cultivars grown in the country with well-adapted, sustainable, and productive ones, a series of expeditions have been conducted throughout Bulgaria under the frame of project KP-06-N46/3 funded by the National Science Fund of the Bulgarian Ministry of Education and Science.

During these scientific expeditions, several local cherry forms were discovered without visual symptoms of brown rot or cherry leaf spot. The objective of this paper is to confirm the degree of field resistance/low susceptibility of the specimens by conducting laboratory tests with artificial inoculations of fruits and leaves. The results would contribute to both the conservation of local genetic resources and enhancement of the sweet cherry breeding programme in the Fruit Growing Institute in Plovdiv, Bulgaria (FGI).

2. Materials and Methods

In 2020, the Fruit Growing Institute (FGI) in Plovdiv started a three-year project funded by the National Science Fund of the Bulgarian Ministry of Education and Science. The scope of the project is conservation and inclusion of local traditional cultivars and forms in the collections of scientific institutes and universities and their use in selection programs for the creation of new varieties with valuable biological and economic qualities. During the development of Project KP-06-PN46/3, FGI and partners from the Maritsa Vegetable Crops Research Institute (MVCRI), Plovdiv; the University of Forestry (UF) in Sofia; the Regional Natural History Museum of Plovdiv; and the Research Institute of Mountain Stockbreeding and Agriculture (RIMSA) in Troyan conducted scientific expeditions resulting in a collection of specimens from different fruit crops including cherries.

In the current study, two of the discovered specimens found in the Damyanovo region (a village in the municipality of Sevlievo, Gabrovo Province, in northern-central Bulgaria) were included. They were compared to two old local cherry cultivars 'Volsko sartse' and 'Ranna cherna edra' and two new selections of FGI-Plovdiv—'Asparukh' (El.17-90) and 'Kossara', using 'Bigarreau Burlat' as the reference cultivar for resistance to economically important diseases.

To confirm the degree of resistance/low susceptibility of the specimens, laboratory tests with artificial inoculations of fruits and leaves were conducted. The isolates, which were used for the studies, are part of the phytopathological collection of FGI and originate from the region of Plovdiv.

2.1. Artificial Inoculations of Leaves

Detached leaves harvested from sweet cherry trees without visible symptoms were used for an in vitro assay of resistance to *Blumeriella jaapii* (Rehm) Arx. The method of inoculation was the leaf disc infiltration technique using syringe. The detached leaves were surface sterilized and inoculated with previously isolated strains of cherry leaf spot or sterile distilled water (0.8% KCL) as control. An inoculum concentration of 18×10^6 cfu/mL was used for typical symptom development. Inoculated leaves were placed in sterile petri dishes with water agar (10 g/L) and incubated at constant 25 °C and 60% humidity with a 12- to 16-h photo period. Symptom development was monitored every 12 h from 0 to 408 h by measuring the expanding chlorotic and necrotic leaf tissue [25–27].

2.2. Artificial Inoculations of Fruits

When assessing the resistance to *Monilinia laxa* (Aderh. and Ruhl.) Honey, the methodology previously described by Kaluzna and Sobiczewski [27,28] was used. Fresh, mature, sweet cherry fruits from cultivars and hybrids of interest were collected. Fruits were disinfected by dipping in 50% ethanol for 3 min and then rinsed in sterile distilled water and excess water was removed. Each fruitlet was inoculated by pricking in two places to the depth of 2 mm with a sterile needle previously immersed in suspension of inoculum with a concentration of 4×10^6 cfu/mL. After inoculation each fruit was immediately placed on moist filter paper in a sterile Petri dish and incubated at a constant 25 °C and 60% humidity. Sterile distilled water was included as negative control.

2.3. Data Collection and Analysis

Inoculations were performed in 12 replicates plus 2 negative controls for every variant. Data was collected on every 24 h and results were subjected to statistical analysis using the multiple range test with the software products "MS Excel 365 Analysis ToolPak Add-Ins" and "R-4.1.2" in combination with "RStudio Desktop 2021.09.1+372" and the installed package "agricolae 1.3-5" [29].

3. Results

3.1. Plant Sampling and Field Observations

In the remote parts of Bulgaria, there are still single trees in small settlements and old monastery or school gardens that are unaffected by urban changes, though they are constantly decreasing and irretrievably disappearing. Between December 2020 and June 2022, the KP-06-H46/3 project team carried out a number of field surveys mainly in areas with prior information about available local varieties and forms of single trees or trees in old gardens. Most of them grow as single trees in yards and field properties, with some being 85–100 years old or more. Others have been preserved to this day, due to the rough terrain and the favourable soil and climate conditions in the orchards, created in the 1950s before the cooperative use of the land. Next to already deserted hamlets, old, vibrant trees still grow, and in separate isolated habitats there are varieties with 6–8 forms, distinguished by the qualities of the fruits, ripening periods, etc. Representatives of fruit and vine species were found at an altitude of up to 1000 m.

In the first stage of the development of the KP-06-H46/3 project, 148 specimens of local varieties and forms were discovered and marked. The habitats were described, and an archive was created with photographic material and GPS coordinates. Biometric, chemical, colorimetric, sensory, phytopathological, and entomological analyses were performed [30–32]. Plant material was taken for study, in vitro propagation, and preservation of the specimens. During these scientific expeditions, several local cherry forms were discovered without visual symptoms of brown rot or cherry leaf spot. The two most promising specimens were found in the Damyanovo region (a village in the municipality of Sevlievo, Gabrovo Province, in northern central Bulgaria). Their designations are SM-57 (found at 43°00′06.0′′ N 24°55′31.9′′ E) (Figure 1), and SM-58 (found at 43°00′08.5′′ N 24°55′26.1′′ E), respectively (Figure 2).

(**a**) (**b**)

Figure 1. Cherry specimen number SM-57 found in Damyanovo region: (**a**) original tree found at 43°00′06.0′′ N 24°55′31.9′′ E; (**b**) fruit of SM-57 at full maturity.

(**a**) (**b**)

Figure 2. Cherry specimen number SM-58 found in Damyanovo region: (**a**) original tree found at 43°00′08.5′′ N 24°55′26.1′′ E; (**b**) fruit of SM-58 at full maturity.

3.2. Laboratory Tests

The reaction of cherry cultivars and specimens SM-57 and SM-58 from local cherry varieties to the pathogens *Monilinia laxa* and *Blumeriella jaapii* under in vitro conditions has been studied. Very good results for both pathogens are demonstrated by the selected hybrid 'Asparukh' (El.17-90) and local cultivar 'Volsko sartse'.

3.2.1. Artificial Inoculations of Fruits

A typical symptom of the causative agent of brown rot *M. laxa* is a brown, slightly concave spot, which quickly grows and covers the entire fruit. With a high attack and a sensitive host on the attacked parts, tufts of conidiophores and conidia are formed (Figure 3).

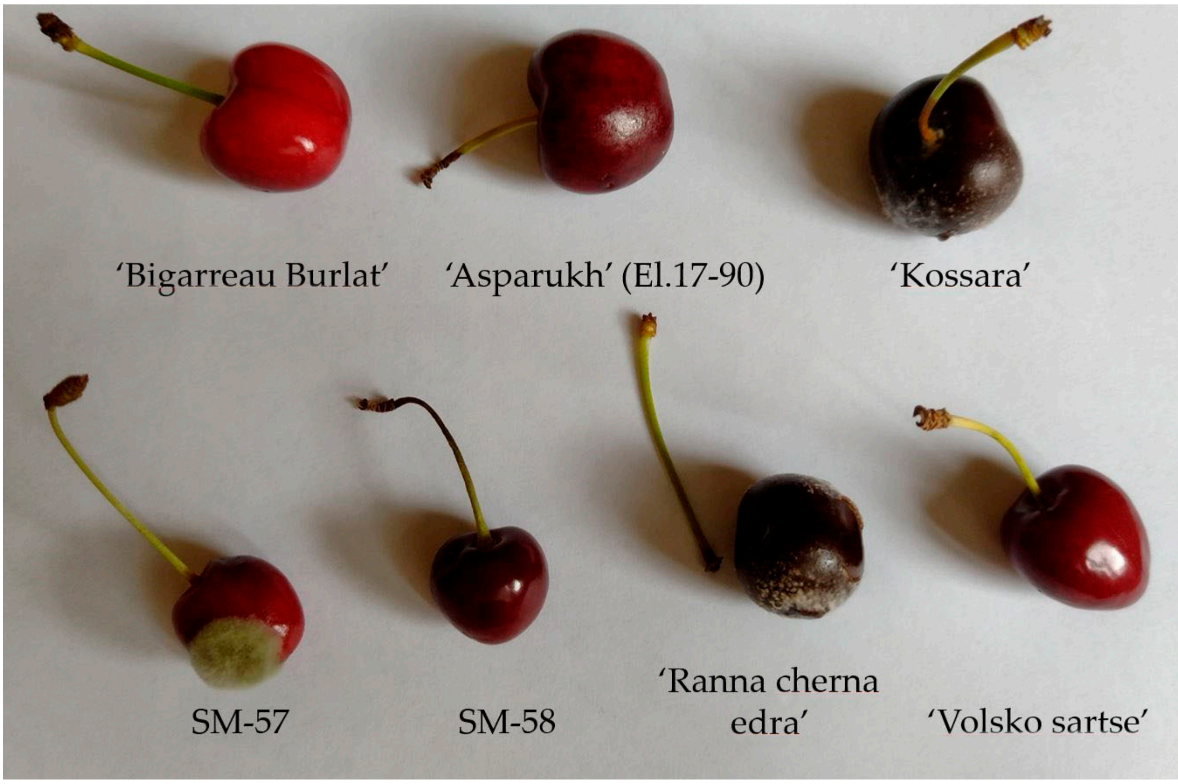

Figure 3. The reaction of cherry cultivars and specimens SM-57 and SM-58 from local cherry varieties to the pathogen *Monilinia laxa* at the 144 h after inoculation.

The studies the have been conducted to establish the reaction of different cherry cultivars to the pathogen *Monilinia laxa* are divergent. Initially, the symptoms of the disease in all studied cultivars are observed after 48h of inoculation. The typical reactions during this period are established in the cultivars 'Kossara', 'Ranna cherna edra', 'Biggarreau Burlat', and specimen number 'SM-57′. The strongest attack is reported in 'Ranna cherna edra', followed by 'Kossara' and 'SM-57′. The dynamics of development of the disease is between 144 and 336 h (Figure 4). The selected hybrid 'Asparukh' (El.17-90) and local cultivar 'Volsko sartse' are very weakly attacked, respectively 0.58 and 5.00 mm of average spread of the infection. In these varieties, the symptoms of the disease are very weak, even at the end of the reporting at 336 h, and are statistically proven (Table 1). The cherry specimen SM-58 was immune when inoculated with the test pathogen and did not manifest any visible symptoms.

3.2.2. Artificial Inoculations of Leaves

The pathogen forms numerous dark red dots on the upper side of the leaves, which subsequently turn into brown necrotic spots.

Initial symptoms after artificial inoculation of the test cultivars with *Blumeriella jaapii* were observed at 72 h. The dynamics of the disease occurred between 288 and 408 h (Figure 5). The strongest attack was recorded on specimen number SM-57 (18.85 mm). The varieties 'Biggarreau Burlat', 'Ranna cherna edra', and SM-58 were weakly attacked, and the spread of the disease was in the range between 4.08 and 8.70 mm. Statistically, the pathogen slightly affected the selected hybrid 'Asparukh' (El.17-90) and cultivar 'Kossara', with spread diameters of 0.25 and 1.12 mm, respectively (Table 2). The old local cultivar 'Volsko sartse' demonstrated an immune reaction to the disease.

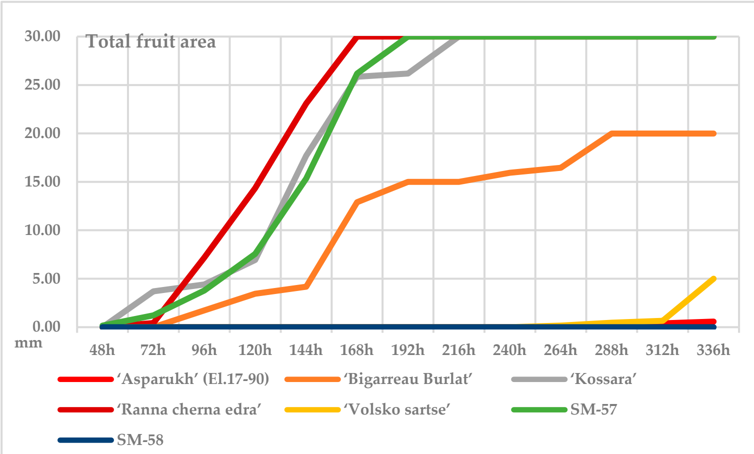

Figure 4. Dynamics of development of the disease brown rot on cherry fruits.

Table 1. Statistical comparison of the diameter [mm] of infection spread.

Cultivars/Accession Numbers	Hours after Inoculation													
	24 h		48 h		72 h		96 h		120 h		144 h		168 h	
'Asparukh' (El.17-90)	0.00	A **	0.00	a	0.00	b	0.00	c	0.00	b	0.00	b	0.00	c
'Bigarreau Burlat'	0.00	a	0.00	a	0.00	b	1.73	bc	3.45	b	4.18	b	12.89	b
'Kossara'	0.00	a	0.00	a	3.70	a	4.41	ab	6.93	b	17.70	a	25.85	a
'Ranna cherna edra'	0.00	a	0.00	a	0.42	b	7.18	a	14.36	a	23.09	a	* TFA	a
'Volsko sartse'	0.00	a	0.00	a	0.00	b	0.00	c	0.00	b	0.00	b	0.00	c
SM-57	0.00	a	0.17	a	1.21	ab	3.78	abc	7.56	ab	15.35	a	26.18	a
SM-58	0.00	a	0.00	a	0.00	b	0.00	c	0.00	b	0.00	b	0.00	c
Cultivars/Accession Numbers	**Hours after Inoculation**													
	192 h		**216 h**		**240 h**		**264 h**		**288 h**		**312 h**		**336 h**	
'Asparukh' (El.17-90)	0.00	c	0.00	c	0.00	c	0.00	c	0.17	c	0.38	c	0.58	c
'Bigarreau Burlat'	15.00	b	15.00	b	15.93	b	16.45	b	20.00	b	20.00	b	20.00	b
'Kossara'	26.18	a	* TFA	a	* TFA	a	* TFA	a	* TFA	a	* TFA	a	* TFA	a
'Ranna cherna edra'	* TFA	a	* TFA	a	* TFA	a	* TFA	a	* TFA	a	* TFA	a	* TFA	a
'Volsko sartse'	0.00	c	0.00	c	0.00	c	0.17	c	0.45	c	0.65	c	5.00	c
SM-57	* TFA	a	* TFA	a	* TFA	a	* TFA	a	* TFA	a	* TFA	a	* TFA	a
SM-58	0.00	c	0.00	c	0.00	c	0.00	c	0.00	c	0.00	c	0.00	c

* different letters in same column means significant difference at $p = 0.05$, ** TFA—total area of the fruit was affected.

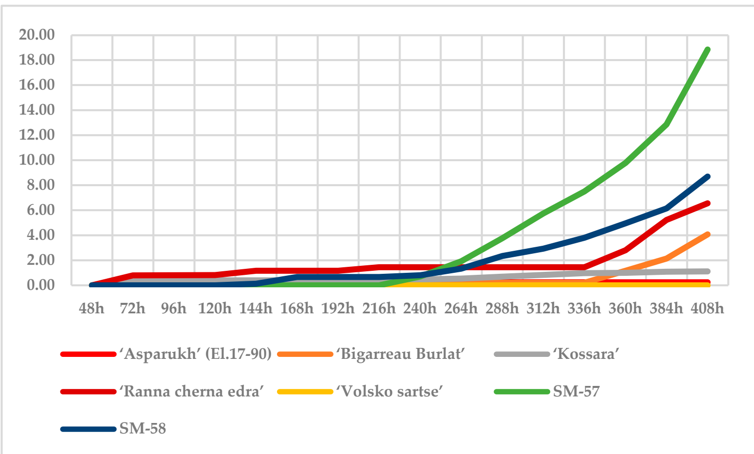

Figure 5. Dynamics of development of the disease cherry leaf spot on cherry leaves.

Table 2. Statistical comparison of the diameter [mm] of infection spread.

Cultivars/Accession Numbers	Hours after Inoculation									
	72 h		96 h		120 h		144 h		168 h	
'Asparukh' (El.17-90)	0.17	a	0.19	a	0.20	a	0.20	b	0.20	ab
'Bigarreau Burlat'	0.00	a	0.00	a	0.00	a	0.20	b	0.20	ab
'Kossara'	0.38	a *	0.39	a	0.40	a	0.40	ab	0.40	ab
'Ranna cherna edra'	0.79	a	0.81	a	0.82	a	1.16	a	1.16	a
'Volsko sartse'	0.00	a	0.00	a	0.00	a	0.00	b	0.00	b
SM-57	0.00	a	0.00	a	0.00	a	0.00	b	0.00	b
SM-58	0.00	a	0.00	a	0.00	a	0.13	b	0.67	ab
Cultivars/Accession Numbers	**Hours after Inoculation**									
	192 h		**216 h**		**240 h**		**264 h**		**288 h**	
'Asparukh' (El.17-90)	0.20	ab	0.24	ab	0.24	ab	0.24	ab	0.24	ab
'Bigarreau Burlat'	0.20	ab	0.20	ab	0.20	ab	0.20	ab	0.20	ab
'Kossara'	0.40	ab	0.42	ab	0.48	ab	0.54	ab	0.69	ab
'Ranna cherna edra'	1.16	a	1.45	a	1.45	a	1.45	a	1.45	a
'Volsko sartse'	0.00	b	0.00	b	0.00	b	0.00	b	0.00	b
SM-57	0.00	b	0.00	b	0.70	ab	1.92	a	3.77	a
SM-58	0.67	ab	0.67	ab	0.80	ab	1.34	a	2.34	a
Cultivars/Accession Numbers	**Hours after inoculation**									
	312 h		**336 h**		**360 h**		**384 h**		**408 h**	
'Asparukh' (El.17-90)	0.24	ab	0.25	ab	0.25	ab	0.25	b	0.25	b
'Bigarreau Burlat'	0.20	ab	0.20	ab	1.17	a	2.14	ab	4.08	b
'Kossara'	0.83	ab	0.97	ab	1.00	ab	1.08	b	1.12	b
'Ranna cherna edra'	1.45	a	1.45	a	2.81	a	5.23	ab	6.57	ab
'Volsko sartse'	0.00	b	0.00	b	0.00	b	0.00	b	0.00	b
SM-57	5.75	a	7.51	a	9.78	a	12.85	a	18.85	a
SM-58	2.94	a	3.80	a	4.96	a	6.14	ab	8.70	ab

* different letters in same column means significant difference at $p = 0.05$.

4. Discussion

Sweet cherry is a major structural species in Bulgaria. According to the Bulgarian Ministry of Agriculture and Food, in 2021, it occupied 23% of the fruit tree areas with a total production of 52,615 tones, which defined it as a second leading fruit crop in the country [33]. The increased interest in establishing new cherry plantations necessitates the provision of new market-oriented cultivars with a better sensory profile of those fruits that are resistant to biotic and abiotic stress factors.

One of the major breeding aims set in the breeding programmes globally is resistance to economically important diseases [34–36]. The sweet cherry breeding programme of the Fruit Growing Institute (FGI), Plovdiv started in 1987 with the creation of the F1 hybrid generation originating mainly from widely spread commercial cultivars and later included the local cultivar 'Ranna cherna edra' as an early ripening donor [37]. The updating of the hybridization programme in 2016 necessitated adding new breeding objectives complying with the market tendencies and changing consumer preferences, including cultivars and rootstocks resistant to biotic and abiotic stressors [38]. The inclusion of traditional varieties in the FGI collections and their implementation in the breeding programs as donors of valuable biological and economic qualities, while preserving the traditional and unique taste and aroma, will contribute enormously to the enhancement of biodiversity and the sustainability of Bulgarian agriculture.

The new selection of FGI-Plovdiv 'Asparukh' (El.17-90) demonstrated low susceptibility to both economically important diseases, brown rot and cherry leaf spot, proving the advantages of using local cultivars in the parent combinations.

One of the two promising specimens found in Damyanovo region, SM-58 (found at 43°00′08.5′′ N 24°55′26.1′′ E) did not manifest any visible symptoms of brown rot Monilinia laxa, both in the field and when inoculated with the test pathogen under laboratory conditions. It can be used in breeding programs and to enrich the gene pool of the FGI-Plovdiv collections in order to solve the problems of integrated and organic fruit production, a prerequisite for participation in future projects and programs.

5. Conclusions

Under in vitro conditions, good levels of tolerance to both pathogens, *Monilinia laxa* and *Blumeriella jaapii*, were demonstrated by the selected hybrid 'Asparukh' (El.17-90) and local cultivar 'Volsko sartse'.

The cherry specimen SM-58 is immune when inoculated with the test pathogen *Monilinia laxa* (Aderh. and Ruhl.) Honey from the phytopathological collection of FGI-Plovdiv and therefore is recommended as a donor of resistance in the sweet cherry breeding programme in the institute.

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Conflicts of Interest: The authors declare no conflict of interest.

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