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BIOLOGIZED CONTROL OF THE MAIN DISEASES OF CHERRY PLUM IN HUMID SUBTROPICS OF THE KRASNODAR REGION

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Abstract

Chemical fungicides are usually used to combat phytopathogens in stone fruit crops, in particular cherry plum (*Prunus cerasifera* Ehrh.). Biofungicides are an alternative to chemicals. Biofungicides preparations are based on saprotrophic bacteria and fungi. Most of these drugs suppress the reproduction of fungal plant pathogens thus reducing the infectious load. These drugs induce immune responses in plants, increasing their resistance to pathogens and unfavorable environmental factors. In this work, for the first time in humid subtropics, we have given a comparative assessment of the biological effectiveness of the biofungicides Baktofit, Vitaplan, Gamair, Fitosporin-M, used together with the chemical fungicides Skor and Horus at half the rate of application. Our goal was to develop systems of biologic protection of cherry plum from the main diseases (clusterosporium disease, moniliosis, and gray rot of fruits) based on biological products in combination with reduced doses of chemical fungicides in conditions humid subtropics of the Krasnodar Territory. The work also aimed to assess the effect of such systems on the yield and annual growth of axial shoots of cherry plum. The research was carried out in 2015-2017 in plantings of cherry plum variety Obilnaya at the production sites of the State Unitary Enterprise of the Krasnodar Territory "Oktyabrsky" (Sochi). We compared the effect of various *Bacillus subtilis* bacteria-based fungicides, namely Baktofit, SP (Sibbiopharm, Russia), Vitaplan (AgroBioTechnology, Russia), SP, Gamair, SP (AgroBioTechnology, Russia), and Fitosporin-M, Zh (BashInkom, Russia) in mixes with half the norms of Horus (Horus®, Syngenta AG, Switzerland) and Skor (Skor®, Syngenta AG, Switzerland), as well as the *Trichoderma harzianum*-based preparation Glyokladin, Zh (Agrobiotekhnologiya, Russia) without mixing with Horus and Skor. Trees were sprayed with fungicide solutions twice during the spring season, in the bud-swelling phase and in the phase of active shoot growth after flowering. The water was control during the treatment. The chemical fungicides Horus (1st treatment) and Skor (2nd treatment) were as a reference. The intensity of the development of clusterosporiosis, monilios and gray rot of fruits and the biological effectiveness of the preparations used, the yield and the value of the annual growth of axial shoots were assessed. According to a three-year experiment, the bacterial biofungicide Fitosporin-M in combination with half the consumption rates of Horus and Skor and the fungal biofungicide Glyokladin without chemical fungicides showed the maximum statistically significant biological effectiveness in protecting cherry plum from clusterosporia, gray rot and brown monilial rot. The efficiency of Gamair turned out to be slightly lower, but it still exceeded the indicators of the reference treatment with chemical fungicides. The efficiency of Baktofit in most cases was lower than the reference. Vitaplan showed the lowest efficiency in all variants. The biological effectiveness of all tested preparations against monilial brown rot was lower than in the case of gray rot and clusterosporiosis. The yield of cherry plum when treated with Fitosporin-M, Glyokladin and Gamair was practically the same in all tests (9.8-11.5 t/ha), being approximately 1.8-1.9 times higher than the control values (5.4-5.7 t/ha) and 1.1-1.2 times higher than the reference (8.9-9.1 t/ha). When using Baktofit, the yield of cherry plum was almost equal to the reference (8.7-9.4 t/ha), while when treated with Vitaplan it was regularly below the reference (6-7 t/ha). The preserved yield upon treatment with biological preparations reached 9.7-11.5 t/ha. The increase in the growth of the axial shoots of cherry plum compared to the control turned out to be maximum (1.7 times) for Glyokladin and slightly less for Fitosporin-M and Gamair, while when using Baktofit and Vitaplan it was 15-25 % lower than the reference values and only 1.1-1.2 times higher than the control values. Thus, biological plant protection products based on *Bacillus subtilis* and *Trichoderma harzianum* can be successful against the main diseases of cherry plum in the subtropics of Krasnodar Territory. It is

acceptable to use bacterial preparations in a mixture with lower rates of chemical fungicides. This approach is more environmentally friendly and reduces the cost of plant protection.

Keywords: biological plant protection, biofungicides, fungal plant pathogens, stone fruit crops, biological effectiveness, *Bacillus subtilis*, *Trichoderma harzianum*

The natural and climatic conditions of the subtropics of the Krasnodar Territory make it possible to obtain high yields of stone fruit crops, in particular cherry plum (*Prunus cerasifera* Ehrh.). However, they also favor the intensive development of a number of harmful phytopathogenic fungi [1]. The most significant causative agents of stone fruit diseases in Sochi are *Monilinia fructigena* (Pers.) Honey and *M. laxa* (Aderh. & Ruhland) Honey, as well as recently discovered invasive species *M. fructicola* (G. Winter) Honey [2], which cause moniliosis (i.e., specific fruit rot and burns of flowers and young shoots of cherry plum). *Botrytis cinerea* Pers., the causative agent of gray rot, and *Wilsonomyces carpophilus* (Lev.) Adask., J.M. Ogawa & E.E. Butler (= *Clasterosporium carpophilum* (Lev.) Aderh., = *Stigmina carpophila* (Lev.) M.B. Ellis), the causative agent of clusterosporium disease, a complex disease of stone fruits resulted in the death of shoots, damage to fruits, and perforated leaf spot are important [3].

Chemical fungicides are usually used [4, 5] to combat the listed plant pathogens of cherry plum, despite the negative consequences of their use, e.g., toxicity, indiscriminate action, and the risk of resistance of plant pathogens [6]. An alternative to chemical fungicides are biofungicides, the preparations based on saprotrophic bacteria and fungi [7, 8]. Currently, their use has already become one of the most promising areas in the protection of agricultural crops [9, 10]. Most of these drugs simultaneously suppress the reproduction of plant pathogenic fungi, reducing the infectious load, and induce immune responses of plants, increasing their resistance to plant pathogens and other unfavorable environmental factors [11, 12]. In recent years, biofungicides have been produced in Russia based on highly effective strains of the bacterium *Bacillus subtilis* and the fungus *Trichoderma harzianum*, known as natural antagonists of plant pathogenic fungi and inducers of the immune response of plants [13-15]. Such drugs are increasingly used for plant protection, since they are able to suppress various plant pathogens and are characterized by high environmental safety [16-20]. However, so far there are only a few studies devoted to their use for the protection of stone fruit crops in Russia [21, 22].

In this work, for the first time in humid subtropics of the Russian Federation, we have given a comparative assessment of the biological effectiveness of the biofungicides Baktofit, Vitaplan, Gamair, and Fitosporin-M used together with the chemical fungicides Skor and Horus at half the rate of application on plantings of Cherry plum Obilnaya.

Our goal was to develop systems for biologic protection of cherry plum from the main diseases (clusterosporium disease, moniliosis, and gray rot of fruits) based on biological products in combination with reduced doses of chemical fungicides, and to assess the effectiveness and effect of such systems on the yield and annual growth of the axial shoots of cherry plum in the context of humid subtropics of the Krasnodar Territory.

Materials and methods. The research was carried out in plantings of cherry plum variety Obilnaya (production sites of the Oktyabrsky State Unitary Enterprise, Sochi, a 5 km distance from the Black Sea coast, 2015-2017). Trees were treated with ready-made fungicide solutions.

The following chemical fungicides were used: Horus (Horus®, WDG, 750 g/l, 0.3 kg/ha, Syngenta AG, Switzerland) and Skor (Skor®, EC, 250 g/l, 0.2 l/ha, Syngenta AG, Switzerland); bacterial fungicides based on *Bacillus subtilis* — Baktofit, SP (strain IPM 215, BA-10000 IU/g, titer of at least 2 billion spores/g;

Production Association Sibbiopharm Ltd, Russia), Vitaplan, SP (strain VKM-B-2604D, titer no less than 10^{10} CFU/g; AgroBioTechnology, Russia), Gamair, SP (strain M-22 VIZR, titer no less than 10^{11} CFU/g; AgroBioTechnology, Russia), Fitosporin-M, Zh (strain 26 D, titer no less than 1 billion live cells and spores/m; BashInkom, Innovation & Research Enterprise, Ltd, Russia); fungal biofungicide based on *Trichoderma harzianum* fungus Glyokladin, Zh (strain 18 VIZR, titer no less than 10^9 CFU/ml; AgroBioTechnology, Russia).

The experimental design was as follows. Control plants were sprayed with water (2 applications); standard with Horus, 0.3 kg/ha (1st application), Skor, 0.2 l/ha (2nd application); treatment 1 with Baktofit, 2 kg/ha + Horus, 0.15 kg/ha (1st application), Baktofit, 2 kg/ha + Skor, 0.1 l/ha (2nd application); treatment 2 with Vitaplan, 0.12 kg/ha + Horus, 0.15 kg/ha (1st application), Vitaplan, 0.12 kg/ha + Skor, 0.1 l/ha (2nd application); treatment 3 with Gamair, 0.15 kg/ha + Horus, 0.15 kg/ha (1st application), Gamair, 0.15 kg/ha + Skor, 0.1 l/ha (2nd application); treatment 4 with Fitosporin-M, 2 l/ha + Horus, 0.15 l/ha (1st application), Fitosporin-M, 2 l/ha + Skor, EC, 0.1 l/ha (2nd application); treatment 5 with Glyokladin, Zh, 3 l/ha (2 applications).

Since bacterial preparations are quite well compatible with chemical fungicides [1, 21, 22], they were used in the form of tank mixtures with half the consumption rates of chemical fungicides (Horus for the 1st application, Skor for the 2nd application).

The control, the standard, and each treatment were performed in 4-fold replication with a randomized location of plots of a 20 m² area each [23]. The first spraying was carried out during the bud swelling phase, the second after flowering, in the phase of active growth and development of shoots and leaves. The dates of sprayings with biologicals were shifted for a period from 2 to 11 days, depending on annual weather conditions. Spraying with solutions was carried out in the morning at an air temperature not higher than +24 °C. Since the effect of biofungicides is long-term [24], the intensity of the development of clusterosporiosis in all variants of the experiment was assessed 10 days after each spraying, and gray and brown rot of fruits 1 month after the termination of the treatments.

The degree of development of clusterosporium disease, moniliosis, and gray rot in fruits was determined according to the generally accepted method [23]. The intensity of disease development was assessed on a 5-point scale with the following gradations: 0 means no damage, 1 stands for up to 10% the leaf surface affected, 2 for 11-25%, 3 for 26-50%, and 4 for over 50% of the surface is affected.

The disease intensity index R (%), reflecting the severity of damage to each plot (variant of the experiment), was calculated by the Abbott's formula [25]:

$$R = \frac{\Sigma(a \cdot b)}{N \cdot K},$$

where $\Sigma(a \cdot b)$ is the sum of the number of diseased leaves (a) multiplied by the corresponding lesion score (b), N is total number of leaves (healthy and diseased), K is the highest score of the scale.

Biological efficiency (BE, %) was calculated by the formula:

$$BE = \frac{K - b}{K},$$

where K is the disease intensity in the control, b is the disease intensity in the test variant.

For a more complete assessment of cherry plum protection schemes, the yield (measured at the time of harvest) and the growth of shoots during the growing season (measured at the end of the growing season) were determined.

The obtained data were processed using the descriptive statistics in Microsoft Excel and Statistica 10 programs (StatSoft, Inc., USA) (26). The arithmetic mean (M) and standard errors of the means (\pm SEM) were calculated. A linear univariate analysis was used to compare several independent groups of data (experimental variants) combined by one attribute (processing variants) [23]. When assessing the ratio of intergroup variability, the Fisher's test (F -test) was used to test the null hypothesis of equality of means for samples – experimental variants, for a significance level of $p < 0.05$ [23]. The least significant difference (LSD₀₅) was calculated, i.e., a value indicating the border of possible random deviations in the experiment, that is, the minimum difference between the mean values of the degree of disease development and yield for each variant of the experiment and control.

Results. Weather conditions in the spring and summer months of 2015–2017 in Sochi did not significantly differ from the climatic norm [27] and contributed to the intensive development of the main diseases of the cherry plum. The maximum degree of development of clusterosporiosis and fruit rot was noted in 2015 (Table 1), which was facilitated by high air humidity and early onset of spring. The results indicate a fairly high efficiency of biofungicides (59.84–96.8%) in the protection of cherry plum from clusterosporiosis (Table 1).

1. Intensity of clusterosporiosis (DI, %) and biological effectiveness (BE, %) of protection schemes for cherry plum (*Prunus cerasifera* Ehrh.) cv. Obilnaya plants ($N = 4$, $M \pm$ SEM, production sites of the Oktyabrsky State Unitary Enterprise, Sochi)

Variant	2015		2016		2017	
	DI	BE	DI	BE	DI	BE
1	6.1±0.15	74.9±0.91	4.5±0.91	77.1±1.64	2.5±0.22	79.7±2.67
2	9.6±0.21	60.1±1.39	7.8±0.31	59.8±4.00	4.6±0.14	63.2±1.71
3	4.2±0.23	82.6±1.02	2.9±0.21	85.0±1.73	1.5±0.08	88.0±0.93
5	2.6±0.21	89.2±1.02	1.6±0.08	92.0±0.40	0.8±0.13	93.6±1.22
4	1.7±0.18	93.0±0.65	0.8±0.13	95.9±0.54	0.4±0.06	96.8±0.43
Standard	4.4±0.21	81.8±0.90	3.1±0.26	84.2±2.48	1.6±0.23	97.0±2.27
Control	24.1±0.49		20.0±1.98		12.6±0.77	
LSD ₀₅	0.56	2.99	0.27	8.40	0.96	5.12
	$F_0 = 1611.3 >$ $> F_t = 2.57$	$F_0 = 134.82 >$ $> F_t = 2.77$	$F_0 = 74.09 >$ $> F_t = 2.57$	$F_0 = 2.092 >$ $> F_t = 2.77$	$F_0 = 172.24 >$ $> F_t = 2.57$	$F_0 = 49.04 >$ $> F_t = 2.77$

Note. For a description of the variants, see the section "Materials and methods". The differences are statistically significant ($F_0 > F_t$) at the 95% level.

Over a 3-year period, the bacterial biofungicide Fitosporin-M showed the best result in protecting cherry plum from clusterosporiosis, the fungal biofungicide Glyokladin was in the second place (see Table 1), but it should be noted that it was used without a mixture with chemical fungicides. BE when treated with mixtures of bacterial biofungicides Fitosporin-M and Gamair with half the rates of chemical fungicides, as well as the use of Glyokladin in its pure form, gave a better effect than treatment with a full rate of chemical fungicides in the reference (Table 2). Even with the highest intensity of clusterosporiosis in June 2015, after the use of Fitosporin-M, Glyokladin, and Gamair, it was no higher than with the reference application of chemical fungicides, while Baktofit and Vitaplan were somewhat inferior to them in efficiency.

When using Glyocladin, even 1 month after the cessation of treatments, the intensity of clusterosporiosis was lower than when trees were treated only with chemical fungicides. This implies a prolonged protective effect of the fungal biofungicide, which is caused not only by the suppression of the causative agent of clusterosporiosis, but also by the activation of plant defense mechanisms [28].

For Stanley plum, the best protection against clusterosporiosis was also achieved when using Fitosporin-M and Glyokladin [1]. Similar results were obtained when testing biological products based on *B. subtilis* and *T. lignorum* in the

piedmont zone of the Krasnodar Territory to protect plums from clusterosporiosis, although in the latter case, the first treatment before bud blooming was carried out only with chemical fungicides, and after flowering, only biological products were used in pure form [22].

Biofungicides also showed good results against gray and monial brown fruit rot: the degree of fruit rot development significantly ($p < 0.05$) decreased compared to the control (Table 2), although BE of all preparations against monial brown rot was lower than for clusterosporiosis (see Table. 1) and gray rot (Table 3).

2. Biological effectiveness (BE) of various protection schemes for cherry plum (*Prunus cerasifera* Ehrh.) cv. Obilnaya plants against monial brown rot ($N = 4$, $M \pm SEM$, production sites of the Oktyabrsky State Unitary Enterprise, Sochi)

Variant	2015	2016	2017
1	62.2±3.03	68.5±2.69	71.6±3.74
2	55.4±1.79	46.0±2.28	56.6±3.24
3	75.7±2.54	77.9±2.74	81.0±2.24
5	76.5±2.65	78.8±3.01	85.0±2.41
4	80.1±3.23	82.8±4.53	87.1±3.14
Standard	69.9±2.55	72.2±3.25	80.3±3.32
LSD ₀₅	9.73	7.83	7.01
	$F_0 = 11.06 > F_t = 2.77$	$F_0 = 29.80 > F_t = 2.77$	$F_0 = 23.04 > F_t = 2.77$

Note. For a description of the variants, see the section "Materials and methods". The differences are statistically significant ($F_0 > F_t$) at the 95% level.

3. Biological effectiveness (BE) of various protection schemes for cherry plum (*Prunus cerasifera* Ehrh.) cv. Obilnaya plants against gray rot ($N = 4$, $M \pm SEM$, production sites of the Oktyabrsky State Unitary Enterprise, Sochi)

Variant	2015	2016	2017
1	67.6±2.60	74.5±3.23	77.8±2.35
2	58.4±2.74	51.1±1.64	61.5±2.55
3	82.3±3.85	84.7±1.97	87.1±2.37
5	83.1±4.19	85.7±1.78	88.5±3.11
4	87.1±13.4	90.0±2.56	92.4±1.71
Standard	76.0±2.77	78.5±3.86	87.3±1.78
LSD ₀₅	9.73	7.83	7.01
	$F_0 = 11.06 > F_t = 2.77$	$F_0 = 29.80 > F_t = 2.77$	$F_0 = 23.04 > F_t = 2.77$

Note. For a description of the variants, see the section "Materials and methods". The differences are statistically significant ($F_0 > F_t$) at the 95% level.

As in the case of clusterosporiosis, all tested biological products showed a sufficiently high biological effectiveness across a three-year experiment. For Fitosporin-M, Glyokladin, and Gamair, it exceeded at least 1.1-1.2 times ($p < 0.05$) that observed in the standard (see Tables 2 and 3). The maximum effect was observed when Fitosporin-M was used in a tank mixture with chemical fungicides, while Glyokladin and Gamair were inferior in effectiveness. Bactofit and Vitaplan have shown the lowest efficiency in the protection of cherry plum from fruit rot in comparison with other biofungicides: for both biological products, BE was always lower than the standard by 20-40% (see Tables 2 and 3).

The best result in the fight against gray rot was obtained when using half rates of Horus and Skor in combination with Fitosporin-M (87.1-92.4%). Biofungicides Glyokladin and Gamair showed higher biological effectiveness than the reference, especially compared to Vitaplan, which also had the least effectiveness.

In general, the yield of cherry plum reflected the degree of the protective effect of each tested drug: it turned out to be the highest when using Fitosporin-M; however, Glyokladin and Gamair were inferior to it. So, in 2015, the highest yield was in trees treated with Gamair (Table 4).

Due to the intensive growth of plant pathogenic fungi, the yield of cherry plum in the control was significantly (1.3-2 times, $p < 0.05$) lower than in all

experimental variants (see Table 4), and the indicators of preserved yield in variants of application of biological preparations reached 9.7-11.5 t/ha. With Fitosporin-M, Gamair and Glyokladin, the yield exceeded 1.7-1.9-fold ($p < 0.05$) the standard, with Baktofit, the results were almost the same, and with Vitaplan, the yield was lower than in the standard, but 1.1-1.3 times higher than in the control (see Table 4).

4. Yield (t/ha) of cherry plum (*Prunus cerasifera* Ehrh.) cv. Obilnaya plants depending on treatment with biofungicides ($N = 4$, $M \pm SEM$, production sites of the Oktyabrsky State Unitary Enterprise, Sochi)

Variant	2015	2016	2017
1	8.7±0.55	9.2±0.23	9.4±0.18
2	7.0±0.25	6.6±0.27	6.0±0.29
3	9.8±0.35	10.0±0.25	10.2±0.33
5	9.7±0.13	10.9±0.35	11.5±0.20
4	9.7±0.30	10.5±0.30	11.0±0.35
Standard	8.9±0.29	9.1±0.27	9.0±0.27
Control	5.4±0.26	5.7±0.27	5.6±0.24
LSD ₀₅	0.96	0.82	0.81

$$F_0 = 26.02 > F_i = 2.57 \quad F_0 = 49.82 > F_i = 2.57 \quad F_0 = 71.77 > F_i = 2.57$$

Note. For a description of the variants, see the section "Materials and methods". The differences are statistically significant ($F_0 > F_i$) at the 95% level.

It is known that the yield of cherry plum significantly depends on the intensity of development of annual shoots during the growing season, and the studied diseases, especially clusterosporiosis, inhibit the growth of shoots [1]. Consequently, the value of the annual growth of shoots can be an important indicator for assessing both the protective and growth-stimulating effects of the tested protection agents. Based on the results of measurements of the length of axial shoots of the current year in the autumn season after the completion of growth (Table 5), biofungicides Fitosporin-M, Gamair and especially Glyokladin in the experimental variants showed better results than chemical fungicides in the reference: the use of biological products led to a 1.2-1.7-fold ($p < 0.05$) increase in the average length shoots of the current year compared the control (see Table 5).

5. Shoot growth (cm/year) of cherry plum (*Prunus cerasifera* Ehrh.) cv. Obilnaya plants depending on treatment with biofungicides ($N = 4$, $M \pm SEM$, production sites of the Oktyabrsky State Unitary Enterprise, Sochi)

Variant	2015	2016	2017
1	62.2±3.00	67.6±2.60	74.9±0.90
2	55.4±1.80	58.4±2.70	60.1±1.40
3	75.7±2.50	82.3±3.90	82.6±1.00
5	76.5±2.70	83.1±4.20	89.2±1.00
4	80.1±3.20	87.1±13.40	93.0±0.70
Standard	69.9±2.60	76.0±2.80	81.7±0.90
Control	45.9±2.30	51.1±1.60	55.4±1.80
LSD ₀₅	0.27	0.56	0.82

$$F_0 = 74.09 > F_i = 2.57 \quad F_0 = 1611.3 > F_i = 2.57 \quad F_0 = 49.82 > F_i = 2.57$$

Note. For a description of the variants, see the section "Materials and methods". The differences are statistically significant ($F_0 > F_i$) at the 95% level.

Particularly noteworthy was the effect of Glyokladin which provided maximum growth of shoots over a three-year experiment. The length of annual shoots was approximately 1.7 times greater than in the control ($p < 0.05$). Fitosporin-M showed the best results, although the length of shoots were close to those for Glyokladin. In our opinion, it is due to not only a pronounced immunomodulatory, but growth-stimulating effect of this fungal biological product [29]. With Baktofit and Vitaplan, the increase exceeded the control values only 1.1-1.2 times and was 15-25% lower than the reference, while the treatment with chemical preparations alone in the reference gave an average increase of 1.5 times as

compared to the control.

Therefore, biofungicides Baktofit, Vitaplan, Gamair, and Fitosporin-M based on *Bacillus subtilis* and *Trichoderma harzianum* could be successfully used against the main cherry plum diseases in the subtropics of the Krasnodar Territory in mixtures with half the norms of chemical fungicides. Our findings confirm the high efficiency of biological preparations, in 2015–2017, the highest efficiency against clusterosporiosis was 93–97%, against moniliosis 80–87%, and against gray rot 87–92%. The highest yield of cherry plum in these years was 9.8–11.5 t/ha, the annual growth of the shoots reached 80–93 cm.

Particularly noteworthy was the effect of Glyokladin, with the use of which the growth of cherry plum shoots was maximal according to the results of three-year experiment: the length of annual shoots was approximately 1.7 times greater than the length of similar shoots in the control ($p < 0.05$). The best results were shown by Fitosporin-M, although the length of shoots were close to that with the use of Glyokladin. In our opinion, it is due to not only a pronounced immunomodulatory but also growth-stimulating effect of this fungal biological product [29]. In the experimental variants with Baktofit and Vitaplan, the increase exceeded the control values only by 1.1–1.2 times and was 15–25% lower than the reference, while the treatment with chemical preparations alone in the reference gave an average increase of 1.5 times as compared to the control.

Therefore, biofungicides Baktofit, Vitaplan, Gamair, and Fitosporin-M based on *Bacillus subtilis* and *Trichoderma harzianum* could be successfully used against the main cherry plum diseases in the subtropics of the Krasnodar Territory in mixtures with half the norms of chemical fungicides. The studies carried out make it possible to judge the high efficiency of biological preparations. In 2015–2017, the highest efficiency against clusterosporiosis was 93–97%, against moniliosis 80–87%, and against gray rot 87–92%. The highest yield of cherry plum in these years was 9.8–11.5 t/ha, the value of the annual growth of the shoots of cherry plum trees reached 80–93 cm.

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