Microbiological and bacterial diseases of apple fruits during storage

Elena Balykina[1], *Olga* Ivanova[1], *Larisa* Yagodinskaya[1], *Dmitry* Korzh[1,*], and *Tatiana* Rybareva[2]

[1]Federal State Funded Institution of Science "The Labor Red Banner Order Nikitsky Botanical Gardens – National Scientific Center of the Russian Academy of Sciences", Yalta, Republic of the Crimea, Russian Federation
[2]Federal State Funded Institution "All-Russian Plant Quarantine Center", branch in the Republic of the Crimea, Simferopol, Republic of the Crimea, Russian Federation

Abstract. During storage, fruits are affected by a complex of pathogens, from which one pathogen of bacterial origin and 11 of fungal etiology have been isolated. It was determined that the treatment of apple fruits before dispatch for storage with fungicides with the following rates of application: Luna Tranquillity, SC –1.0 l/ha, Geox, WDG–0.4 kg /ha, Zato, WDG–0.15 kg /ha allows to save up to 98% of the crop yield. At the same time, the waste contained fruits with mechanical damage affected by the mold fungus *Penicillium roseum* LK. Biological efficacy of Luna Tranquillity, SC, Zato, WSG and combinations of these fungicides against *Penicillum roseum* LK. were at the level of 81-95%, no other diseases were detected in these variants. It was found that fungi were found on fruits treated with a combination of fungicides Topsin - M, WP–1.5 kg/ha in combination with Merpan, WP–2.5 kg/ha and Medea preparation, ME–1.0 l/ha; such fungi were *Fusarium avenaceum* (fr.) Sacc, *Gloeosporium fructigenum* Berk, *Penicillum roseum* LK.

1 Introduction

The demand for fresh fruit increases by more than 10% annually. Increasing production rate does not ensure a stable and uniform supply of fresh products to the consumer. Solving the problem requires not only equipping the horticulture industry with modern equipment, but also the use of advanced fruit storage technologies. The storability (shelf life) is determined by the ability of fruits to remain for a certain time without significant weight loss and degradation of commercial qualities [6].

The quality and storability of fruit batches are formed under the influence of a complex of environmental, biological, agrotechnical factors that can act as sources and activators of fruit growth and development, as well as triggers for the development of physiological diseases and injuries, or contribute to an increase in the degree of manifestation of damages. Diseases and damages “acquired” by fruits during the pre-harvest period are most often intensified during storage, resulting in losses of up to 30% or more.

* Corresponding author: ent.protection@yandex.ru

The key factors determining the vital activity of fruits in the period after harvesting are temperature (T°C), as well as the content of oxygen (O_2) and carbon dioxide (CO_2) and relative humidity (%) in the storage atmosphere [8, 11, 12, 13, 14, 15, 16, 17]. According to the results of studies by domestic and foreign authors, the main cause of diseases development and a decrease in product quality is the excessive accumulation of ethylene (C_2H_4) inside fruits and the environment. It is synthesized by fruits or comes from the environment and in extremely low concentrations activates their maturation, overripe and aging, which leads to the development of many physiological and fungal diseases, loss of quality. The most effective inhibition of ethylene is provided in a dynamic controlled atmosphere. In the last period, the dynamic regulated atmosphere has been mastered in some countries. However, this technology imposes a number of special requirements for the tightness of the chambers, equipment that ensures the creation and maintenance of the specified atmosphere parameters, the qualifications of technical personnel, the quality of fruits, including their physiological uniformity, which increases the costs of its implementation [5, 9, 10].

The quality and storability are influenced by the activities carried out during the pre-harvest period and the technology of harvesting. To reduce the infectious background and improve the preservation of the pomaceous fruits, their chemical treatment is often used in the last weeks before harvesting. The percentage of crop preservation by chemical method is 16-20% [3, 4].

As follows from the publications of domestic and foreign authors, climatic changes have led to significant changes in the species structure of mycopathocomplexes on many agricultural crops. In this regard, some species of the genus *Alternaria* have acquired traits of facultative parasitism and complete parasitism on various agricultural crops, including apples. One of the most widespread and harmful diseases of agricultural crops are fungi of the genus *Fusarium* Link, affecting more than 200 species of cultivated and wild plants. On the culture of apple trees, it causes heart sickness of fruits. This is not a complete list of pathogens that cause fruit rot [1, 2, 7].

The objective of the research: to establish a complex of pathogens that causes rot of apple fruits during storage and to determine the effectiveness of fungicides to manage their development.

2 Materials and methods of research

The research was conducted in 2019-2021 in the Laboratory of Entomology and Phytopathology of the Nikitsky Botanical Gardens - National Scientific Center of the Russian Academy of Sciences. Before dispatching for storage, the fruits of apple cultivars ‘Ligol’, ‘Renet Simirenko’, ‘Fuji’ and ‘Champion’ were treated, 10 days before harvesting, with fungicides with application standards: Luna Tranquillity, SC–1.0 l/ha, Geox, WDG–0.4 kg/ha, Zato, WDG–0.15 kg/ha, Topsin-M, WP–1.5 kg /ha in combination with Merpan, WP–2.5 kg /ha, Medea, ME–1.0 l/ha and Delan, WSG–0.75 kg/ha. Control – two containers with non-treated fruits. Apple tree fruits were stored in industrial refrigerating chambers in a controlled atmosphere at a temperature of 1.0-4.0°C and an oxygen content of 3-4% and carbon dioxide – 3-5% and a relative humidity of 94-96% of AO "Pobeda" (stock company) of the Nizhnegorsky district and OOO SO "Kurskoe" (limited liability company, agricultural association) of the Belogorsky district of the Republic of the Crimea.

Apple fruit samples were selected three times to determine the species composition of pathogens and the effectiveness of fungicides: 1 - 30 days after the placement in storage; 2 - 60 days later and 3 - 90 days later, 100 fruits from each variant of the experiment and control. After each selection, the number of healthy and affected apples was counted.

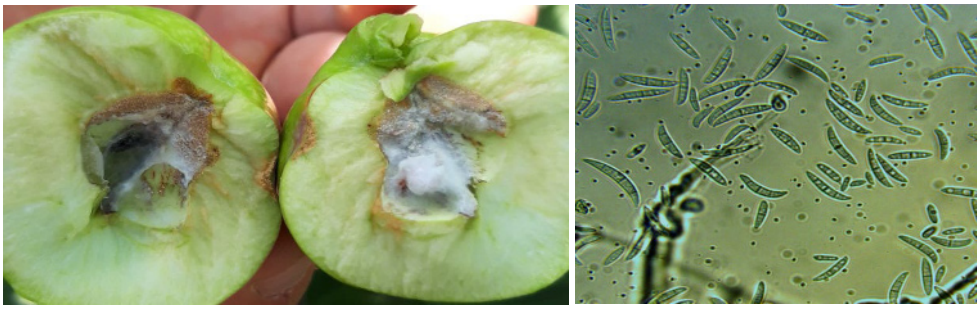
The selected material was analyzed in laboratory conditions by inoculation on nutrient media of washing-off from 10 apples in 200 ml of sterile water. Then the suspension was replanted on agar culture media of Chapek (for fungi) and CDA (for bacteria). In the presence of damage on the fruit, a segment on the edge of the diseased/healthy tissue was cut off from this part of the apple, sterilized in alcohol and laid out in a wet chamber and on a nutrient medium in Petri dishes. Cultivation was carried out in a thermostat at T = 28°C for 24-96 hours. Thus, a mixed culture of microorganisms formed on the medium was obtained, separate colonies of the same type of bacteria or mycelium of fungi were moved out to obtain a pure culture of pathogens. Morphological, cultural and physiological properties were studied in the isolated strains of microorganisms, and later they were identified by generally accepted phytopathological methods.

3 Results and discussion

In the process of studying the material affected during storage (apple fruits), the following pathogens were isolated: bacteria *Erwinia amylovora* (Burrill) Winslow et al. (brown spots on the skin of apples without smell) - 4%; *Penicillium expansum* (Lk.) Thom (wet rot with a strong smell) - 37%; fungi *Botrytis cinerea* Pers (dry gray rot) - 8%; *Gloeosporium fructigenum* Berk, *Cryptosporiopsis curvispora* (Peck) Gremmen., (bitter rot, anthracnose) - 5%; *Alternaria tenuis* Nees, *Alternaria alternata* (Fr.:Fr.) Keissl (finely-spotted dry, black rot with penetration into the fruit) - 11%; *Fusarium avenaceum* (Fr.) Sacc (white rot of the core) - 7%; *Rhizopus nigricans* Her. - 10%; *Trichothecium roseum* (Pers.:Fr.) (pink rot) - 3%; *Monilia fructigena* Pers.:Fr. (mummified fruits) - 7%; *Sphaeropsis malorum* Pk. (diplodia, black rot) - 6%; *Fusicladium dendriticum* (Wallr.) Fckl, (warehouse scab) - 5%; *Gloeodes pomigena* (Schwein.) (cloud of pome fruits) - 4%. From the data obtained, it follows that only the causative agent of penicilliosis rot infects apples in the storage, while other pathogens make it into together with fruits from the garden. At the same time, they can destroy from 30% to 50% of the fruits.

As a result of three-year observations, it was found that during the storage of fruits, pathogens of bacterial and fungal diseases contribute to the development of rot and lead to the death of 20 to 50% of the harvested crop. In wet years, the development of diseases increases, pathogens form phytopathogenic complexes. In the absence or improper use of fungicides during the years of excessive moisture, not only the quality of the fruits decreases, but also the probability of their damage by rot during storage increases. Often, infection of the future crop with a complex of pathogens occurs during the flowering period. It was found that at this time the pathogens *Fusarium avenaceum* (Fr.) Sacc., *Monilia cinerea* Pers., *Trichothecium roseum* Lk, *Alternaria tenuis* Nees, *Alternaria alternate*, *Cryptosporiopsis curvispora* (Peck) Gremmen penetrate into the apple blossoms.

Fusarium affect most often occurs during maturation and storage. Nevertheless, in 2021, it appeared already at the end of June. Green, deformed fruits had traces of suberification in the seed chamber and well-developed mycelium *Fusarium avenaceum* (Fr.) Sacc (Fig.1).



A **B**

Fig. 1. Mycelium *Fusarium avenaceum* (Fr.) Sacc inside the seed chamber of an immature apple (**A**). pathogen (**B**). Crimea, Belogorsky district, OOO SO "Kurskoe", June 2021

Trichothecium roseum Lk causes pink mold-like rot of pears and apples stored. In the garden, the pathogen penetrates into the forming and growing fruits by drying pistils and stamens, later through mechanical injuries of the fruit skin or through damage by pests. A rotting brown spot forms around the place of attachment of the peduncle on the apple. Then it becomes whitish (because of mycelium), and then pink - when the spores mature. The fungus produces toxic substances. About one hundred trichotecin mycotoxins are known. The mechanism of toxic action is based on the ability to inhibit protein synthesis.

Over the past three years, anthracnose has been observed in the fruits of apple cultivars ‘Ligol’ and ‘Champion’. A characteristic sign of the disease on fruits is a "red bull's eye" (Fig. 2). It manifests itself during maturation and storage. Infection occurs in rainy years through damage to leaves and shoots, through flowers during the flowering period and for a month after it. In years with warm and humid winters, it can be carried out throughout the winter and in early spring through cracks and frost cracks.

A **B** **C**

Fig. 2. Manifestation of *Cryptosporiopsis curvispora* on the fruits of the cultivars ‘Ligol’ (**A**), ‘Champion’ (**B**), mycelium and anthracnose spores (**C**). Crimea, Belogorsky district, OOO SO "Kurskoe", 2021

In addition to the above diseases, during storage, lesions of fungi by pathogens of monilia -*Monilia fructigena* Pers (Fig. 3-A), black cancer - *Diplodia malorum* Fuck (Fig. 3-B), botrytis – *Botrytis cinerea* Pers (Fig. 3-C), Alternaria spot – *Alternaria tenuis* Nees (Fig. 3-D), the "warehouse" form of scab were detected. It has been established that the infection on apples often has a mixed character. *Alternaria spp* (Fig. 3-D) or *Rhizopus spp.* (Fig. 3-F) can be detected simultaneously on bacterial lesions during storage.

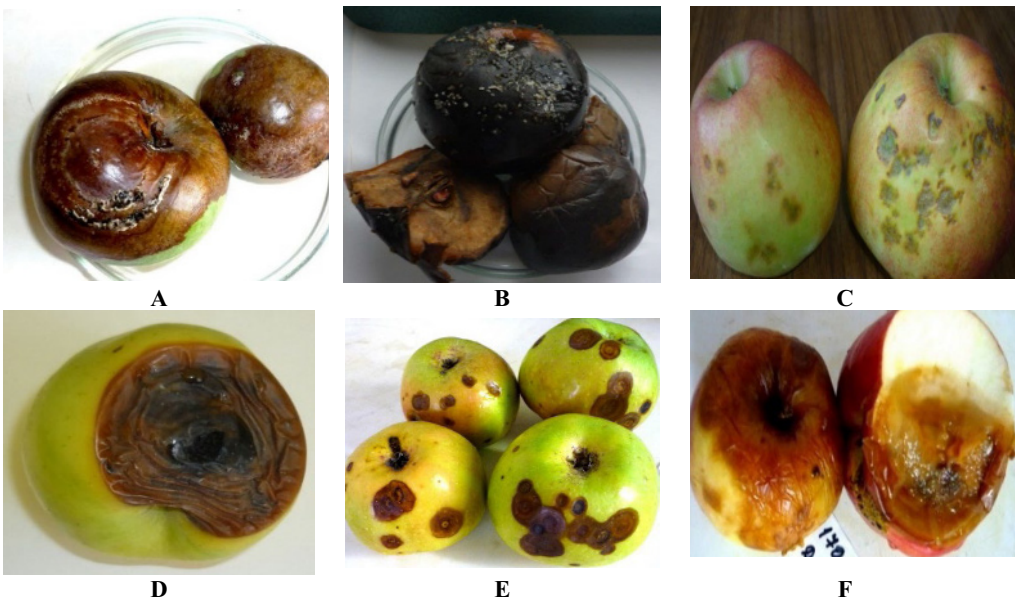


Fig. 3. Symptoms of apple damage during storage with *Monilia fructigena* Pers (**A**), *Diplodia malorum* Fuck (**B**), *Botrytis cinerea* Pers (**C**), *Alternaria tenuis* Nees (**D**), and mixed infection (**E**) and (**F**).

To prevent the development of storage diseases before harvesting, the fruits were treated with fungicides Luna Tranquillity, SC, Geox, WDG, Zato, WDG, Topsin-M, WP in combination with Merpan, WP, Medea, ME and Delan, WSG (Table 1). When placing in storage, fruits from all variants of experiment and control had no visible rot damage.

30 days after the dispatching for storage, the lesion of the fruits in the control containers amounted to 10% of the weight of the fruits stored. The pathogens of alternariosis and moniliosis were detected on fruits treated with the fungicide Medea. The fruits treated with the fungicide Delan were most severely affected, and spores of 7 pathogens were present (Table 1).

Table 1. Dynamics of sporulation of pathogens of apple fruit diseases during storage (day after placing in storage). Crimea, Nizhnegorsky district, AO "Pobeda", 2019-2020.

Preparation, rate of use, l, kg/ha	*Alternaria alternata* Fc.)	*Venturia inaegualis* Cocke,	*Cladosporium herbarum* (Pers.) Lk	*Monilia fructigena* Pers	*Fusarium avenaceum* (Fr.) Sacc	*Rhizopus nigricans* Her.	*Gloeosporium fructigenum* Berk.,	*Stemphilium sp.*,	*Penicillum roseum* LK.	*Botrytis cinerea* Pers
	Spore/cm^2 surface, after 30 days (Dec, 2019)									
Luna Tranquillity, SC1.0	0	0	0	0	8	0	0	0	14	0
Zato, WSG 0.15	7	0	0	0	16	0	0	0	16	0
Luna Tranquillity, SC + Zato, WSG 1.0 + 0.15	0	0	0	0	0	0	0	0	0	0
Geox, WDG 0.4	0	0	0	0	0	0	0	0	32	0
Topsin-M, WP 1.5 kg/ha + Merpan, WP 2.5 kg/ha	0	0	0	0	33	3	18	0	15	0
Medeya, ME 1.0	23	0	0	32	0	0	0	0	0	0

Delan, WSG 0.75 kg/ha	15	22	12	16	37	0	0	2	27	0
Control	129	306	93	69	72	39	107	0	77	38
	after 60 days (Jan, 2020)									
Luna Tranquillity, SC1.0	0	0	0	0	0	0	0	0	7	12
Zato, WSG 0.15	0	0	0	0	19	0	0	0	25	18
Luna Tranquillity, SC + Zato, WSG 1.0 + 0.15	0	0	0	0	0	0	0	0	6	14
Geox, WDG 0.4	0	0	0	0	0	0	0	0	42	26
Topsin-M, WP 1.5 kg/ha + Merpan, WP 2.5 kg/ha	5	0	0	0	28	0	39	0	45	50
Medeya, ME 1.0	18	0	0	60	0	0	0	0	23	30
Delan, WSG 0.75 kg/ha	62	27	64	36	72	0	0	12	107	29
Control	129	306	93	69	72	39	107	0	77	38
	after 90 days (Feb, 2020)									
Luna Tranquillity, SC 1.0	0	0	0	0	0	0	0	0	9	10
Zato, WSG 0.15	0	0	0	0	0	0	0	0	32	16
Luna Tranquillity, SC + Zato, WSG 1.0 + 0.15	0	0	0	0	0	0	0	0	10	12
Geox, WDG 0.4	0	0	0	0	0	0	0	0	42	37
Topsin-M, WP 1.5 kg/ha + Merpan, WP 2.5 kg/ha	9	0	0	0	26	0	10	0	59	47
Medeya, ME 1.0	22	0	0	68	0	0	0	0	32	38
Delan, WSG 0.75 kg/ha	70	59	19	46	63	86	0	29	16	173
Control	378	451	265	192	242	126	207	0	165	63

0 - no pathogen; **up to 30 spore/cm^2** – low degree; **from 30 to 100 spore/cm^2** – average degree; **more than 100 spore/cm^2** – high degree of infestation

On the 60th day after the placing in storage, single spores of pathogens appeared on apples treated with a combination of fungicides Topsin-M, WP 1.5 kg / ha + Merpan, WP 2.5 and Zato, WSG 0.15. The infectious background also increased when treated with Delan, WSG and Medea, ME. 3 months later, on the 90th day after the dispatch for storage, apples remained relatively clean after the use of fungicides Luna Tranquillity, SC, Zato, WSG and Geox, WDG. In the control for three months, half of the fruits stored were affected.

It should be noted that none of the preparations used completely restrained the development of *Penicillum roseum* LK. It was present in all variants of the experiment. The strongest causative agent of this disease affected fruits with mechanical damage from the fingers, "incisions" from the petioles of fruits, pressed-in "wounds" from containers. Biological efficacy of fungicides Luna Tranquillity, SC, Zato, WSG and combinations of these fungicides in relation to *Penicillum roseum* LK. was at the level of 81-95%. Other preparations have shown slightly lower efficacy, with the exception of Delan, WSG, whose has negative efficacy against these diseases (Fig. 4).

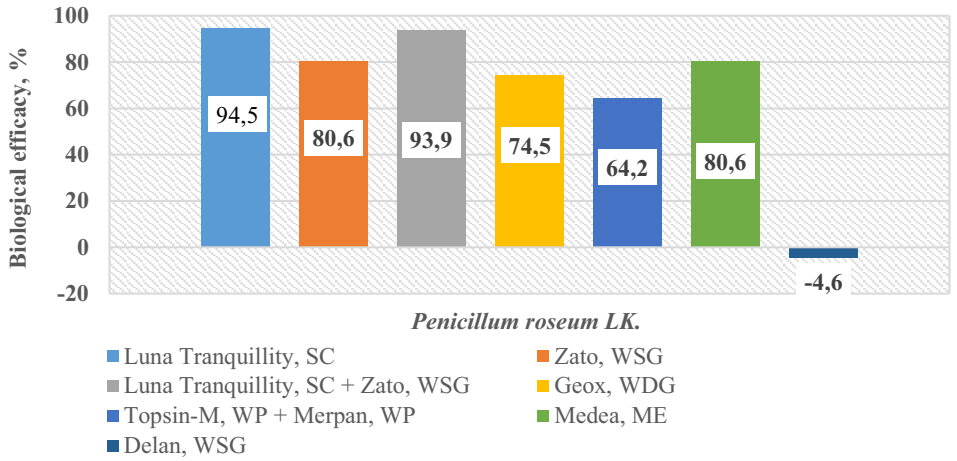


Fig. 4. Biological efficacy of fungicides against *Penicillium roseum* LK. Crimea, Nizhnegorsky district, AO "Pobeda", 2019-2020.

The final accounting of fruit infestation at the end of storage showed that crop losses without pretreatment with fungicides can reach 60.0% or more. The best preservation is ensured by the use of fungicides Luna Tranquillity, SC, Zato, WSG and Geox, WDG (Table 2).

Table 2. Percentage of waste in the experiment variants after storage. Accounting in March 2020 Crimea, Nizhnegorsky district, AO"Pobeda", 2020.

Variant	Total weight of fruits, kg	Amount of affected fruits	
		kg	%
Luna Tranquillity, SC 1.0	600.0	9.6	1.6
Zato, WSG 0.15	600.0	12.3	2.1
Luna Tranquillity, SC + Zato, WSG 1.0 + 0.15	600.0	8.3	1.4
Geox, WDG 0.4	600.0	10.5	1.75
Topsin-M, WP 1.5 kg/ha + Merpan, WP 2.5 kg/ha	600.0	19.5	3.3
Medeya, ME 1.0	600.0	18.6	3.1
Delan, WSG 0.75 kg/ha	600.0	187.0	31.2
Control	200.0	122.0	61.0

Another important cause of fruit rot during storage is mechanical damage that occurs during harvesting and transportation of the crop. This particular factor often provokes manifestations of latent infection that accumulates in fruits during the growing season. An essential factor that ensures long-term and high-quality fruit storage is the cleanliness of refrigerators, containers and storage rooms, where apples are placed for storage for a long period.

4 Conclusions

The results of laboratory studies of apple fruits treated with six different fungicides before their storage allow us to draw the following conclusions:

- ✓ During the storage period, apples are affected by a complex of pathogens, from which we have isolated one pathogen of bacterial origin and 11 of fungal etiology. The causative agent of penicilliosis rot infects apples in storage, while the rest are brought

along with fruits from the garden. At the same time, *Penicillium expansum* (Lk.) Thom can destroy from 30% to 50% of fruits.

- ✓ It has been established that the treatment of apple fruits before dispatching for storage with fungicides with application standards: Luna Tranquillity, SC – 1.0 l/ha, Geox, WDG – 0.4 kg/ha, Zato, WSG – 0.15 kg/ha allows us to save up to 98% of the crop. At the same time, the waste contained fruits with mechanical damage affected by the mold fungus *Penicillum roseum* LK. Biological efficacy of Luna Tranquillity, SC, Zato, WSG and combinations of these fungicides against *Penicillum roseum* LK. were at the level of 81-95%, no other diseases were detected in these variants. A number of pathogens were found on fruits treated during storage with a combination of fungicides Topsin-M, WP – 1.5 kg/ha in combination with Merpan, WP – 2.5 kg/ha and Medea, ME – 1.0 l/ha during storage. Such fungi are the following: *Fusarium avenaceum* (Fr.) Sacc, *Gloeosporium fructigenum* Berk, *Penicillum roseum* LK. The fungicide Delan, WSG – 0.75 kg/ha showed weaker fungicidal protection against storage diseases. Nine pathogens were detected on the fruits, storage losses amounted to 31.4%.
- ✓ At the end of storage in the control, the waste amounted to more than 50%, in the variants with the use of Luna Tranquillity, SC, Zato, WSG and Geox, WDG, the percentage of waste was 1.4–1.75%, in the variants with Medea, ME and the combination Topsin-M, WP + Merpan, WP the percentage of waste was 3.1 – 3.3%.
- ✓ An important cause of fruit rot during storage is mechanical damage that occurs during harvesting and transportation of the crop. This factor is often provoked by manifestations of latent infection accumulating in fruits during the growing season.

References

1. I.L. Astapchuk, G.V. Yakuba, A.I. Nasonov, Fruit growing and viticulture of the South of Russia, **68(2)**, 255-271 (2021) (in Russian) DOI: 10.30679/2219-5335-2021-2-68-255-271
2. I.L. Astapchuk, G.V. Yakuba, A.I. Nasonov, Fruit growing and viticulture of the South of Russia, **71(5)**, 219-233 (2021) (in Russian) DOI: 10.30679/2219-5335-2021-5-71-219-233
3. E.B. Balykina, L.P. Yagodinskaya, O.V. Ivanova, D.A. Korzh, *Systems of protection of fruit crops from pests and diseases (guidebook)*, 40 (Simferopol, 2017) (in Russian)
4. E.B. Balykina, L.P. Yagodinskaya, O.V. Ivanova, NOVATION, **3-3,** 4-9 (2016) (in Russian)
5. V.A. Gudkovsky, L.V. Kozhina, Yu.B. Nazarov, R.B. Gucheva, Achievements of science and technology of agriculture **30 (9)**, 105-108 (2016) (in Russian)]
6. V.I. Kopylov, E.B. Balykina, I.B. Berenstein, V.A. Burlak, N.E. Opanasenko, V.A. Ryabov, et al., *Modern intensive fruit growing in the Crimea,* 548 (Simferopol: PH "ARIAL", 2017) (in Russian)]
7. G.V. Yakuba, I.L. Astapchuk, A.I. Nasonov, Fruit growing and viticulture of the South of Russia, **60(6)**, 148-162 (2019) (in Russian) DOI: 10.30679/2219-5335-2019-6-60-148-162
8. V.C. Florian, C. Puia, R. Groza, A. Suciul, T. Florian, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, **46(2)**, 538-545 https://doi.org/10.15835/nbha46211194
9. V.A. Gudkovsky, L.V. Kozhina, Y.B. Nazarov, A.E. Balakirev, R.B. Gocheva Dostizheniya nauki i tekhniki APK, **33 (2),** 61-67 (2019) (in Russ.) DOI: 10.24411/0235-2451-2019-10215.

10. V.A. Gudkovsky, L.V. Kozhina, Yu.B. Nazarov, A.V. Sutormina, D.V. Akishin, IOP Conf. Series: Earth and Environmental Science **845,** 012099 (2021) doi:10.1088/1755-1315/845/1/012099
11. Ifigeneia Mellidou, Kim Buts1, Darwish Hatoum, Quang Tri Ho, Jason W Johnston, Christopher B Watkins, Robert J Schaffer, Nigel E Gapper, Jim J Giovannoni, David R Rudell, Mellidou et al. BMC Plant Biology, **14**, 328 (2014)
12. Marcel Wenneker, Bart P. H. J. Thomma, European Journal of Plant Pathology, **156**, 663–681 (2020) https://doi.org/10.1007/s10658-020-01935-9
13. J.C. Onik, Y. Xie, Y. Duan, X. Hu, Z. Wang, Q. Lin, PLoS ONE, **14(4)**, e0215472 (2019) https://doi.org/10.1371/journal.pone.0215472
14. Paul Abi Tarabay, Hala Chahine-Tsouvalakis, SalwaTohmé Tawk, Nabil Nemer, Wassim Habib, Annals of Agricultural Sciences, **63** (2), 207-213 https://doi.org/10.1016/j.aoas.2018.11.006
15. Yu.V. Plugatar, A.I. Sotnik, O.A. Denisova, R.D. Babina, N.N. Gorb, BIO Web of Conferences **39**, 07002 (2021) https://doi.org/10.1051/bioconf/20213907002
16. J.M. Sjöstrand, I.I. Tahir, Hovmalm H. Persson, H. Stridh, M.E. Olsson, Acta Hortic, **1325**, 7-12 (2021) DOI: 10.17660/ActaHortic.2021.1325.2
17. Vitalijs Radenkovs, Karina Juhnevica-Radenkova, International Journal of Fruit Science, **18 (3)**, 268-286 (2018) https://doi.org/10.1080/15538362.2017.1422451